UNIVERSITÄT BERN

Graduate School for Cellular and Biomedical Sciences

University of Bern

Zika virus: causality, open science and risk of emerging infectious diseases

PhD Thesis submitted by

Michel Jacques Counotte

from **The Netherlands**

for the degree of

PhD in Biomedical Sciences

Supervisor
Prof. Dr. Nicola Low
Institute of Social and Preventive Medicine
Faculty of Medicine of the University of Bern

Co-advisor
Prof. Dr. Julian Higgins
Population Health Sciences
Bristol Medical School of the University of Bristol

Contents

Abstract

The Zika virus (ZIKV) outbreak in the Americas between 2015 and 2017 took the world by surprise. Within two years, over 1.5 million suspected or confirmed cases were reported. However, the true incidence is likely much higher, due to under-reporting and asymptomatic infections that are undetected. As of July 2019, 87 countries had reported ongoing or past circulation of ZIKV. ZIKV infection results generally in mild and transient symptoms. The disease caused by ZIKV is often asymptomatic or mild. However, infection during pregnancy can result in severe adverse congenital outcomes with microcephaly as most prominent. This was first noted in clusters of infants born with disabilities linked to ZIKV infection in Brazil in 2015, making ZIKV a disease with a serious public health impact. In this thesis, I explore different aspects of the ZIKV epidemic. I use different epidemiological methods to provide insight in the Zika virus as a cause of adverse outcomes, ZIKV as a sexually transmitted disease and the risk of future ZIKV outbreaks.

In Chapter 1, I provide an introduction to the history of emerging infections and the emergence of ZIKV specifically. I describe the investigation of causality, the use and accumulation of evidence during disease outbreaks, and how disease transmission can be investigated using mathematical models.

In Chapter 2, I provide insight in how evidence accumulates during an outbreak and more in general during new causal questions. Case reports and case series were the first studies to appear, followed by basic research (*in vivo* and *in vitro* studies). It took more than a year after the onset of the ZIKV outbreak for robust epidemiological studies to be published. Establishing early public health guidance thus requires a broad approach taking into account all evidence available. We have to make do with the low quality evidence. To minimize further delays, evidence should be accessible as soon as it becomes available through rapid and open access dissemination.

In Chapter 3, I extend a systematic review that was conducted earlier, and turn it into a living systematic review. I introduce the concept and implementation of living systematic reviews in the context of an emerging disease. I assess the evidence on the causal relation between ZIKV infection and adverse congenital and auto-immune neurological outcomes, published between May 30, 2016 and January 18, 2017, using a framework based on the causality dimensions of Bradford Hill. During this period, the evidence expanded that ZIKV was indeed a cause of congenital abnormalities and Guillain-Barré syndrome (GBS). I provide a proof of concept for the use of living systematic reviews to synthesize evidence about an emerging pathogen such as ZIKV.

In Chapter 4, I assess the evidence published between January 18, 2017 and July 1, 2019. I quantify the strength of association of the relation between maternal ZIKV infection and

congenital adverse outcomes and between ZIKV infection and GBS. I found that the strength of association between ZIKV infection and adverse outcomes from case-control studies differs according to whether exposure to ZIKV is assessed in the mother (odds ratio (OR) 3.8, 95% CI: 1.7–8.7, I^2=19.8%) or the foetus/infant (OR 37.4, 95% CI: 11.0–127.1, I^2=0%). In cohort studies, the risk of congenital abnormalities was 3.5 times higher after ZIKV infection (95% CI: 0.9–13.5, I^2=0%). The strength of association between ZIKV infection and GBS was higher in studies that enrolled controls from hospital (OR: 55.8, 95% CI: 17.2-181.7, I^2=0%) than in studies that enrolled controls at random from the same community or household (OR: 2.0, 95% CI: 0.8–5.4, I^2=74.6%). The heterogeneity between the studies could be partly explained by the heterogeneity in methods and sampled populations. Studies suffered from bias and uncontrolled residual confounding.

In Chapter 5, I present a framework to systematically assess the evidence for ZIKV as a sexually transmitted disease. I reviewed all available literature and concluded that the risk of sexual transmission of ZIKV is likely small, but relevant for certain risk groups. I found that in semen viral RNA could be detected for a median period of 34 days (95% CI: 28–41 days) and 35 days (no CI given) based on two cohort studies. Aggregated data about detection of ZIKV RNA from 37 case reports and case series indicate a median duration of 40 days (95% CI: 30–49 days) and a maximum duration of 370 days in semen. In human vaginal fluid, the median duration was 14 days (95% CI: 7–20 days) and the maximum duration was 37 days. Infectious virus in human semen was detected for a median duration of 12 days (95% CI: 1–21 days) and a maximum of 69 days. I highlight the poor quality of the evidence and the need for systematic observational studies that evaluate the risk of sexual transmission of ZIKV.

In Chapter 6, I present predictions on the future risk of ZIKV, based on data from Managua, Nicaragua, using mathematical modelling. The risk of a new outbreak in the next decades is low due to herd immunity. However, a next outbreak will disproportionally hit people in the young reproductive age hardest (age 15–29 years). Vaccination could curb this risk: Early introduction of vaccination in 15-year-old girls has the capacity to extend the herd immunity and be of benefit to the whole population. Introduction of a vaccine needs to happen within a decade after the 2016 outbreak to achieve this protection. The duration of immunity following ZIKV infection has impact on the speed at which outbreaks will reoccur.

In Chapter 7, I present an overview of the main findings and I discuss the interpretation and implications of these results. I discuss the strengths and limitations of the work, and outline follow-up questions emerging from the work.

In this thesis, I establish and use different frameworks and methods that help to make sense of the limited evidence that is available during disease outbreaks. ZIKV has been introduced on the American continent, and it is likely there to stay, thus we have to accept that ZIKV will continue to re-emerge. At the same time, due to the climate change, the European temperate region also becomes more suitable for vector-borne disease such as ZIKV. With the ZIKV epidemic on the wane, we now have time to consolidate findings and implement the lessons learnt. We need to be prepared for the re-emergence of ZIKV but also for the emergence of new diseases. The tools and methods I present in this thesis, will help us to be more prepared for a next outbreak.

Chapter 1

Introduction

"The man of science who cannot formulate a hypothesis is only an accountant of phenomena."

– **Pierre Lecomte du Noüy** (December 20, 1883 – September 22, 1947)

The Zika virus (ZIKV) outbreak in the Americas between 2015 and 2017 took the world by surprise. Within two years, over 1.5 million suspected or confirmed cases were reported [1]. However, the true incidence is likely much higher, due to under-reporting and asymptomatic infections that go undetected [2]. As of July 2019, 87 countries had reported ongoing or past circulation of ZIKV [3]. ZIKV infection results generally in mild and transient symptoms. The true burden of the disease lies in the adverse outcomes it causes. Infection during pregnancy can result in adverse congenital outcomes with microcephaly as most prominent. This was first noted in clusters of infants born with severe disabilities linked to ZIKV infection in Brazil in 2015 [4], making ZIKV a disease with a serious public health impact. This raised the question whether ZIKV was the cause of these adverse outcomes and catalysed research interest. From the beginning of 2016, over 300 scientific publications started to appear per month, where before 2016 that was the total size of all the published research. In this thesis I explore different aspects of the ZIKV epidemic.

With the research described in this thesis, I use different epidemiological methods to provide insight in the Zika virus as a cause of adverse outcomes, the risk of Zika virus as a sexually transmitted disease and the risk of future Zika virus outbreaks. This introductory chapter gives an overview of five topics: In the first section, I introduce the history of emerging infections. In the second, I describe the emergence of Zika virus and the outbreak in the Pacific region and the Americas. In the third section, I describe the investigation of causality, in the fourth section I provide insight in how evidence accumulation during disease outbreaks and the use of evidence. In the fifth section, I describe the investigation of disease transmission using mathematical models. The chapter ends with an outline of the aims of the subsequent chapters of this thesis.

1.1 The history of emerging infections

Infectious disease and disease outbreaks have likely always been part of human history. Throughout the centuries, emergence and re-emergence of infectious disease have had substantial impact [5]. One of the first documented outbreaks is the Plague of Athens (430 BC) of which the exact aetiology is still disputed today; first hypothesized to be caused by a wide variety of pathogens ranging from smallpox and typhus to Ebola [6], but most likely caused by typhus based on ancient microbial DNA samples [7, 8]. The impact of the outbreak was vast with a mortality rate of at least 25% [9]. The outbreak likely altered the outcome of the Peloponnesian War by depleting the Athenian military of its personnel and some of its most important leaders [10]. Disease outbreaks shaped history, where larger outbreaks have economic and political consequences. The introduction of disease into populations that have not been in contact with these, means that infections can spread without being restricted by pre-existing immunity. Examples are the introduction of infectious disease that were common in Europe – such as measles and smallpox – following the Spanish discovery of America [11]. Until the beginning of the 1900s, infectious disease caused around half of the total human mortality [12].

Recent history is similarly filled with examples of emergence and re-emergence of infectious disease on a large scale. In the last decades, we have seen the emergence of severe acute respiratory syndrome (SARS) [13], the H1N1 Influenza pandemic [14], Middle East Respiratory Syndrome (MERS) [15], and the re-emergences of Ebola [16] (Figure 1.1). Disease that all posed their unique challenges, but all had substantial global impact. For example, SARS infected over 8,000 individuals, with a mortality rate reaching 10% [17]. The macro-economic

impact of SARS was estimated at US$30–100 billion, or around US$3–10 million per case [17]. The frequency and scale on which outbreaks occur does not seem to diminish: Between 2011-2017, the World Health Organization (WHO) described a total of 1,307 epidemic events, in 172 countries [18]. Where the ZIKV outbreak was the most recent pandemic.

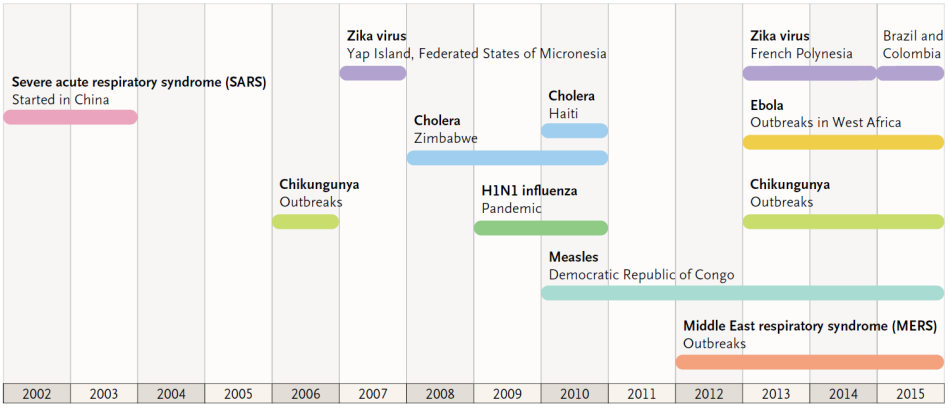


Figure 1.1: Important infectious disease outbreaks that occurred between 2002 and 2015. Reproduced with permission from [19], Copyright Massachusetts Medical Society.

1.2 Zika virus

ZIKV was discovered in 1947. The virus was isolated from a captive, sentinel rhesus monkey in the Zika forest of Uganda during routine surveillance for yellow fever [20]. In 1948, the virus was isolated from the *Aedes africanus* mosquito; the first human cases were detected in Uganda and Tanzania in 1952, in a study that showed the presence of neutralizing antibodies against ZIKV in sera [21]. Before 2007, ZIKV caused sporadic outbreaks in Asia and Africa [22].

ZIKV is a flavivirus, closely related to dengue virus (DENV) and from the same family as the yellow fever virus. The virus is mainly transmitted through mosquitoes from the *Aedes* family. ZIKV isolates have been grouped into two major lineages, African and Asian, where the African lineage is ancestral to the Asian lineage [23].

1.2.1 The Zika virus epidemic

The first documented outbreak of ZIKV occurred on Yap Island, Micronesia, in 2007. Here, after the outbreak, 5005 of the 6892 (73%) sampled individuals had detectable antibodies against ZIKV [24]. In 2013, ZIKV caused a larger outbreak in French Polynesia, in the south pacific, where the outbreak affected up to 49-66% of the people [25]. Here an increase in cases of Guillain-Barré syndrome was observed that coincided with the outbreak [26]. The virus spread to several countries in the Pacific Ocean: Easter Island, the Cook Islands and New Caledonia [27]. From there, the virus spread to the Americas.

Between 2015–2017, ZIKV caused an epidemic in the Americas. The reported number of cases in the Americas peaked between February and March 2016 (Figure 1.2). This peak was mainly driven by the large number of cases from Brazil; in other countries the outbreak occurred later [28]. Subsequent large outbreaks in the next year were not observed, likely due to the occurrence of herd immunity (Section 1.5.1). Localized outbreaks did occur [28].

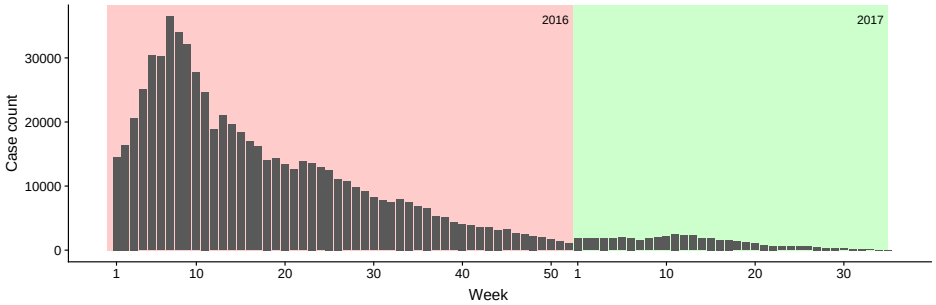


Figure 1.2: Reported cases of Zika virus in 2016 and 2017. Suspected and confirmed cases according to case definitions implemented by countries. The total number of cases per week is aggregated from the Caribbean, Central America and South America and based on data from PAHO and https://github.com/andersen-lab/zika-epidemiology/tree/master/paho_case_numbers.

During the epidemic in the Americas, it became clear that ZIKV infection during pregnancy causes congenital abnormalities, with a reduced neonatal head circumference (microcephaly) as most prominent [29] (Chapter 3 and 4). The WHO convened a working group with experts from different disciplines, and on the 31st of March 2016, based on assessment of the available evidence, this 'Zika Causality Working Group' determined that sufficient evidence existed to indicate that ZIKV infection during pregnancy causes congenital abnormalities and that ZIKV infection can trigger GBS [30]. During the outbreak, sexual transmission of the virus was also observed (Chapter 5).

1.2.2 Transmission routes

ZIKV is primarily transmitted by mosquitoes of the *Aedes* family (Figure 1.3). Direct human-to-human transmission occurs either through vertical transmission – in utero from mother to embryo or foetus – (Chapter 3 and 4) or through sexual transmission (Chapter 5).

Mosquito transmission

A. aegypti is mainly responsible for the current outbreak in the Americas [31, 32]. The mosquito is well adapted to urban settings, tends to bite indoors and feeds multiple times per cycle of egg production, increasing the probability of transmission. The time spent at home, and the body surface area of children was found to be correlated with risk of mosquito bites [33]. *A. albopictus* has been shown to be able to transmit Zika virus in Africa and in laboratory settings. *Aedes* mosquitoes also transmits CHIKV and DENV.

The *A. aegypti* that is found throughout the Americas, originated from Africa [34]. Humans have shaped the evolution and spread of the mosquito. The species was likely introduced to the New World by slave trade ships between the sixteenth and eighteenth centuries. A secondary wave of invasion to the Asian-pacific region likely occurred in the 20th century [34].

Reservoirs. In Africa and Asia, ZIKV can circulate between non-human primates and mosquitoes [31]. This sylvatic cycle forms a reservoir for the disease and is likely responsible for periodic reintroduction of the virus in the human population (Figure 1.3). These spill-over events result in low-level endemic circulation. In Africa or Asia, no outbreaks of the scale

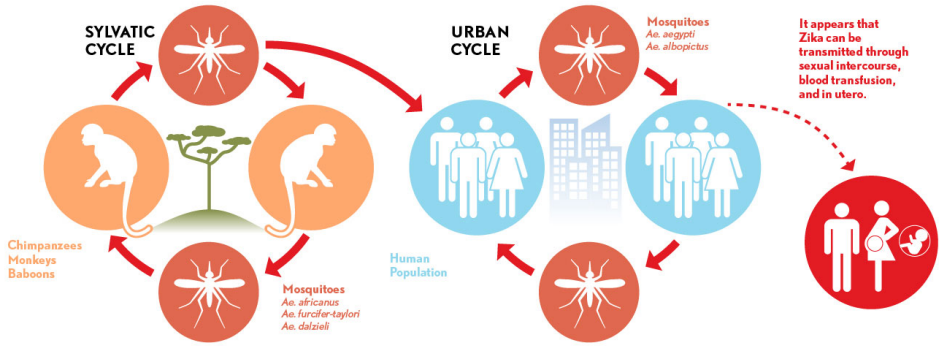


Figure 1.3: The urban and sylvatic cycle of the Zika virus. Source: https://www.cfr.org/backgrounder/zika-virus.

of the one in the Americas have been documented. Likewise, no adverse outcomes were reported until recently (Chapter 4). For example, in 2017, Angola experienced a ZIKV outbreak with adverse congenital outcomes, linked to the outbreak in Brazil [35]. The presence of a disease reservoir in the Americas has not been established yet. New world monkeys, residing in tropical regions of Central and South America, have been shown to potentially sustain ZIKV infection and thus contribute to a sylvatic cycle in the Americas [36, 37].

Vertical transmission

In pregnant women, ZIKV can pass the blood-placenta barrier and infect the embryo or foetus. Here, the virus has a tropism for the developing brain and disrupts the normal growth [38]. ZIKV antigen and viral RNA have been detected in amniotic fluid and placental tissues from ZIKV-infected women and in fetal and newborn tissues diagnosed with congenital Zika syndrome [39, 40]. *In vivo* and *in vitro* evidence has corroborated this (Chapter 3). Live virus has been detected in breast milk [41]. However, so far, transmission related to breast feeding has not been reported [41, 42]. ZIKV can potentially be transmitted via blood transfusion [43].

Sexual transmission

Unique to human arthropod-borne flaviviruses, ZIKV can be transmitted through sexual intercourse as well (Chapter 5). A first case of sexual transmission of ZIKV was reported in 2008. A male traveller was infected in Senegal, and transmitted the disease to his partner in the United States of America through sexual contact [44]. During the outbreak in the Americas, the Centers for Disease Control and Prevention (CDC) and the European Centre for Disease Prevention and Control (ECDC) reported increasing numbers of cases of ZIKV that were apparently transmitted through sexual intercourse [45, 46]. The majority of cases were men traveling back from ZIKV endemic countries, infecting their female sexual partner who did not travel. Both in the American and European region, one percent of the cases originated from sexual transmission via travellers [45, 46]. The exact impact of sexual transmission in an endemic setting remains difficult to quantify, since infection through mosquito-borne and sexual transmission are indistinguishable [47] (Chapter 8.2). In animal models, ZIKV has been demonstrated to persist in the testes and semen [48]. Similarly, persistence of viral RNA has been demonstrated in semen in men (Chapter 5, [49]).

1.2.3 Pathogenesis and clinical disease

ZIKV is neurotropic in the developing brain and has a tropism for reproductive tissues and cells, such as the testes and semen in men and the placenta in pregnant women [50]. Some of these properties of ZIKV have been observed in other flaviviruses. Like ZIKV, the flavivirus bovine viral disease virus causes congenital abnormalities such as malformation and/or demyelination of the central nervous system in cows [51]; Japanese Encephalitis virus, which is transmitted by mosquitoes and causes encephalitis in humans, is sexually transmissible in pigs and causes reproductive disorders there [52]. The combination of the potential for sexual transmission and adverse congenital outcomes in a mosquito-borne disease are unique to ZIKV. Phylogenetic analysis revealed that epidemic strains have accumulated multiple mutations, potentially resulting in an increased virulence of the virus [53].

Signs and symptoms

Infection with Zika virus is often asymptomatic, with up to 50–73% of the infected individuals not experiencing any symptoms, depending on the geographical location of the outbreak [54]. The median incubation period for symptomatic infection is 6 days (95% CI 4–8) (Figure 1.4) [55]. Symptomatic infection results in a transient flu-like illness with maculopapular rash, often with conjunctivitis. Symptoms resolve naturally after 2–7 days and can be similar to those caused by DENV and chikungunya virus (CHIKV) infection. However, DENV can cause serious complications with rapid onset of capillary leakage with or without haemorrhage that can lead in some cases to death [56]. Symptomatic CHIKV infection can be accompanied by severe joint pain [56].

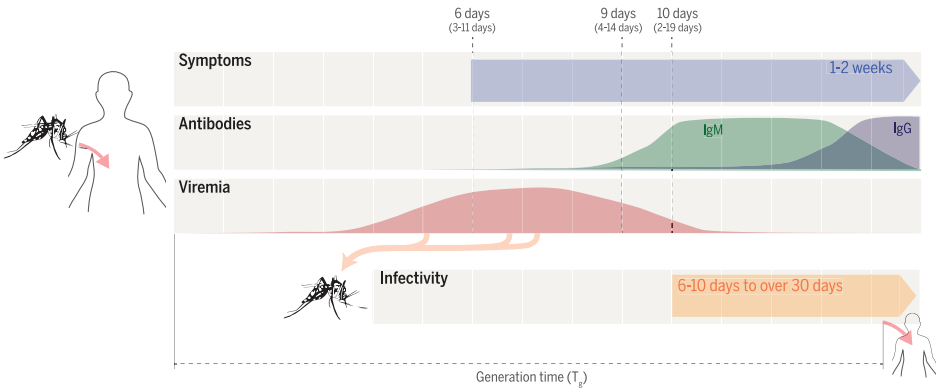


Figure 1.4: Infection dynamics of Zika virus (ZIKV). If symptoms develop after ZIKV infection, this is typically after 6 days (95% range: 3–11). Approximately 9 days (95% range: 4–14) after infection antibodies start increasing; first IgM, followed by IgG antibodies. Viremia starts to increase before potential symptoms appear. Susceptible mosquitoes will be infected by feeding during viremia. After an incubation period, infected mosquitoes are able to transmit the infection to susceptible humans. Reprinted with permission from the American Association for the Advancement of Science (AAAS) from [57].

In rare cases ZIKV triggers Guillain-Barré syndrome (GBS), an auto-immune neurological diseases [58]. Infection during pregnancy can result in vertical transmission and adverse congenital outcomes. In November 2015, in Brazil, the first clusters of congenital abnormalities that coincided with maternal ZIKV infection were reported [4].

Adverse outcomes

Adverse congenital outcomes. Infection during pregnancy, and particularly early pregnancy, is linked with several congenital outcomes, and possibly adverse pregnancy outcomes. ZIKV has been compared to the 'TORCH'- pathogens. TORCH pathogens are infectious agents that cause congenital abnormalities, when infection occurs during pregnancy; The acronym is an abbreviation of the pathogens *Toxoplasma gondii*, other, rubella virus, cytomegalovirus and herpes simplex virus. Some have opted that ZIKV belongs in this category or have dubbed the group of disease 'TORCH-Z' pathogens [59].

Foetal infection has been linked with adverse pregnancy outcomes, and a wide spectrum of congenital abnormalities. Microcephaly, or a reduced head circumference, is the most prominent adverse outcome. However, vision and hearing abnormalities, joint contractures, epilepsy, heart abnormalities have been linked to infection as well [29]. The spectrum of disease has been described as congenital Zika syndrome and consists of 1) severe microcephaly with partially collapsed skull; 2) thin cerebral cortices with subcortical calcifications; 3) macular scarring and focal pigmentary retinal mottling; 4) congenital contractures; and 5) marked early hypertonia and symptoms of extrapyramidal involvement [60]. The risk of congenital Zika syndrome in infant born to women infected with ZIKV during pregnancy ranges from 5 to 14% [61].

Guillain-Barré syndrome. GBS is an auto-immune disease resulting in an acute peripheral neuropathy and is the most common cause of acute flaccid paralysis [62]. Antibodies attack the axolemmal or Schwann cells and affect function of the neurons [62]. Symptoms last a few weeks to several months. Most people make a full recovery, some have permanent damage. Treatment is aimed at reducing the number of antibodies attacking these cells, by performing plasmapheresis and administering intravenous immunoglobulins. Additional treatment is symptomatic: Supportive care, sometimes with mechanic ventilation is provided. The global baseline incidence of GBS has been reported between 0.6–4 cases per 100,000 per year [62]. Approximately two-thirds of the GBS patients had an infection within the previous 6 weeks that caused a flu-like illness or gastroenteritis. It is believed that infections trigger an immune response that cross-reacts with antigens of axolemmal or Schwann cells.

Throughout the Americas, during the ZIKV epidemic, an increase in GBS incidence was reported [63]. At a population level, Mier-Y-Teran-Romero et al. (2018) showed that the estimated incidence of GBS ranged between 1.4 (0.4–2.5) and 2.2 (0.8–5.0) per 10,000 ZIKV infections comparing surveillance/reported cases from Brazil, Colombia, Dominican Republic, El Salvador, French, Honduras, Puerto Rico, Suriname, Venezuela, and Micronesia [63].

1.2.4 Immunity

In immunocompetent individuals, the host's immune system recognizes and processes a ZIKV infection. The infection induces a cellular and a humoral immune response [64]. Humoral immunity is immunity from serum antibodies produced by plasma cells. Antibodies, or immunoglobulins (Ig), bind to the virus and facilitate clearing it from the body. IgM eliminates pathogens in the early stages of B cell-mediated immunity before there is sufficient IgG (Figure 1.4) [65]. IgG can persist for years to decades following infection [66].

Protective immunity against flaviviruses is often assumed to be life-long. This was the case for DENV, until recent evidence showed the possibility of homotypic reinfection [67]. It is currently unclear whether protective immunity after ZIKV infection is indeed life-long.

Early evidence from French Polynesia and Fiji might suggest that immunity wears off [68]. However, reinfection within the same individual has not been described so far.

ZIKV shares a large part of its genetic identity and structural homology with other flaviviruses, including DENV, resulting in cross-reactivity between infections. In an experimental setting where animals have been pre-exposed to DENV, antibody-dependent enhancement occurs, meaning that a secondary infection with ZIKV is more severe than in DENV-naive animals [69, 70]. In humans, a large cohort showed that prior DENV exposure might result in less severe ZIKV disease, contrary to the *in vivo* findings in animal models [71]. However, this might be dependent on the time between the two infections; a shorter time between DENV and ZIKV infection may be protective, a longer time could result in enhancement of the disease severity [72]. Currently, much of our knowledge comes from animal models, some of which lack innate immune signalling [73].

1.2.5 Diagnosis

ZIKV infection can be diagnosed by detecting the pathogen directly in bodily fluids or by detecting antibodies against the virus in the serum of infected individuals. During active replication of the virus, one can detect the viral ribonucleic acid (RNA) using reverse transcription polymerase chain reaction (RT-PCR) or one can isolate and culture the virus (Figure 1.4). When the infection causes an immune-response in the host, ZIKV-specific antibodies can be measured. Immunoglobulin M (IgM) is the first antibody to appear in serum after infection, followed by an isotype-switched IgG response. A plaque reduction neutralization test (PRNT) can be performed to determine the neutralizing effect of the antibodies in the serum [74]. A serum sample is prepared in a series of dilutions, mixed with a viral suspension and incubated on cells. The number of plaques that form due to infection of the cells at a certain dilution is compared against the number of plaques formed under cells incubated with the viral suspension without serum. The dilution that yields a 50% reduction in plaques – noted as PRNT50 – corresponds with how much antibody is present.

Diagnostic tests for ZIKV are not perfect. The proportion of infections correctly detected by a test (sensitivity) or the proportion of absence of infection correctly detected (specificity) depends on the method used, the timing of the test and other factors such as cross-reactivity.

Viral RNA in the blood can only be detected during viremia. If the patient is sampled during this period the method has a high specificity. The sensitivity is reduced when the sampling is not performed during viremia, meaning we cannot rule out disease with a negative test. Viral culture methods have the capacity to detect infectious virus. However, viral culture is technically challenging and false negative results can occur. The window of detection is like that of viral RNA, and thus also limited to viremia.

The detection of antibodies against ZIKV has limitation as well. Previous circulation of DENV has been shown to cross-react in antibody tests, causing false positive results (a positive test, in absence of a true infection). Test algorithms try to take into account these complicated patterns [75].

Early in the epidemic, the case definition of ZIKV was unclear and, because of limited availability of diagnostic tests, sometimes based on symptomatology or clinical suspicion. The quality and availability of diagnostic test has improved over the last years, but is still far from perfect. Even when the same tests are conducted, between lab heterogeneity decreases comparability of results [76].

ZIKV RNA has been detected in a wide range of bodily fluids: Blood, semen, saliva, fluids of the female genital tract, anal secretions and breast milk [41]. However, the presence of viral RNA does not necessarily mean the presence of infectious virus. Samples that contain viral RNA have often not been culturable (Chapter 5), although the inability to isolate virus does not prove the absence of infectious virus [77].

1.2.6 Treatment and prevention

Currently, there is no vaccine or specific antiviral treatment against ZIKV infection. Treatment is targeted at alleviating and managing symptoms [78]. Interventions are targeted at mitigating the risk of exposure, especially in women of reproductive age and pregnant women.

Vector control

Vector control is one of the most broadly used interventions, due to the lack of vaccines and antiviral treatment. Control strategies are tailored to the behaviour of the vector. For *Aedes aegypti*, mechanical measures, such as reduction of small bodies of standing water can reduce breeding sites. Chemical methods, either with larvicides or adulticides or the use of repellents can be applied. The use of biological methods, such as introducing the *Wolbachia* bacterium to reduce vector competence has been explored with varying results [79]. Early results from surveillance data comparing cities where *Wolbachia* is used to suppress circulation of flaviviruses, and cities where the intervention are not used, are promising [80]. A larger trial is currently under way [81].

A recent control approach used genetically modified (GM) *A. aegypti* mosquitoes designed to contain a dominant lethal gene that results in death of its offspring. Release of these mosquitoes has been effective in reducing populations of *A. aegypti* by up to 85% [82]. However, after large scale release in Brazil it became clear that offspring of the GM mosquitoes survived and produced offspring that also made it to sexual maturity [83]. As a result, unintended incorporation of portions of the transgenic strain genome into the target population occurred [83].

The basis of WHO's Integrated Vector Management (IVM) program is the simultaneous use of multiple strategies for vector-borne disease prevention. It is believed that no single intervention will be sufficient to control the disease, similar to DENV vector control [84]. An increase of insecticide resistance contributes to the challenge of vector control [85].

Vaccine development

Immunity conferred by vaccination that protects against infection, would protect pregnant women against adverse outcomes or through herd immunity the whole population against outbreaks. An ideal vaccine is immunogenic, safe and cost-effective. Promising results from phase I clinical trials showed levels of neutralizing antibody titres that were considered protective against reinfection [86, 87]. Some vaccines have already entered phase II trials [88]. However, there are still several hurdles to take before we have access to a vaccine [89]. First, due to a substantial reduction of circulation of ZIKV, the evaluation of vaccine efficacy has stalled at the moment. Second, it is unclear if neutralizing antibodies induced by vaccination are sufficient to the unborn child against vertical transmission [90]. Last, it remains to be investigated if vaccine-induced antibodies will cross-react with other Flaviviruses. Keeping

in mind the unexpected outcomes observed during a clinical phase III trial of the Sanofi Pasteur's CYD-TDV (Dengvaxia) dengue vaccine: It became clear that the vaccine could put previously uninfected children at a higher risk of a severe case of dengue fever [91, 92].

1.2.7 Public health response

The outbreaks of microcephaly and GBS triggered a large public health response, initially in Brazil. The ministry of health of Brazil responded in November 2015 by declaring a national emergency [93]. Subsequently, on 1 February 2016, the WHO declared a Public Health Emergency of International Concern (PHEIC) [94]. This increased awareness and PHEIC catalysed the amount of resources that became available to counteract and monitor the outbreak, including the causal associations between clinical disease and ZIKV.

During the ZIKV outbreak, the WHO emphasized the necessity of data sharing, and standardization of research efforts [95]. WHO, CDC and several research consortia initiated or supported cohorts that investigated different aspect of the outbreak. They set out a research agenda with efforts to harmonize research, improve data sharing and standardize and improve diagnostic methods [95].

WHO and CDC developed rapid guidance on testing [75, 96], pregnancy [97, 98], mosquito control [99, 100] and sexual transmission [101, 102]. Interim guidance on the prevention of sexual transmission of ZIKV, in 2016, initially advised the use condoms or abstain from sex for six months after return from travel to endemic countries. This was later reduced to three months for men and two months for women, based on evidence presented in Chapter 5.

1.3 Causality

To establish the link between ZIKV infection and congenital abnormalities and adverse neurological outcomes as a 'causal' link requires careful assessment. In daily life, understanding cause and effect is often an intuitive process, where evidence helps us establish causal links. We try to answer the 'Why?' in many instances. In epidemiology and in science as a whole, causal questions are pivotal or as Kenneth Rothman phrases it: "Outside of the physical sciences, much of scientific knowledge comprises of a collection of causal statements" [103].

Since antiquity, philosophers and scientist have tried to understand and explain fundamental concepts of causation. Aristotle introduced inductive and deductive reasoning to come to conclusions, where in the first we move from specific observations to broader generalizations and theories, in the latter we move from the more general to the more specific [104]. He viewed causality as the doctrine of four causes: the material, the formal, the efficient and the final cause. In the 17th and 18th century, Francis Bacon, David Hume and John Locke shaped our modern views on causality. They used inductive reasoning, arriving at generalization from repeated observations. The logical necessity (all A are B, C is A, therefore C is B) is not present in inductive reasoning. Hume challenged inductive reasoning and its fallibility. The 19th century philosopher John Stuart Mill described methods of inductive reasoning to address issues of causation [105]. Early in the 20th century, the philosopher Karl Popper introduced the concept of falsifiability [106]. Popper provided insight in how scientific knowledge grows [107]. Contrary to Bacon, who believed that a crucial experiment may establish or verify a theory, Popper postulated that it can at most refute or falsify a theory [107].

Similarly, the cause of diseases has been subject of investigation for centuries. Before technological advancements were made to detect most pathogens, different theories tried to establish the cause of disease. Up to the nineteenth century people believed the miasma theory that postulated that disease were caused by a miasma (ancient Greek for pollution), a noxious form of 'bad air'. Jakob Henle already suggested in 1840 that a 'Contagium animatum' or 'Contagium vivum' existed [108]: Living microorganisms that caused infections. The germ theory was postulated through observations by Louis Pasteur, Robert Koch and John Snow and replaced the miasma theory. It states that microorganisms known as pathogens can cause disease. Pasteur demonstrated that the growth of microorganisms was responsible for spoilage of beverages, not the air the beverage came into contact with [109]. Snow identified drinking water as the source of cholera in London [110]. Koch was able to isolate anthrax using purified cultures isolated from diseased animal [111]. Koch built upon Jakob Henle's work and provided a framework for identifying acute diseases associated with microorganisms [112]. The Koch's postulates state that: 1) The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms. 2) The microorganism must be isolated from a diseased organism and grown in pure culture. 3) The cultured microorganism should cause disease when introduced into a healthy organism. 4) The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.

Often one cause or one pathogen alone, is not enough to explain an effect or disease. Simply put, not all become ill after infection. Kenneth Rothman proposed the concept of sufficient component causes, the idea that a minimum set of factors and circumstances that, if present in a given individual, will produce the disease [103]. Different individuals have different sets of components that combine to produce a sufficient cause. Causes can be necessary and/or sufficient. The concept of sufficient-component cause is widely used in epidemiology as a framework for teaching and understanding multicausality: The concept that a complete causal mechanism involves a multitude of factors [113]. The complexity of multicausality is described as the web of causation [114].

In the 20th century, Sewall Wright, Jerzy Neyman and Ronald A. Fisher contributed to the conception of the statistical theory of causal inference we know today. Although Neyman and Fisher had conflicting views about statistical models [115], they viewed causation as conceptually different from correlation, and investigated how causal inferences could be made from correlational data [116]. Wright described the use of a schematic representation of a causal system, called a 'path diagram', now used in the form of Directional Acyclic Graphs (DAGs) [117]. The first randomized experiments in agriculture were designed by Neyman and Fisher and are considered the 'gold standard' for causal research [118]. Recent advancements in conceptualizing causality are made by thinking in counterfactuals, and using DAGs to think about causality, but mechanistic explanation of causes remain as important as the probabilistic link between cause and effect [119].

In other disciplines, such as pharmacovigilance research or the investigation whether drugs cause side effects, causality is assessed using a variety of methods such as 1) expert judgement, where professional opinions are based on all available evidence, 2) probabilistic methods and 3) scales and algorithms [120]. None of these methods is free of flaws and hampered by a certain level of subjectivity.

1.3.1 Bradford Hill viewpoints

The statistician Sir August Bradford Hill proposed a set of criteria to help assess a causal relation. Hill's criteria were an expansion of a set of criteria formulated in a landmark report by the Advisory Committee to the US Surgeon General on Smoking and Health [121, 122], which, in turn, were inspired by the work of Hume and John Stuart Mill.

An inventory of an association's 1) strength, 2) consistency, 3) specificity, 4) temporality, 5) biological gradient, 6) plausibility, 7) coherence, 8) experimental evidence, and 9) analogy, helps to provide insight in its causality. The viewpoints were not intended to be strict criteria but are often interpreted as such [123]. The viewpoints illustrate that one should consider a plethora of evidence which is in line with that the statement that "Robust causal inference comprises a complex narrative, created by scientists appraising, from diverse perspectives, different strands of evidence produced by myriad methods" [124] or what others describe as "triangulation with different sources of evidence" [125].

1.3.2 Epidemiological studies that investigate causation

One of the main objectives in epidemiological research, is to find causal relations [113]. The randomized controlled trial is considered the best study design to discover these causal relations, especially when we can assign the exposure as is the case with interventions. By correctly randomizing exposure and assuming a sufficient sample size, the difference in outcome is caused by the exposure. In a perfect RCT, in the absence of other sources of bias, confounding factors are distributed at random. Unfortunately, many causal relations are not feasible to explore in RCTs, either due to ethical or practical constraints. For example, the distribution of the exposure to ZIKV infection is assigned by a non-random process. These exposures can be studied in observational studies.

In observational studies, the researcher does not randomly assign the exposure, he or she merely observes the outcome as a result of the exposure [126]. The internal validity – the ability to measure what the study sets out to measure – can be compromised by bias. In this context bias refers to a deviation from the truth [127]. Bias is often grouped in three categories: selection bias, information bias and confounding [127, 128]. Selection bias originates from issues with the comparability of groups of subjects. Information bias arises from the incorrect assessment of the exposure or outcome. Confounding occurs due to an extraneous variable that is associated with both the outcome and the exposure, but not part of the causal pathway. Due to non-random assignment of the exposure, observational studies are more sensitive to bias and confounding compared to RCTs. Controlling for confounding assumes we understand and are able to measure confounders. We are always at risk of uncontrolled unidentified confounders [129].

Confounding can be controlled through the study design or in the analysis of the data. As discussed above, randomization reduces the probability that an uneven distribution of confounders occurs. Matching groups by confounder, or restricting to a certain stratum within the confounder are other approaches of controlling confounding during the study design [103]. During the analysis, confounding and bias can be controlled using stratification or multivariable modelling approaches [103]. More advanced methods use propensity scores or instrumental variables [130, 131].

1.4 Evidence accumulation and synthesis

As disease outbreaks emerge, data is collected. The scientific community responds with an increased interest and research effort, resulting in an increase in peer-reviewed publications about the disease (Figure 1.5). Often the first source of epidemiological data is from surveillance systems. Cases or suspected cases are actively or passively collected and reported to health authorities, who periodically report these through websites or other channels. The detail of description is limited and sampling is often not systematic. However, the speed with which new cases arise is informative, and can inform mathematical modelling efforts (Section 1.5.1). Case reports and case series enable discovery of new associations [132, 133]. As outbreaks propagate and the capacity to respond is increased, the systematic collection of data improves. Anecdotal observations are reported in case reports or case series. These generate hypotheses that often inspire the conduct of studies designed to challenge these hypotheses. In the ZIKV outbreak, an observed increase in congenital abnormalities and a hypothesized causal link with maternal ZIKV infection sparked much of the research from 2016 onwards. In parallel, basic research investigates the biology and pathogenesis of the disease. Most research is disseminated through publication in peer-reviewed journals.

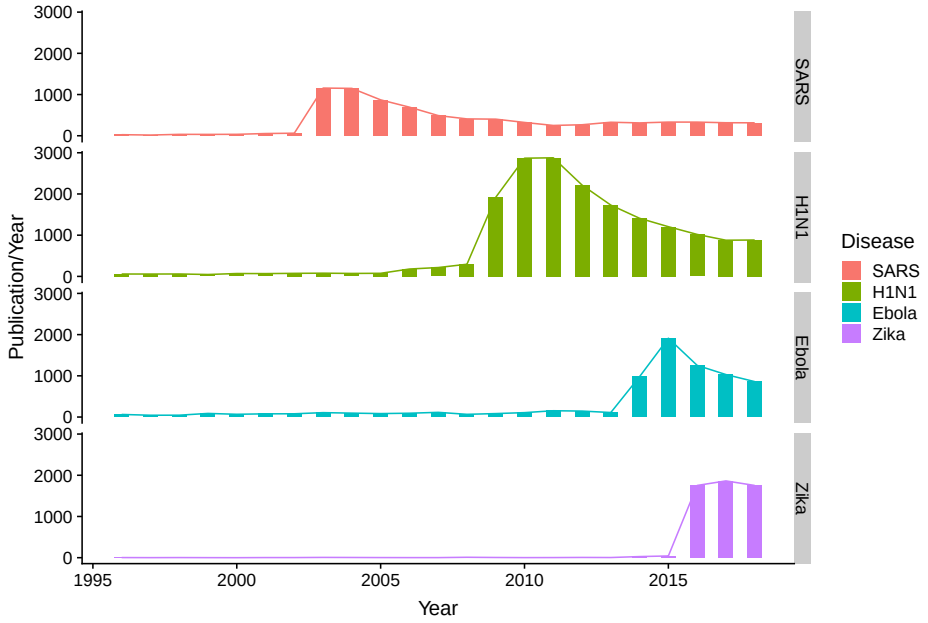


Figure 1.5: The increase of peer-reviewed publications indexed in MEDLINE after large disease outbreaks. The Severe acute respiratory syndrome (SARS) outbreak started November 2002, the H1N1 flu epidemic started early 2009, the most widespread Ebola outbreak in Africa started December 2013 and the Zika epidemic started end of 2015. MEDLINE indexed publications are retrieved using the search terms 'Ebola', 'Zika', 'SARS' and 'H1N1'.

1.4.1 Collation of evidence: systematic reviews

To summarize the large body of (medical) research and to assess the best available evidence for public health decisions, systematic reviews are conducted. These are collations of

empirical evidence according to predefined protocols, driven by research questions, often formulated as PICOs, specifying the Population, Intervention or exposure, Control, and Outcome of interest [134]. Methods are applied to attempt to identify or minimise bias [135]. Systematic reviews can include a meta-analysis: a statistical analysis of the results of independent studies, aimed at producing a single estimate of an effect [135].

The workflow of a systematic review consists of 1) formulating review question, 2) defining inclusion and exclusion criteria, 3) locating studies, 4) selecting studies, 5) assessing study quality, 6) extracting data, 7) analysing and presenting results, and 8) interpreting results [135]. The analysis and description of the results can either be qualitative and quantitative.

1.4.2 The living systematic review

Systematic reviews in fields where evidence accumulates rapidly such as research on emerging diseases, quickly become outdated. A solution is keeping systematic reviews up to date in the form of living systematic reviews. Elliot et al. (2014) defined living systematic reviews as "high quality, up-to-date online summaries of health research that are updated as new research becomes available" [136]. The rapid accumulation of evidence requires a streamlined approach of data collection and extraction [137]. Living systematic reviews require reviewers to define its properties on forehand in a protocol, such as the frequency of searching, screening and extraction. Criteria for updates of the publication are determined and stopping criteria are defined [138].

Automation and machine learning

Current technology facilitates establishing and maintaining living systematic reviews [137]; application programming interfaces (API) allow computers to access databases with medical literature and this enables us to perform searches automatically. Software can index and deduplicate searches, and provide reviewers periodic updates of this information (Chapter 3). Machine learning can facilitate screening by classifying on potential relevance based on earlier decisions. These methods rely on pattern recognition and inference. Typically, an algorithm is 'trained' using a dataset compiled by humans. The algorithm 'learns' a set of rules and applies these to future data. Training of the algorithm can be iterative as output is verified and used to improve the algorithm. Specific eligibility criteria, such as a certain study design, can be translated into an algorithm classifying studies. Algorithms have been successfully trained and applied to detect RCTs [139]. In systematic reviews it is crucial to correctly reject publications that are not eligible for inclusion and to avoid falsely rejecting relevant information. Experiments with classifiers have shown promising results: The RCT classifier was shown to be able to exclude 60–80% of irrelevant records retrieved from a database search while maintaining a sensitivity of over 99% [139]. However, for other study designs results are less promising.

The current consensus is that automation is unable to perform the full eligibility assessment [137], but it can facilitate the process and decrease the workload. An even more challenging task for automation is data extraction, which is hindered by the way information is published. There is no uniform form, enormous heterogeneity in how information is presented within a published article, and research is often shielded off by paywalls or unlicensed for text-mining [140].

The fields of living systematic reviews and bioinformatics are evolving rapidly, and promising results show advancement in automation of data extraction and bias assessment [141].

Marshall et al (2016) showed that "Risk of bias assessment may be automated with reasonable accuracy. Automatically identified text supporting bias assessment is of equal quality to the manually identified text in the Cochrane Database of Systematic Reviews." [141].

1.4.3 Quality of the evidence: risk of bias and certainty of evidence

Not all publications hold the same amount of evidence. The quality of the evidence of individual studies or the certainty of the conclusions, depends on the study design, the sample size, and validity of the study. Imperfect study design, conduct, analysis, or reporting can cause the measured effect to be underestimated or overestimated [142]. Risk of bias tools are designed to assess the extend of bias and reflect the confidence we have in the results.

For outcomes from the aggregation of multiple studies, the certainty of evidence can be considered as the certainty that a true effect lies within a chosen range [143]. In the context of recommendations, the certainty of evidence reflects our confidence that the estimates of effect are adequate to support the decisions [143]. One of the most widely adopted frameworks to assess the certainty of evidence has been developed by the grading of recommendations assessment, development and evaluation (GRADE) working group [143]. By judging the risk of bias, inconsistency, indirectness, or publication bias of outcomes of a systematic review, we classify the quality of the evidence of outcomes as very low, low, medium or high (Chapter 8.1). Quality or certainty of evidence is correlated with the study design. Depending on the public health problem, different study designs might be appropriate and valued most. To evaluate the effect of interventions, typically meta-analyses of RCTs are considered as the most trusted source of information.

1.4.4 Evidence aggregation: meta-analyses

In the quantitative accumulation of evidence, we summarize the effect measures of multiple studies in one measure of effect with its corresponding uncertainty. In these meta-analyses, statistical techniques are used to summarize the result of multiple studies [144]. By combining information from all studies, meta-analyses provide more precise estimates of the measures of effect than those derived from the individual studies. However, precise estimates do not represent the true effect per se, in presence of bias or confounding, which plays especially a role in observational studies [135].

The simplest way to aggregate multiple studies would be to calculate the mean of the effect sizes. However, more precise studies are considered to carry more information and thus should have more influence in the analysis. This is expressed through assigning a weight, for example the inverse of the variance. The effect size and the weight are used to calculate a weighted average [145]. If all the weights are the same then the weighted average is the same as the mean effect size. Depending on the anticipated heterogeneity between the conducted studies we choose an appropriate model. In a fixed-effect model we assume that there is one true effect size which is shared by all the included studies. We assume that the differences among study results occur only due to chance. The random-effect model is appropriate if the true effect could vary from study to study and the model allows addressing heterogeneity that cannot be explained by other factors [145, 146].

Heterogeneity

Studies are never exactly the same: The underlying true effects in each study are often not identical, populations and methods can vary and random variation occurs. This variation can cause heterogeneity between the studies. Heterogeneity is any kind of variability among studies in a systematic review [135, 147]. The amount of heterogeneity can be formally quantified by calculating the weighted sum of squared differences between individual study effects and the pooled effect across studies, or Cochran's Q. The I^2 is the percentage of variation across studies that is due to heterogeneity rather than chance, and is calculated from the Q and the degrees of freedom [147]. The I^2 can be interpreted as the percentage of variation across studies that is due to heterogeneity rather than chance. Identifying the factors that cause the heterogeneity and stratifying the results by these factors or performing meta-regression using co-variates [148], allows us to quantify, visualize, and explain the heterogeneity.

1.4.5 Translating evidence into guidance

Evidence from systematic reviews is often used to inform public health guidance. For example, a systematic review provides evidence on which intervention is preferred and this evidence is incorporated in guidelines by recommending the use of the intervention. Evidence translates into decisions through guidelines. Not only the evidence on the effect measure is used, evidence on the impact of the decision or recommendation are incorporated as well [149]. The broadly used 'evidence-to-decision framework' described by Alonso-Coello and colleagues, helps to use evidence in a structured and transparent way to inform decisions in recommendations [149]. The evidence is viewed in a broader perspective, judging whether the problem is a priority, the magnitude of the desirable and undesirable effects, the certainty of the evidence, consideration of how stakeholders value the main outcomes, the balance between desirable and undesirable effects, resource use, acceptability, and feasibility [149].

As with systematic reviews, guidelines become outdated as new evidence becomes available. New evidence might change decisions and timely incorporation is crucial. Living systematic reviews could result in living guidelines, making the whole cycle of evidence from discovery to implementation dynamic and capable to incorporate changes [136]. Establishing public health guidance in an emerging disease setting presents additional challenges due to a lack of evidence and a need for a rapid response [150].

1.5 Risk of transmission

In order to properly respond to disease outbreaks, we need to understand and quantify the risk of disease transmission. One of the tools to achieve this is a mathematical model.

1.5.1 Mathematical models

Mathematical models are descriptions of systems using mathematical concepts and language. In infectious disease modelling, a distinction is made between models that describe a population as compartments (compartmental models) or as individuals (individual or agent-based models). In the compartmental model, an interrelation of a set of equations describes the overall behaviour of a disease in a population. In one of the simplest forms the population is divided in different states, represented by compartments: susceptible,

infectious and recovered, the 'SIR' model [151]. Movement between compartments occurs at specific rates. In the individual based model or agent-based model, the properties and behaviour of individuals is modelled. Both approaches simulate the behaviour of an infectious disease in a population and help increase the understanding of the disease transmission dynamics and interventions.

Models can be dichotomized based on whether a model allows randomness: Deterministic models function in the absence of randomness in the development of future states of the system; stochastic models take into account randomness. Randomness or chance plays an important role when the number of infectious individuals is small [152]. The model parameters can be inferred by fitting the model to the data (theory or data driven) or by assuming parameter values based on external knowledge (hypothesis or assumption driven) [153]. The function of a model can be to describe observations or the forecast or predict events in the future [153].

A central measure in infectious disease modelling is the reproduction number ($\mathcal{R}_0$) [154]. The reproduction number is defined as the average number of secondary cases caused by an infectious individual in a fully susceptible population [155]. The $\mathcal{R}_0$ is partly inherent to the disease, but also depends on the region, the environment, and the population's size, age-distribution and density. The value of $\mathcal{R}_0$ is as Klaus Dietz describes: "The magnitude of $\mathcal{R}_0$ allows one to determine the amount of effort which is necessary either to prevent an epidemic or to eliminate an infection from a population, it is crucial to estimate $\mathcal{R}_0$ for a given disease in a particular population" [154]. If the $\mathcal{R}_0$ is ≤ 1, we will only see minor outbreaks that go extinct; with values above 1, large outbreaks are likely to occur.

As soon as infections occur, the number of susceptible individuals declines. The effective reproduction number is the average number of secondary cases per infectious case in a population with both susceptible and non-susceptible individuals. The effective reproduction number is the product of the basic reproduction number and the fraction of the host population that is susceptible. As an outbreak progresses and people become immune after infection, the effective reproduction number will decline. The outbreak will stop when the effective reproduction number is equal or smaller than 1. When a sufficiently large proportion of the population is immune either by previous infection or vaccination an outbreak will not occur; this phenomenon is called herd immunity [156].

Mathematical models of infectious disease can be used to increase the understanding of the behaviour of a system, to infer counter-factual scenarios, to evaluate the effect of interventions and to predict future behaviour of the system. Transmission parameters that cannot be directly measured in observational studies due to practical or ethical constraints can be inferred from a model [157]. For the sexual transmission of ZIKV, the duration of infectiousness is an important parameter that drives the required duration of protection [47]. However, it can not be measured directly. By extending models with economic outcomes, we can explore whether interventions are worth implementing. Models balance between complexity and usability. An ideal model is simple yet informative and captures the properties of a system that are for the research question relevant. We apply the principle of choosing the simplest, valid model to answer complex questions. This principle is referred to as Occam's razor or that unnecessarily complex models should not be preferred to simpler ones [158].

Since models are a simplification of the reality, they require us to make assumptions. In the simplest models, we assume for example homogeneous mixing, or that the probability of transmission is the same for everyone. The validity of the assumptions can either be formally

tested by testing model fit, by performing sensitivity analyses, or by reasoning. Seino (2005) underlines that 'the awareness of assumptions' bridges the 'real world' to the 'mathematical world' [159].

1.5.2 Modelling of ZIKV

ZIKV is a vector borne disease and, depending on the context, this vector can be explicitly modeled. A common way to model vector transmission are Ross-Macdonald models [160], originally designed to investigate the transmission of malaria. These are compartmental models where the infection of the host and the vector is modeled. The distribution of vectors varies over time and space, adding to the complexity of the problem [161]. However, depending on the purpose of the model, simpler models ignoring the vector can fit to the data equally well [162].

In ZIKV research, mathematical models have been applied for a variety of purposes. The examples below illustrate the use of modelling during the ZIKV outbreak for different purposes:

Inferring $\mathcal{R}_0$

The basic reproduction number for ZIKV has been inferred from data from the South Pacific, Brazil and Colombia using different models. Estimates range from 1.4-1.7 for the French West Indies, 1.9-2.2 for French Polynesia, 2.1 for Salvador Brazil to 4.3-5.8 for Yap Island [163–166]. Differences in $\mathcal{R}_0$ are a result of the spatial heterogeneity but also model structure and assumptions [163].

Explaining observed transmission patterns and the risk of adverse outcomes

Zhang et al. (2017) modeled the spread of ZIKV at a larger scale, throughout the Americas. They used a meta-population model consisting of a set of local populations connected by the movement of individuals between the different populations based on data. The model provided insight in the timing of introduction of ZIKV in Brazil, and the projected number of newborns from women infected by ZIKV [167].

Inferring the risk of sexual transmission

Gao et al. (2016) and Towers et al. (2016) were the first to model sexual transmission using ODE based models [168, 169]. They concluded that the contribution of sexual transmission to the total transmission of ZIKV was limited. Moghadas et al. modeled ZIKV in Colombia using an ABM and parameters described in literature to explore the impact of transmissibility of asymptomatic individuals and sexual transmission [170]. They estimated that the fraction of cases due to sexual transmission is estimated below 4% of the cumulative incidence.

Predicting future risk of ZIKV epidemics

Several authors studied the time to a next ZIKV outbreak. Kucharski et al. concluded that it would take 12-20 years before ZIKV would re-emergence in French Polynesia [164]. Netto et al (2017) used a SEIR model to show that in Salvador, Brazil, the effective reproduction number was insufficient to cause a new outbreak during the subsequent years [171]; Lourenço et al. (2017) showed the same for the whole of Brazil: herd immunity should protect the population

from a new outbreak in 'the coming years' [172]. Fergurson et al. (2016) concluded that the herd immuntiy caused by the widespread epidemic would provide a multiyear window before new large-scale outbreaks occur [173].

1.6 Aims and outline of the thesis

The overall aim of this thesis is to provide more insight in the epidemiology of the Zika virus in the Americas, and how evidence emerges during disease outbreaks and informs public health decisions. I discuss ZIKV as a cause of adverse outcomes (Chapter 2-4), ZIKV as a sexually transmitted disease (Chapter 5 and supplementary Chapters 8.1-8.3) and the future risk of ZIKV outbreaks (Chapter 6). At the end of the thesis I discuss the findings and provide an outlook for future research (Chapter 7).

Specific objectives of the thesis are:

- To describe how the evidence of ZIKV as a cause of adverse outcomes accumulates and what lessons can be learned from this (**Chapter 2**).
- To investigate ZIKV as cause of adverse outcomes and to establish methods for a living systematic review (**Chapter 3**).
- To continue the living systematic review and focus on a quantification of the strength of association and the bias and heterogeneity in the evidence (**Chapter 4**).
- To investigate the risk of sexual transmission of ZIKV (**Chapter 5**).
- To investigate the future risk of transmission of ZIKV, using a mathematical model based on data from Managua, Nicaragua (**Chapter 6**).
- To put the results in context and provide an outline for future research (**Chapter 7**).

Supplementary Chapters 8.1-8.3 provide context for Chapter 5 and aim:

- To use the evidence of sexual transmission to formulate guidelines to prevent sexual transmission (**Chapter 8.1**).
- To describe the research agenda to investigate the sexual transmission of ZIKV (**Chapter 8.2**).
- To place ZIKV as sexually transmitted infection in the context of emerging and re-emerging sexually transmitted infections (**Chapter 8.3**).

Chapter 2

Emergence of evidence about causality: case study from the Zika virus outbreak

Michel Jacques Counotte[1]
Kaspar W. Meili[1,2]
Nicola Low[1]

[1] Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland
[2] Department of Epidemiology and Global Health, Umeå University, Sweden

This article is **in preparation**.

Contribution: I contributed to the study design, performed the analysis, made the figures and wrote the first draft of the manuscript and incorporated all feedback.

2.1 Abstract

Causality is a principal theme in epidemiological research. Establishing that an exposure causes a specific health outcome is based on evidence and may inform guidance about public health measures. The Zika virus (ZIKV) outbreak in the Pacific and the Americas between 2013–2016 presented with the aetiological causal questions whether ZIKV infection causes congenital abnormalities and Guillain-Barré syndrome. Earlier conducted systematic reviews collected evidence to answer these questions. The objective of this study was to examine the body of evidence that was used to establish the causal relation between ZIKV infection and adverse outcomes. We hypothesised that the temporal sequence would follow a certain structure, where case reports and series come first, followed by *in vivo* and *in vitro* studies. Case-control studies are followed by cohort studies and eventually trials. We assessed 1) how long it takes before findings from a specific study design appear, 2) how publication of preprints could reduce the time to publication and 3) how time to publication evolves over time.

We included 346 publications published between March 6, 2014 and January 1, 2019. In the 2013–2016 ZIKV outbreak, case reports and case series were the first study designs to emerge. Basic research studies appeared rapidly after this. Publication of more robust epidemiological study designs, such as case-control and cohort studies, appeared between 400–700 days after ZIKV was first detected in the region of the study origin. The delay due to the publication process of basic research and epidemiological research was lower at the beginning of the outbreak. A year after the declaration of the PHEIC, the publication delay rose to 150 days. Only a small proportion of publications was available as preprints (16/346).

The accumulation of evidence over time in new causal problems seems to follow a hierarchy where case reports and case series are rapidly followed by basic research. During the ZIKV outbreak, robust epidemiological studies, such as case-control studies and cohort studies, took 400-700 days to appear. Causal inference based on a wide spectrum of evidence is therefore essential for early public health guidance in emerging causal problems. Publishing preprint does reduce the delay, and especially in epidemiological research this is an underused tool.

2.2 Introduction

Causality is a principal theme in epidemiological research. Establishing that an exposure causes a specific health outcome is based on evidence and may inform guidance about public health measures. The concepts and types of evidence required to conclude that an association is causal are the subject of ongoing debate. Vandenbroucke proposed a hierarchy of evidence based on the best chance for discovery and explanation of phenomena [133]. Observations published in case reports and case series, or findings in data and literature drive discovery. Verification of these discoveries happens in observational studies and in randomized controlled trials, given that exposures can be randomized.

The Zika virus (ZIKV) outbreak in the Pacific and the Americas between 2013–2016 presented several aetiological causal questions. In 2013–2014, ZIKV caused an outbreak in French Polynesia [58, 174]. During this period, investigators documented some severe neurological conditions, including 40 people with Guillain-Barré Syndrome (GBS). GBS is usually a rare sporadic condition. Often triggered by infection, an autoimmune response affects the peripheral nerves, leading ascending paralysis, which can be fatal if it involves the respiratory

nerves [62]. At the time, the reports did not attract much attention and the investigators refrained from making a causal connection because dengue was also circulating at the time [174]. Then, in November 2015, the ministry of health in Brazil reported a cluster of births affected by microcephaly in the north east, where ZIKV was circulating [93]. Microcephaly is a birth defect, indicative of impaired brain development, which can be caused by congenital infection. In December 2015, the Pan American Health Organization announced heightened surveillance owing to an "increase of congenital anomalies, Guillain-Barré syndrome, and other neurological and autoimmune syndromes in areas where Zika virus is circulating" [175]. The World Health Organization (WHO) declared a Public Health Emergency of International Concern (PHEIC) on 1 February 2016 because of the severity of these clinical conditions and their temporal association with ZIKV circulation [94]. Retrospective assessment of the French Polynesia outbreak identified an increase in adverse congenital outcomes as well [176]. The PHEIC and the extensive outbreak catalysed the research on ZIKV. Early public health guidance about the prevention of ZIKV infection and its potential consequences was based on limited evidence, however [101].

Systematic reviews were developed to address the PHEIC recommendation for research about the causal relationships between ZIKV infection and adverse congenital outcomes, including microcephaly and between ZIKV and autoimmune outcomes, including GBS [29]. The reviews organised the findings around a 'causality framework' with ten dimensions derived from those proposed by Bradford Hill [29, 123]. An expert committee reviewed the evidence collected by these systematic reviews up to May 2016 and reached the conclusion that "the most likely explanation of the available evidence" was that ZIKV is a cause of adverse congenital outcomes and a trigger of GBS [30]. This review has been kept up to date as a living systematic review, by periodically incorporating new results [177, 178]. The additional evidence has reinforced the conclusions of causality.

A temporal sequence for the emergence of evidence was already hypothesised during the planning of the systematic reviews in early 2016 (Figure 2.1). Acknowledging that 'astute observations' of new causes of disease often start an aetiological investigation [132], case reports and case series were eligible for inclusion in the systematic reviews. These study designs are often excluded from systematic reviews because they are the lowest level of the "hierarchy of evidence". That hierarchy applies to evaluation research but Vandenbroucke proposed a reverse hierarchy for discovery in which 'anectodal' forms of evidence are at the top [133]. Cross-sectional, case-control and retrospective follow-up studies follow because they are quickest study designs that include a control group. Prospective cohort studies take longer to set up and RCTs only provide additional information if a treatment or vaccine is available. In addition to epidemiological studies, basic and clinical laboratory science start early in the search for causes.

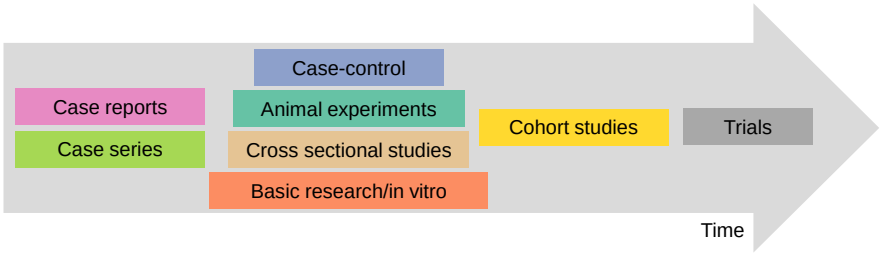


Figure 2.1: Hypothetical accumulation of evidence over time, by study design.

The objective of this study was to examine the body of evidence that was used to establish the causal relation between ZIKV infection and adverse outcomes. We hypothesised that the temporal sequence would follow Figure 2.1. We assessed 1) how long it takes before findings from a specific study design appear, 2) how publication of preprints could reduce the time to publication and 3) how time to publication evolves over time.

2.3 Methods

2.3.1 Included studies/Review methods

We analysed records of studies that were included in published systematic reviews [29] and two updates [177, 178] of the relationships between ZIKV and congenital abnormalities and GBS. The methods of the original review and updates are described elsewhere [29, 177]. The included studies reported evidence about any of the questions of the causality framework, based on the Bradford Hill dimensions of causality. Until January 18, 2017, we included epidemiological and basic research study designs; after that date we continued the review of evidence from epidemiological study designs only (Figure 2.2). Here we analyse studies collected until January 1, 2019.

2.3.2 Extracted information

For all included studies, we retrieved the received and published date, the location of the study and the study design (Table 2.1). For epidemiological studies, we extracted the study location and the number of patients with both exposure and the outcome according to the case definition provided in the publication. We excluded modelling studies or surveillance and outbreak reports.

Table 2.1: Information used in the analyses: Variables retrieved from PAHO, extracted from included studies (publications), and variables calculated from the data. Abbreviations: PAHO, Pan American Health Organization; PHEIC, Public Health Emergency of International Concern; ZIKV, Zika virus.

Variables	Explanation	Source
Introduction date	Date the state (for Brazil) or country reported the first case or cases of ZIKV according to PAHO [179]	PAHO
Publication date	The first date a publication was available	Publication
Received date	The date the publication was received by the journal the publication appeared in	Publication
Accepted date	The date the publication was accepted for publication by the journal the publication appeared in	Publication
Study design	Epidemiological studies: Case report, case series, case-control study, cohort study; Basic research: *in vivo* or *in vitro* studies	Publication
Outcome	Guillain-Barré syndrome or congenital abnormalities	Publication
Sample size (N)	For epidemiological studies: The total number of patients with the outcome and positive ZIKV exposure according to criteria of publication	Publication
Total time to publication from introduction	The time between introduction date and publication date in days	Calculated
Total time to publication from PHEIC	The time between February 1, 2016 and the publication date in days	Calculated
Publication delay	The time between the received date and the publication date in days	Calculated

2.3.3 Introduction date

We considered the date the first case of endemic ZIKV was reported in each state for Brazil, or country for the rest of the region (Figure 2.2) [179]. We assigned 16 July, 2015 as the date of ZIKV introduction if the state in Brazil was not explicitly reported. We assigned 1 October 2013 as the introduction date for French Polynesia [180].

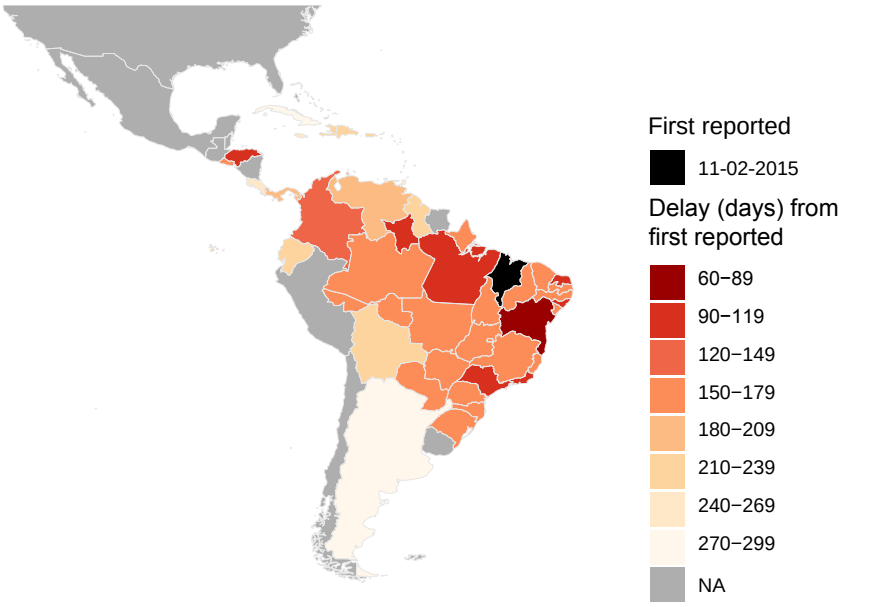


Figure 2.2: Map of South and Central America showing the timing of the first reported case of Zika virus according to the Pan-American Health Organization [179] by state for Brazil, by country for all other regions. NA: Not available.

2.3.4 Publication date

We defined the publication date as the earliest date the publication was available. If the publisher's website did not state an exact date, we assigned the 'epub' date from MEDLINE via PubMed or 'page created' date for specific online journals (EID and MMWR). We also recorded the date the manuscript was received by the publisher (received date) and the date of acceptance for publication (accepted date).

2.3.5 Total time to publication

We defined the time to publication as the time between introduction of ZIKV virus in the region and the publication date. For basic research studies, many of which were done in countries unaffected by ZIKV, we assigned the time to publication as the time between 1 February 2016 (the PHEIC declaration) and the first available publication date.

2.3.6 Publication delay

The delay resulting from the publication process (publication delay) was defined as the time between the 'received date' and the first available publication date.

2.3.7 Analysis

We provide a descriptive analysis of the total time to publication and the publication delay by publication. Of these durations, we provide the median and interquartile range (IQR) by study design and over time. We compare the publication delay by three month period (quarter).

2.4 Results

During the period of the first review [29] and subsequent update [177], we screened 2,847 publications. During the remaining period, between January 7, 2017 and January 1, 2019, we screened an additional 2,594 publications. Figure 2.3 shows the evolution of the volume of the published ZIKV research over time is provided.

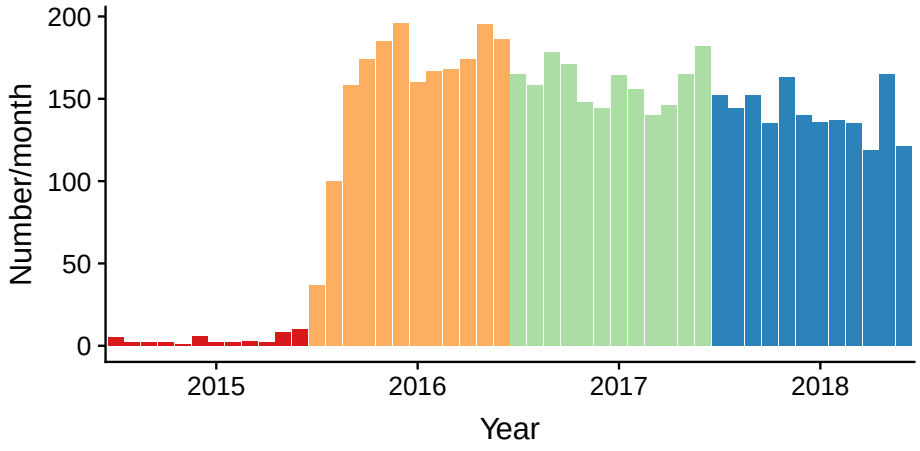


Figure 2.3: Research volume by month between 2016 and 2019, retrieved from MEDLINE via PubMed using the keywords 'Zika' and 'Zika virus'.

2.4.1 Included studies

We included 346 publications published between March 6, 2014 and January 1, 2019 (Table 2.2 and Figure 2.4). Up to January 18, 2017, we included 171 publications. Most publications were epidemiological study design (94/171), 77 out of 171 studies were basic research studies (either research on animal models or *in vitro* laboratory-based research). Between January 18, 2017 and January 1, 2019, we restricted our search to publications of epidemiological study design, and included another 175 publications. For 220/269 epidemiological studies, a date of ZIKV introduction was known. The time between received and published was reported in 204 out of 346 studies. In 16/204 of these studies there was no publication delay, usually because the first publication date was as a preprint.

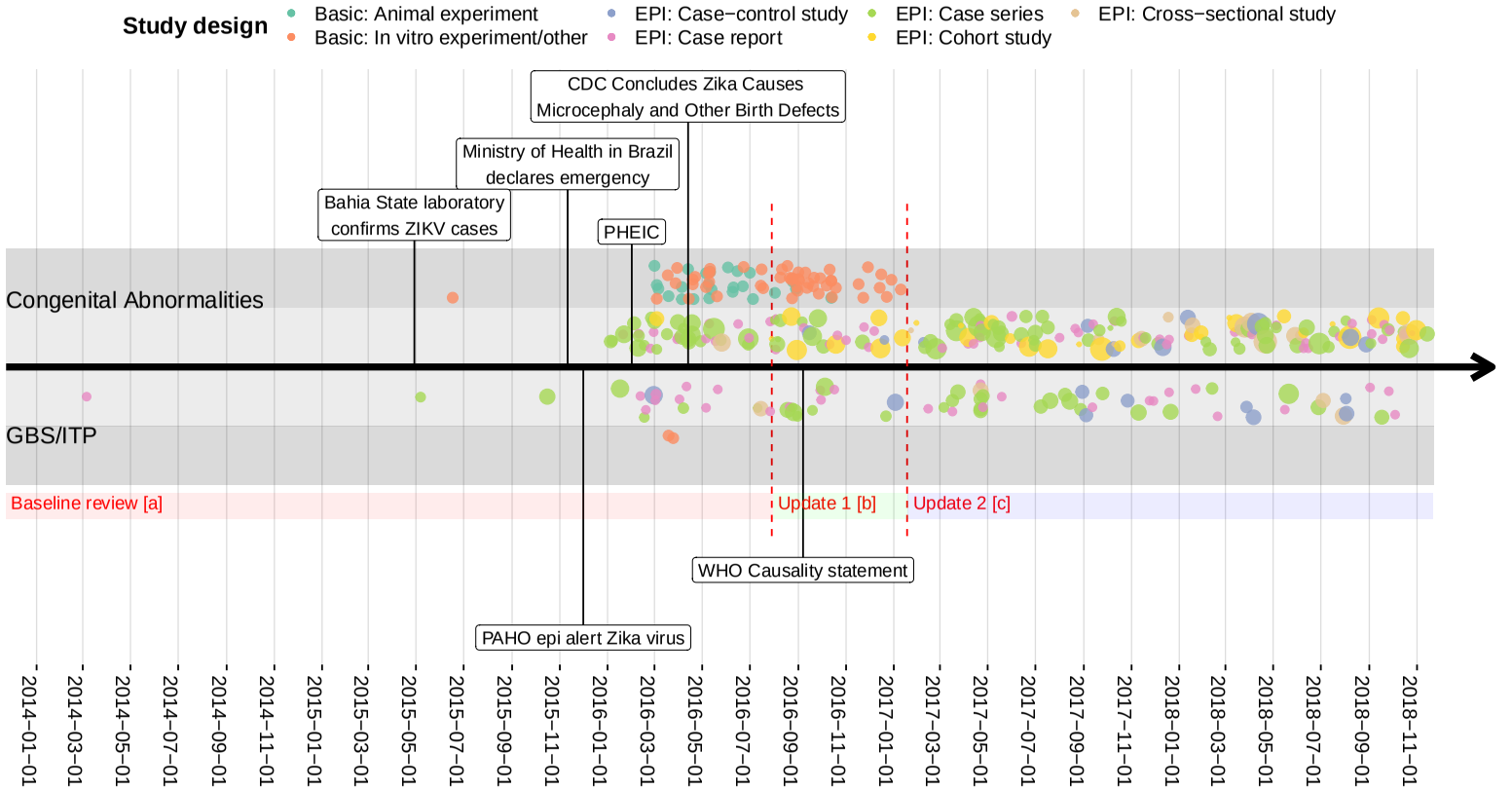


Figure 2.4: Publications by publication date, study design and outcome. For epidemiological studies, the size of the points correspond with the number of individuals with a positive outcome (Congenital abnormalities or GBS/ITP) and a positive exposure (Zika virus infection). Basic research studies were included up to the end of the first update (red dotted line Abbreviations: EPI, of epidemiological study design; CDC, Centers for Disease Control and Prevention GBS, Guillain-Barré syndrome; ITP, Immune thrombocytopenic purpura; PAHO, Pan American Health Organization; WHO, World Health Organization;, PHEIC, Public Health Emergency of International Concern; ZIKV, Zika virus. Different review periods: Baseline review [a][29], update 1 [b][177], update 2 [c][178].

Table 2.2: Overview of counts and completeness of data per study design and outcome. Studies published up to January 1, 2019 for epidemiological studies reporting on at least one individual with ZIKV exposure and outcome of interest, and up to January 17, 2017 for basic research studies. Abbreviations: CA, congenital abormalities; GBS, Guillain-Barré syndrome.

Study design/parameter	CA	GBS	Total
Epidemiological studies			
Case report	54	28	82
Case series	85	30	115
Case-control study	10	9	19
Cohort study	35	0	35
Cross-sectional study	14	4	18
Total epidemiological studies:	198	71	269
Introduction date available	159/198	61/71	220/269
Basic research studies			
Animal experiments	24	0	24
In vitro experiment/other	51	2	53
Total basic research:	75	2	77
Total:	273	73	346
Publication delay available	167/273	37/73	204/346
Studies with a zero publication delay	16/167	0/37	16/204

2.4.2 Total time to publication

Figure 2.5A shows the comparison of publications published between the PHEIC and the end of the second review period (January 18, 2017). We saw the first case reports and case series published after 44 and 77 days, respectively. Basic research emerged rapidly after the PHEIC. In this period a limited number of case-control studies was available. The earliest publication of a case-control study for GBS, was a result of a retrospective study looking back at the French Polynesia outbreak [176]. The median total time to publication was longer for more robust study designs (cohort studies, case-control studies). We see a similar pattern for epidemiological studies if we consider the data up to January 1, 2019 and consider the time to publication between the regional introduction of ZIKV and the publication date (Figure 2.5B).

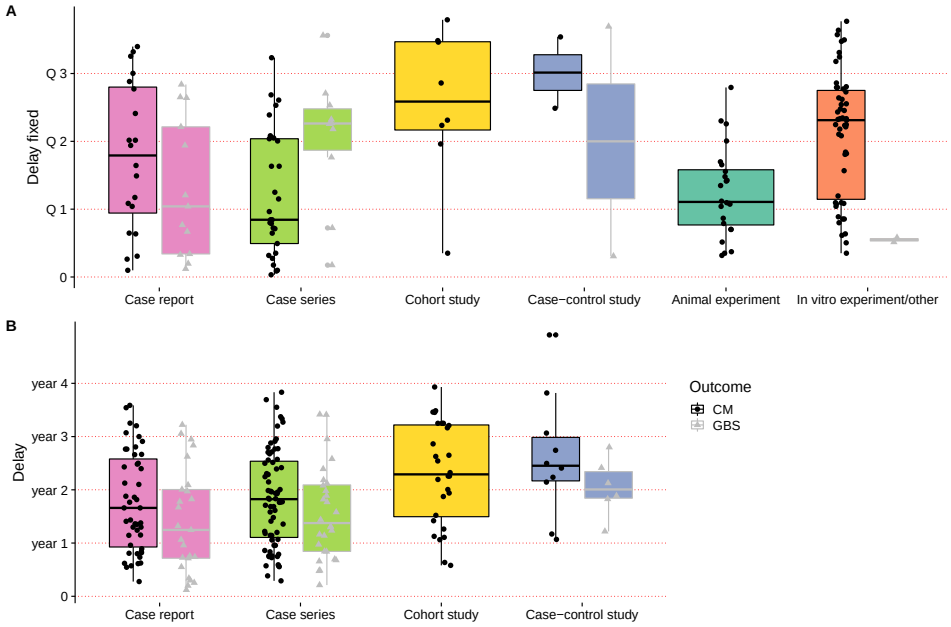


Figure 2.5: Time to publication, by study type. **A**. The time from the PHEIC declaration (1 February 2016) to the date of publication for studies included up to January 18, 2017. **B**. The time from introduction of ZIKV in the region in which a study was conducted to the date of publication for epidemiological studies included up to January 1, 2019. The box plots show the median an interquartile range, solid black shapes are studies of congenital abnormalities, grey shapes are studies of GBS.

2.4.3 Publication delay

Figure 2.6 shows the delay between receipt of manuscripts by a journal and the data of first publication, from the first quarter of 2016 to the end of 2018. Four out of seven basic research studies published in the first quarter of 2016 appeared as preprints (on www.bioRxiv.com) and had a median publication delay of zero days. The median publication delay increased with time to 107 days (IQR: 66–107 days) by the fourth quarter of 2016. During the same period for epidemiological study designs, the median publication delay increased from 19 days (IQR: 14–22) in the first quarter of 2016 to above 97 days (IQR: 63–138) from the fourth quarter of 2016. In the last quarter of 2018, the publication delay rose to 264 days (IQR: 144–392). On average, the publication delay accounted for 20% (IQR: 11–33%) of the total time from the PHEIC to first publication.

2.5 Discussion

In the 2013–2016 ZIKV outbreak, case reports and case series are the first study designs to emerge. Basic research studies appeared rapidly after this. Publication of more robust epidemiological study designs, such as case-control and cohort studies, appeared between 400–700 days after ZIKV was first detected in the region of the study origin. The delay due to the publication process of basic research and epidemiological research was lower at the beginning of the outbreak. A year after the declaration of the PHEIC, the publication delay

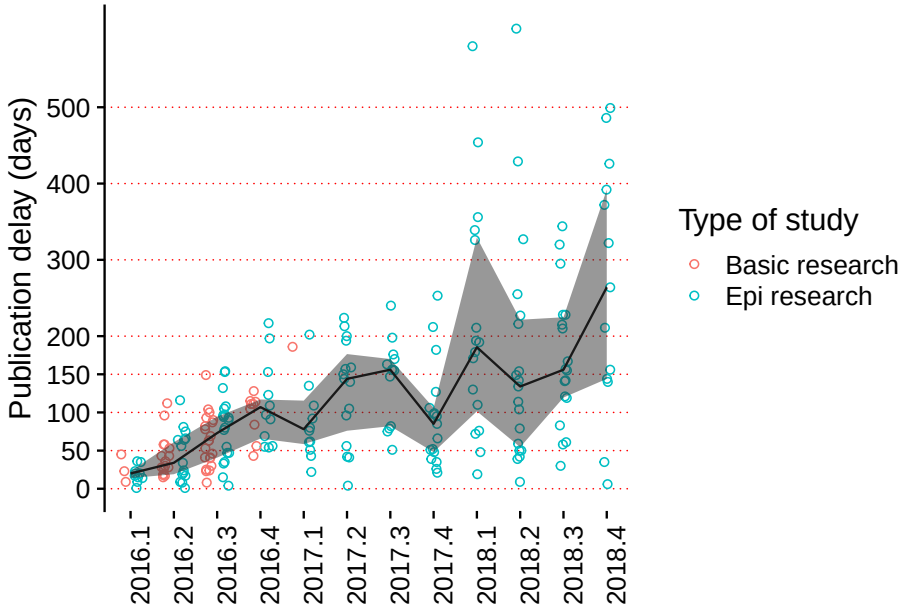


Figure 2.6: Publication delay, by quarter and by study design. The black line connects the median publication delay (black dots) and the interquartile range (grey ribbon) for all included studies.

rose to 150 days. Only a small proportion of publications was available as preprints (16/346).

2.5.1 Strengths and weaknesses of the study

A strength of this study is the pre-specified hypothesis about the time to publication of aetiological research and the use of data from systematic reviews that had screened and selected studies that addressed the causal relationship between ZIKV infection and its adverse outcomes. We calculated additional measures related to the time to publication of research, including delays due to the publication, and thus the time that could have been gained by publishing preprints.

The limited information extracted about each study was a limitation. The time between introduction of ZIKV and the actual publication of a research study is dependent on factors both within and between study designs. There is substantial variation in the time to publication within the study designs. We did not quantify several factors that likely influence this duration such as the size of the outbreak, the research capacity or outbreak preparedness. Small outbreaks or small population sizes limit the opportunity to enrol sufficient patients with adverse outcomes, and unless involved in multi-centre/multi-region studies, these regions are less likely to produce high quality epidemiological studies. The same holds true for regions with limited research capacity, such as appropriate diagnostic facilities and expertise. Outbreak preparedness likely increased over time, with funding increasing after the PHEIC, meaning that initiation of studies started relatively late for regions that were affected earliest by the outbreak. Countries that were affected later in time by ZIKV, might have already had surveillance and diagnostic methodology in place.

The publication delay is a proxy measure, which could not be calculated for all studies; for

only 59% (204/346) studies the "received date" was provided. It is unclear whether these data are missing at random. Furthermore, the recorded publication delay could only be calculated for the journal in which a study was published. The true publication delay includes the time taken up by rejection and resubmission. The publication date also ignores dissemination of the findings at conferences or within collaborations. However, here the information is only available to a limited audience. The timing of ZIKV introduction is also a proxy measure, which does not capture the first actual case, but signals the moment at which the health authorities and the research community noted the introduction in that region and thus serves its purpose as a proxy for when research start intensifying. Phylogenetic data suggest that ZIKV was often introduced months before formal detection and notification [167].

2.5.2 Interpretation of the findings

The sequence of emergence of evidence about causality was not exactly as hypothesised (Figure 2.1). While case reports and case series were the first types of study to be published, findings from animal research were also published quickly. This finding might have been influenced by the more frequent use of preprints to disseminate laboratory research than clinical science [181]. In our study, the time taken to publication of case-control studies and cohort studies was similar, particularly for studies of congenital outcomes. Case-control studies are widely assumed to be quicker to organise and conduct than cohort studies [132, 133]. In the ZIKV outbreak, one case-control study about GBS was published soon after the PHEIC declaration because it used data already collected from the earlier ZIKV outbreak in French Polynesia. An important consideration is the short duration of pregnancy. The cohorts that were fastest to produce results, were cohorts that were already in place for other disease (Dengue, influenza) [182]. Follow up of the outcomes of a disease exposure in pregnancy takes a matter of months. It might therefore not be possible to extrapolate this finding to other conditions in which the outcome takes years to develop.

The rapid, and sustained, publication of a large body of research about ZIKV is a rich resource for meta-research about causality. The declaration of the Ministry of Health of Brazil in November 2015 [93] and the declaration of the PHEIC by WHO in February 2016 [94] catalysed the ZIKV research effort across many different disciplines and resulted in an increase in research funding and a WHO-initiated research agenda [95]. Some of the observed patterns in study design and time publication are influenced by the type of outcome. Investigation of GBS, which is very rare, estimated at 4/10.000 ZIKV infections [63], is likely to be restricted to case-control as cohort studies would take too long to enrol enough participants.

We provide empirical evidence about publication delays during an outbreak of an emerging infection. WHO and others have encouraged rapid dissemination and timely open access to data to help the response during public health emergencies [183]. The ZIKV outbreak and 2013–2016 Ebola virus outbreak in West Africa, emphasised the need for rapid sharing of data. However, the publication delay returned to an average observed in across disciplines within a year after the declaration of the PHEIC; the age of the average preprint before it is published by a journal across different scientific disciplines is 166 days [184]. In medical research, publication of preprints is still underused [181] but the launch of the MedRXiv preprint server (www.medRxiv.org) in June 2019 might signal a change.

2.5.3 Implications for public health, policy and research

Looking back at the ZIKV outbreak and how evidence accumulated on the adverse outcomes will provide guidance for a next outbreak. It provides insight in how evidence accumulates in new causal questions. Specifically for disease outbreaks, we can increase preparedness by the lessons learnt from the Zika virus outbreak. Especially, since disease outbreaks or disease re-emergence continue to happen due to extraneous pressure such as shifts in climate, population growth and increased movement of people either due to displacement or voluntary movement [185].

In a disease outbreak with adverse outcomes that are new or incompletely understood, the full spectrum of evidence needs to be assessed to establish causality. Early in an outbreak, we need anecdotal evidence to drive discovery and explanation [133]. Studies across the different scientific disciplines are informative while we wait for robust epidemiological studies. Here, different frameworks can help us assess the evidence such as Bradford Hill dimensions [123]. We rely on a wide spectrum of evidence in line with how Krieger et al. phrase it: "Robust causal inference instead comprises a complex narrative, created by scientists appraising, from diverse perspectives, different strands of evidence produced by myriad methods." [124]. Rapid consensus on causality is often needed to form public health guidance.

Not one outbreak or emerging causal question is the same, thus deconstructing other causal problems based on study design and timing of evidence will provide more insight in how evidence accumulates. The ZIKV outbreak in the Americas was unique by its size; making rare non-pathognomonic outcomes visible. Also, the 2015–2017 outbreak in the Americas benefited from the outbreak in 2013 in French Polynesia. Much data was collected there, and retrospective analyses confirmed the association between ZIKV infection and adverse outcomes [176]. This resulted in the publication of a case-control study on GBS, rapidly after the declaration of the PHEIC [176]. Likewise, Cauchemez et al. used a modelling approach to estimate the risk of adverse congenital outcomes in French Polynesia retrospectively [186].

2.5.4 Conclusion

The accumulation of evidence over time in new causal problems seems to follow a hierarchy where case reports and case series are rapidly followed by basic research. During the ZIKV outbreak, robust epidemiological studies, such as case-control studies and cohort studies, took 400–700 days to appear. Causal inference based on a wide spectrum of evidence is therefore essential for early public health guidance in emerging causal problems. Publishing preprint does reduce the delay, and especially in epidemiological research this is an underused tool.

Chapter 3

Zika virus infection as a cause of congenital brain abnormalities and Guillain-Barré syndrome: From systematic review to living systematic review.

Michel Jacques Counotte[1]
Dianne Egli-Gany[1]
Maurane Riesen[1]
Million Abraha[1]
Teegwendé Valérie Porgo[2]
Jingying Wang[1]
Nicola Low[1]

[1] Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland
[2] Department of Social and Preventative Medicine, Université Laval, Québec, Canada

This article is published in ***F1000 Research***: Zika virus infection as a cause of congenital brain abnormalities and Guillain-Barré syndrome: From systematic review to living systematic review (2018) DOI: 10.12688/f1000research.13704.1.

Contribution: I contributed to the methodology, and coordinated and participated in the screening and extraction of evidence. I performed the analysis and made the figures and wrote the first draft of the manuscript and incorporated all feedback until the manuscript was published.

3.1 Abstract

Background. The Zika virus (ZIKV) outbreak in the Americas have caused international concern due to neurological sequelae linked to the infection, such as microcephaly and Guillain-Barré syndrome (GBS). The World Health Organization stated that there is "sufficient evidence to conclude that Zika virus is a cause of congenital abnormalities and is a trigger of GBS". This conclusion was based on a systematic review of the evidence published until June 30, 2016. Since then the body evidence has grown substantially, leading to this update of that systematic review with new evidence published from June 30, 2016 – January 1, 2017, version 2.

Methods. We review evidence on the causal link between ZIKV infection and adverse congenital outcomes and the causal link between ZIKV infection and GBS or immune-mediated thrombocytopaenia purpura. We also describe the transition of the review into a living systematic review, a review that is continually updated.

Results. Between June 30, 2016 and January 1, 2017, we identified 2413 publications of which 102 publications were included. The evidence added in this version confirms the conclusion of a causal association between ZIKV and adverse congenital outcomes. New findings expand the evidence base in the dimensions of biological plausibility, strength of association, animal experiments and specificity. For GBS, the body of evidence has grown during the search period for version 2, but only for dimensions that were already populated in the previous version. There is still a limited understanding of the biological pathways that potentially cause the occurrence of autoimmune disease following ZIKV infection.

Conclusions. This systematic review confirms previous conclusions that ZIKV is a cause of congenital abnormalities, including microcephaly and is a trigger of GBS. The transition to living systematic review techniques and methodology provides a proof of concept for the use of these methods to synthesise evidence about an emerging pathogen such as ZIKV.

3.2 Introduction

Outbreaks of Zika virus (ZIKV) infection in the Americas have caused international concern owing to the severity of neurological sequelae linked to the infection (WHO statement IHR 2005). During 2016, the number of countries affected by the ZIKV outbreak had grown from 33 countries (WHO situation report 05.02.2016) to 75 countries (WHO situation report 05.01.2017). By March 9 2017, 31 countries had reported microcephaly or other congenital central nervous system (CNS) abnormalities potentially associated with ZIKV infection and 23 had reported an increase in the incidence of the immune-mediated condition Guillain-Barré syndrome (GBS) or laboratory confirmed ZIKV in persons with GBS (WHO situation report 10.03.2017). The causal association between ZIKV and adverse neurological outcomes has now been examined in many systematic and non-systematic reviews of research [187, 188]. Case reports of other conditions in people with ZIKV infection, including immune-mediated idiopathic thrombocytopaenia purpura (ITP), have also been published [189–192].

The World Health Organization (WHO) based its assessment, that there is "sufficient evidence to conclude that Zika virus is a cause of congenital abnormalities and is a trigger of GBS" (WHO Zika causality statement) [29], on a review of systematically identified studies up to May 30 2016 and nonsystematically identified studies up to July 29, 2016. The review addressed specific questions about 10 dimensions of causal associations, based on the work of Bradford Hill [123] and organised as a causality framework (Supplementary

Table 1) that covers: temporality (cause precedes effect); biological plausibility of proposed biological mechanisms; strength of association; exclusion of alternative explanations; cessation (reversal of an effect by experimental removal of, or observed decline in, the exposure); dose-response relationship; experimental evidence from animal studies; analogous cause-and-effect relationships found in other diseases; specificity of the effect; and the consistency of findings across different study types, populations and times. The review included 108 articles about congenital abnormalities or GBS but there was no, or insufficient evidence to answer questions in several dimensions of the causality framework [29]. The causality framework included questions about ITP, but the review authors judged the number of published articles to be too low to assess causality. Since the WHO statement and accompanying publication, about 200 scientific publications every month are being added to the body of evidence about all aspects of research about ZIKV.

A living systematic review would help to overcome some of the challenges of keeping up to date with the high volume of ZIKV research publications. A living systematic review is a systematic review that is "continually updated, incorporating relevant new evidence as it becomes available" [136], which can help in fields where evidence is emerging rapidly and where new review outcomes might change policy or practice decision [10]. Technical solutions are available to facilitate the reviewing process, such as automated searching and deduplication and computer-assisted screening of article titles and abstracts, increase the efficiency and speed of a review team and transform the review into a living document.

This article aims to fulfil two separate objectives. First, we update our systematic review [29] with new evidence published from June 30, 2016 – January 1, 2017, about all 10 dimensions of the causal associations between ZIKV and (a) congenital brain abnormalities, including microcephaly, in the foetuses and offspring of pregnant women and (b) GBS/ITP in any population. Second, we describe the transition of the review into a living systematic review.

3.3 Methods

3.3.1 Classic protocol

We performed the review according to the protocol registered in PROSPERO CRD42016036693 (PROSPERO protocol). The eligibility criteria, information sources and search strategy, study selection and data extraction are the same as reported in the protocol and in the previous publication [29]. In brief, the search covers PubMed, Embase and LILACS electronic databases; the Pan American Health Organization (PAHO), WHO, the Centers for Disease Control and Prevention (CDC) and the European Centre for Disease Prevention and Control (ECDC) websites; and several preprint databases (BioRxiv, PeerJ and ArXiv). Search terms included 'Zika virus' and 'ZIKV' and corresponding MESH terms. Two reviewers screen and select articles for inclusion and extract data independently. We included publications that held information on at least one of the ten dimensions of the causality framework, regardless of the study design [29]. We gathered publications systematically from June 30, 2016 – January 1, 2017, for this update. We refer to the original publication as version 1 [29] and to this current update as version 2. Reporting of the results follows the Preferred Reporting Items of Systematic reviews and Meta-Analyses (PRISMA) statement (Supplementary File 1) [134].

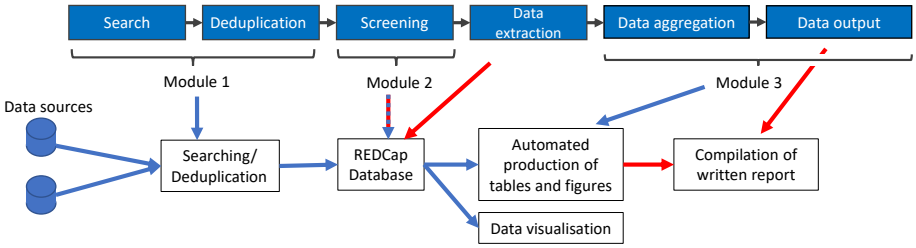


Figure 3.1: Living systematic review automation. Blue boxes and arrows represent the conceptual steps in a systematic review process. Automation is divided in three modules. Module 1 is the automation of the searching and deduplication of information from different data sources. Module 2 partly automates screening. Module 3 automates the production of tables and figures and outputs the data to a web platform (Data visualisation). Blue arrows represent automated information flows; red arrows represent manual input. The blue-red dashes arrow represents a blended form where reviewers verify automated decisions of the system. The white boxes show the practical implementation of the system and the data flow.

3.3.2 From systematic review to living systematic review

To keep up with the quantity of published research, we developed a living systematic review workflow (Supplementary File 2). We have identified three modules that could be automated (Figure 3.1). As of December 2017, module 1, searching and deduplication, and part of module 3, the output of the report have been automated. Reviewers can be notified daily with a list of new unique search results so that screening can be performed instantly. Following manual data extraction and synthesis, the output can be updated semi-automatically. We use the online database Research Electronic Data Capture (REDCap) [19] to maintain the references, perform screening and extract data into piloted extraction forms. We plan to update the review twice per year with formal peer reviewed updates (Figure 3.2), and continually through a web platform.

We synthesised the findings as narrative summaries of the evidence according to causality dimension and outcome, as previously described [29], and compare them with the previous review (version 1). We use the term 'confirmation' to summarise findings of new studies included in version 2 if they report the same findings as those in version 1. We use the term 'expansion' of evidence if studies included in version 2 provide new findings.

3.4 Results

Between June 30, 2016 and January 1, 2017, we identified 2,413 publications. After deduplication, we retained 1700 unique records. Based on screening of title and abstract, we discarded 1025 publications, retaining 675 items; after screening of the full text, 102 publications were included. Figure 3.3 shows the PRISMA flow diagram for this review [134]. Seventy-seven publications held information on one or more dimensions of the causality framework on adverse congenital outcomes and 26 on GBS or idiopathic thrombocytopaenia purpura. Table 3.1 compares the included publications, study types and the causality dimension(s) they address in version 1 [29] and version 2 of the review.

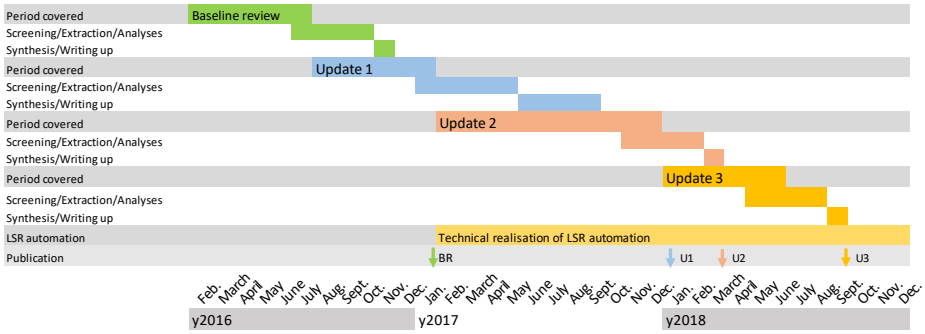


Figure 3.2: Timeline of review conduct, publication and transition to a living systematic review. Version 1 (v1, [29]) and version 2 (v2, this version) classic, manual systematic review. During 2017 automation of the workflow was conducted resulting in a projected version 3 (v3) and 4 (v4) with more rapid throughput. LSR, living systematic review.

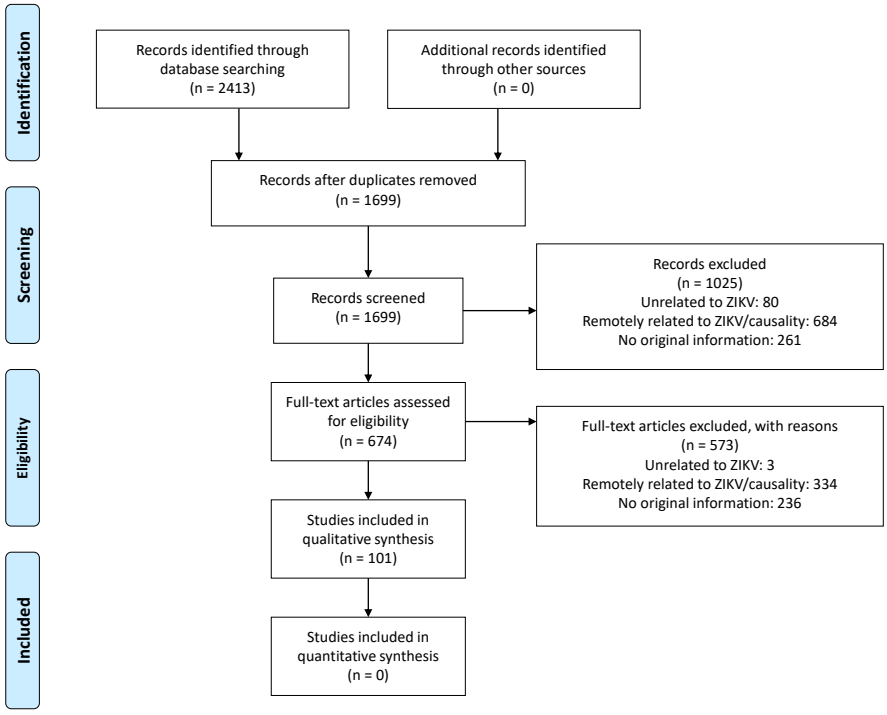


Figure 3.3: PRISMA flow diagram of included studies.

Condition and version number	Adverse congenital outcomes		GBS/ITP	
	v1, N	**v2, N**	**v1, N**	**v2, N**
Study type				
Case report	9	13	9	5
Case series	22	12	5	11
Case-control study	0	3	1	1
Cohort study	1	8	0	0
Cross-sectional study	2	1	0	1
Controlled trials	0	0	0	0
Ecological study/outbreak report	5	4	19	7
Modelling study	2	0	0	0
Animal experiment	18	8	0	0
In vitro experiment	10	22	0	0
Sequencing and phylogenetics	3	3	2	0
Biochemical/protein structure studies	NA	3	NA	0
Total:	**72**	**77**	**36**	**25**
Causality dimensions				
Temporality	21	21	26	21
Biological plausibility	25	42	4	0
Strength of association	3	5	2	4
Alternative explanation	18	23	6	12
Cessation	2	0	6	2
Dose-response relationship	0	0	0	0
Experiment	20	11	0	0
Analogy	NA	NA	NA	NA
Specificity	0	4	0	0
Consistency	NA	NA	NA	NA

Table 3.1: Summary of included publications by study type and on which causality dimension they provide evidence. One publication can address multiple causality dimensions. Comparison between the current (v2) and previous publication (v1, [29]) stratified by outcome. GBS/ITP, adverse autoimmune outcomes (Guillain Barré syndrome/idiopathic thrombocytopaenia purpura).

3.4.1 Adverse congenital outcomes

A detailed overview of the new evidence is provided in Table 3.2 and Supplementary Table 2. In the search period for review version 2, an additional 548 cases of adverse congenital outcomes were described in 30 studies [40, 193–223]. Adverse congenital outcomes described were: clinical microcephaly [40, 194–202, 206, 207, 209–213, 215, 216, 218–220, 223], imaging confirmed brain abnormalities [196–201, 207, 209–216, 218–221, 223], intrauterine growth restriction [196, 198, 212, 213, 218, 221], ocular disorders [196, 197, 201, 207, 212, 218, 220, 221] and auditory disorders [207, 208, 220].

Temporality

This update confirms the previous conclusion that ZIKV infection precedes the adverse congenital outcomes. We found an additional 21 publications in which ZIKV infection preceded the adverse congenital outcome at an individual level [194, 196–198, 201, 203, 207–209, 211–213, 215, 217, 218, 220, 221, 223–225] and at a population level [224, 226]. Infections in the first trimester and second seemed to be related to the most adverse outcomes [196, 212].

Cohort studies of pregnant women from French Guiana and Brazil found a higher proportion of congenital abnormalities in babies born from mothers infected in the first and the second trimester [196, 212].

Biological plausibility

This update includes an additional 42 studies [40, 193, 195, 203, 204, 209, 210, 213, 217–219, 221, 227–256], some of which expand the evidence base. Whereas review version 1 found inconclusive evidence of whether ZIKV particles in infants were capable of replication, both in vivo and ex vivo studies now demonstrate that this is the case [195, 217, 231, 235, 245, 249, 250]. Furthermore, there was a strong expansion of the evidence clarifying how ZIKV causes adverse congenital outcomes. ZIKV uses receptors from the TAM family to enter cells [240, 243–245, 247, 249], where the virus induces cell death, primarily in developing neuronal cells [228, 234, 238, 248, 251, 252, 254, 256].

Strength of association

We included five publications that confirm a strong association between ZIKV infection and adverse congenital outcomes [196, 199, 206, 212, 216]. The strength of association at an individual level was high but imprecise, owing to small sample sizes. Estimates from cohort studies [196, 212] appeared to be lower than those from case-control studies [199, 206, 216]. The definition of the outcomes and the outcomes assessed, varied between studies. The risk of any adverse congenital outcomes was higher and more variable than the risk of microcephaly. The risk ratio for microcephaly between ZIKV unexposed and exposed was 4.4 (95% CI: 0.2-80.8) in a cohort in Brazil [196] and 6.6 (95% CI: 0.8-56.4) in a cohort in French Guiana [212]. In the Brazilian cohort [196], the proportion of any adverse congenital outcomes among ZIKV infected women was high (41.9% [49/117]), compared with the uninfected group (5.2% [3/57]). In a prospective case control study in Brazil, women with laboratory-confirmed ZIKV had 55.5 (95% CI: 8.6-infinity) times the odds of having a baby with microcephaly compared with women without evidence of ZIKV infection [216]. A retrospective case control study in Hawaii found an odds ratio of 11.0 (95% CI: 0.8-147.9) [206]. In the latter, however, exposure was assessed retrospectively using serology.

Exclusion of alternatives

We included 23 new studies in this version [40, 193, 196, 197, 199, 201, 205, 207, 208, 210–219, 221, 223, 224, 257]. Many studies included in this review that reported on adverse outcomes of congenital ZIKV excluded TORCH infections [40, 193, 196, 197, 199, 201, 205, 207, 208, 210–219, 221, 223, 224, 257]; exposure to toxic chemicals [40, 197, 207, 208, 210] or genetic conditions [197, 207, 208, 210, 213, 217, 223]. Maternal or foetal malnutrition, hypoxic-ischaemic lesions and underlying genetic conditions were not excluded. No single alternative explanation could be given to explain the relation between ZIKV and adverse congenital outcomes.

Cessation

We did not find any new publications for this causality dimension. Evidence is still lacking on the effect of intentional removal due to lack of vaccination or elimination of mosquitoes on a large scale.

Dose-response

There is still no direct evidence about the association between Zika viral load and probability of adverse congenital outcome in observational studies, or of an association between symptomatic status and outcome. In a study in the United States, Honein et al. found similar proportions of adverse congenital outcomes in symptomatic and asymptomatic ZIKV-infected mothers [204].

Animal experiments

This version of the review includes an additional 11 studies [227, 230, 258–266]. These studies confirm a consistent relation between a range of contemporary ZIKV and adverse congenital outcomes, including from Brazil [260], Puerto Rico [264] and Mexico [262, 265]. The body of evidence coming from animal studies has grown; both in mice and macaques, congenital anomalies such as intra-uterine growth restriction and signs of microcephaly were observed after ZIKV infection [258, 260, 266].

Analogy

As for version 1, evidence for this dimension was not reviewed systematically because our search strategy did not include terms for other infections or conditions. Studies included in this version of the review confirm the analogy between congenital ZIKV and TORCH infections [267]. Vertical transmission of WNV and DENV were summarised in version 1 of the review. In version 2, we included a case series from El Salvador that reported CHIKV in 169 newborns of women with symptomatic CHIKV infection; a minority had CNS infection, but microcephaly was not reported [268]. For most analogous pathogens, infections earlier in the pregnancy have a higher risk of adverse outcomes [267].

Specificity

We included one study [60], suggesting an expansion of evidence of a distinct congenital Zika syndrome (CZS) [60]. In a review of 34 published reports, the authors suggest five congenital abnormalities that, in conjunction, comprise a pattern that is unique to ZIKV: severe microcephaly with overlapping cranial structures, subcortical location of brain calcifications, macular scarring and retinal mottling, congenital contractures and early pyramidal and extrapyramidal symptoms [60].

Consistency

The studies included in this version of the review confirm the pattern of consistency observed in version 1. ZIKV infection in association with adverse congenital outcomes were reported in a range of study designs from different regions (WHO situation report 05.01.2017), although the proportion of affected infants varies over geographic region and time. ZIKV exposure resulted in adverse congenital outcome in people living in ZIKV endemic areas [40, 193–199, 201, 202, 204–208, 210–214, 216, 218–220, 223–225, 257, 269, 270] and in female travellers who returned to non-endemic countries [193, 203, 209, 215, 217, 221, 271, 272]. Direct evidence from epidemiological studies comparing different lineages is lacking due to circulation of a single strain.

Conclusion

The evidence added in version 2 of the review confirms the conclusion of a causal association between ZIKV and adverse congenital outcomes. New findings expand the evidence base in the dimensions of biological plausibility, strength of association, animal experiments and specificity. In vitro and in vivo studies elucidate pathways on how these outcomes likely occur. Conclusive evidence on the strength of association is lacking. Studies provide crude overall measures of association, not taking into account potential co-factors.

Question	v1, n	v2, n	Summary
Temporality			
1.1a	18	19	Confirmation. Sufficient information to conclude that ZIKV infection precedes the development of congenital abnormalities in individuals [194, 196–198, 201, 207–209, 211–213, 215, 217, 218, 220, 221, 223–225].
1.1b	2	1	The peak of adverse congenital outcomes in Colombia was 24 weeks after infection [224] (similar to Brazil, 34 and 30 weeks [29]).
1.2	18	19	Confirmation. Most mothers of infants with adverse outcomes were exposed to ZIKV during the first or the second trimester of their pregnancy [193, 273].Third trimester exposure can lead to brain malformations as well [214].
Biological plausibility			
2.1	1	6	Confirmation of the role of viral entry factors (receptor-ligand interaction) [240, 243–245, 247, 249].
2.2	1	4	Substantial expansion of the evidence on which cells express the receptors responsible for cell entry of ZIKV [240, 244, 245, 249].
2.3	11	11	Expansion of evidence, sufficient information to conclude that ZIKV particles can be found in the umbilical cord blood and/or amniotic fluid of previously or currently infected mothers [40, 193, 195, 203, 204, 209, 210, 213, 217, 219, 221].
2.4	0	7	The evidence that ZIKV particles found in tissue of the offspring are capable of replication was inconclusive in the previous version. In this update we found that in vitro evidence strongly indicates these ZIKV particles are capable of replication [231, 235, 245, 249, 250]. Ex vivo experiments demonstrate ZIKV capable of replication as well [195, 217].
2.5	6	7	Expansion of evidence, sufficient information to conclude that particles can be found in the brain and other tissues of cases with congenital abnormalities [40, 193, 195, 210, 218, 219, 233].
2.6	7	6	Confirmation. ZIKV particles found in the brain are capable of replication [195, 228, 229, 232, 233, 239].

2.7	9	22	Strong expansion of evidence; Expansion of the understanding of how ZIKV causes congenital anomalies [227, 228, 230, 232, 234–242, 246–248, 251–256].
Strength of association			
3.1	2	5	Expansion of evidence on the strength of association at an individual level [196, 199, 206, 212, 216]. However, the estimation of the effect size remains imprecise.
3.2	1	0	At a population level, confirmation lacks on the strength of association. However, 29 countries reported a relative increase in microcephaly cases during the ZIKV outbreak (WHO situation report 05.01.2017).
Exclusion of alternatives			
4.1	18	23	Confirmation. In many epidemiological studies TORCH infections are assessed [40, 193, 196, 197, 199, 201, 205, 207, 208, 210–219, 221, 223, 224, 257].
4.2	4	5	Confirmation. Exposure to toxic chemicals has been excluded [40, 197, 207, 208, 210].
4.3	0	0	No exclusion of alternative explanation: maternal/foetal malnutrition.
4.4	0	0	No exclusion of alternative explanation: hypoxic-ischaemic lesions.
4.5	3	7	Confirmation of evidence where the role of genetic conditions was excluded [197, 207, 208, 210, 213, 217, 223].
4.6	0	0	No exclusion of alternative explanation: radiation.
Cessation			
5.1	0	0	No publication with evidence that intentional removal of ZIKV infection in individuals leads to a reduction in congenital abnormalities.
5.2	0	0	No publication with evidence that intentional removal of ZIKV infection at population-level leads to a reduction of cases of congenital anomalies
5.3	2	0	Natural removal (end of epidemic) leads to a reduction in microcephaly cases in Brazil; Other countries have shown a decrease in reported microcephaly cases as the cumulative ZIKV incidence plateaued
Dose-response			
6.1	0	0	No publication with evidence that the risk of adverse congenital outcomes is associated with the viral load in the mother.
6.2	0	0	No publication with evidence that the clinical severity of the infection of the mother determines the severity of the congenital anomalies. In one cohort study, symptoms in the mother did not influence the outcome [204].
Animal experiments			

7.1	3	3	Expansion of the evidence that the inoculation of pregnant female animals (mice and macaques) with ZIKV causes congenital anomalies in the offspring [258, 260, 266].
7.2	10	3	Confirmation of the evidence that the intracerebral inoculation of newborn mice with ZIKV leads to ZIKV replication in the CNS [259, 263, 265].
7.3	8	3	Expansion of the evidence that other routes of inoculation of newborn animals with ZIKV leads to ZIKV replication in the CNS (intravaginal infection of adult mice, subcutaneous infection of newborn mice) [262, 264, 266].
7.4	1	8	Expansion of the evidence that other experiments with animals or animal-derived cells support the association of ZIKV infection and congenital anomalies [227, 230, 258, 261–265].
Analogy			
8.1	NA	NA	CHIKV was shown to be vertically transmissible and lead to adverse congenital outcomes [268].
8.2	NA	NA	Confirmation. Congenital ZIKV analogous to other TORCH infections [267].
8.3	NA	NA	For most analogous pathogens, infections earlier in the pregnancy have a higher risk of adverse outcomes.
Specificity			
9.1	0	4	Expansion of evidence for distinct congenital Zika syndrome. Unique pattern of five features suggested: severe microcephaly with overlapping cranial structures, subcortical location of brain calcifications, macular scarring and retinal mottling, congenital contractures and early pyramidal and extrapyramidal symptoms [60].
Consistency			
10.1	NA	NA	Confirmation. ZIKV-related adverse congenital outcomes in different regions (South America, Central America, and the Pacific region). The proportion of cases varies over geographic regions/time.
10.2	NA	NA	Confirmation. ZIKV exposure and adverse congenital outcome in different populations (people living in ZIKV endemic areas and travellers.
10.3	NA	NA	No publication with evidence of consistency across lineages due to circulation of single strain.
10.4	NA	NA	Confirmation. ZIKV exposure and adverse congenital outcomes found in different study types.

Table 3.2: Summary of the evidence on the relation between ZIKV infection and adverse congenital outcomes. Evidence is displayed per dimension of the causality framework and per question. Zika virus (ZIKV); Dengue virus (DENV); West Nile virus (WNV); Chikungunya virus (CHIKV); Toxoplasmosis, Other [Syphilis, Varicella-zoster, Parvovirus B19], Rubella, Cytomegalovirus, and Herpes infections (TORCH); Central Nervous System (CNS).

3.4.2 GBS/ITP

In the search period for version 2 of the review, an additional 155 cases of ZIKV-related GBS [274–287] and 11 ZIKV-related cases of ITP [189–192] were described in 19 studies. Table 3.3 summarises the evidence for specific questions in each of 10 causality dimensions (detailed overview in Supplementary Table 3).

Temporality

We found an additional 17 publications that confirmed that ZIKV infection preceded the GBS or ITP at an individual level [189, 191, 192, 274–278, 280–283, 285, 287–289] or at a population level [279, 283, 290, 291]. ZIKV infections seems to be followed by GBS on average between 5 and 10 days. In one case series from Colombia [283], the authors distinguished between rapid onset of GBS symptoms after ZIKV symptoms (para-infectious) and post-infectious onset, with an asymptomatic period after ZIKV symptoms before the start of GBS symptoms.

Biological plausibility

We did not find any publications about the biological plausibility of ZIKV as a cause of GBS or ITP.

Strength of association

We did not find any comparative observational studies during the search period for version 2. Several surveillance studies confirmed an increase in notified GBS cases during ZIKV outbreaks at the population level [290]. Rate ratios were significantly higher for Brazil, Colombia, the Dominican Republic, El Salvador, Honduras, Suriname and Venezuela when comparing pre-ZIKV GBS incidence and the incidence during the outbreak [290]; this ratio ranged from 2.0 (95% CI: 1.6-2.6) to 9.8 (95% CI: 7.6-12.5).

Exclusion of alternatives

We included 12 publications [189, 190, 192, 275, 277, 280, 281, 283, 284, 286, 287, 290] that expanded the list of alternative causes for autoimmune disease that were excluded, such as infections, vaccines, other system illnesses and medication, drugs or other chemicals. Many GBS cases in these publications had serological evidence of previous exposure to DENV, as seen in version 1. It remains unclear how large the potential role of co-factors such as antibody dependent enhancement are.

Cessation

We did not identify any publications with evidence about the effect of intentional removal/elimination/prevention of ZIKV on either GBS or ITP. An additional publication confirmed evidence that the natural removal of ZIKV resulted in a decrease in GBS cases in Brazil, Colombia, Dominican Republic, El Salvador, Honduras, Suriname and Venezuela [290].

Dose-response

We did not identify any publications about this dimension for either GBS or ITP.

Animal experiments

No additional evidence from animal experiments was identified that support the association between ZIKV infection and GBS/ITP development.

Analogy

As for version 1, evidence for this dimension was not reviewed systematically because our search strategy did not include terms for other infections or conditions. We did not identify any new publications addressing this dimension for either GBS or ITP.

Specificity

We did not identify any new publications addressing this dimension for either GBS or ITP.

Consistency

Studies included in version 2 confirmed the consistency of the evidence for 3 of 4 questions about the association between ZIKV and GBS. By geographical region, ZIKV transmission has been associated with the occurrence of GBS in 2 of 4 regions; increased GBS incidence has been reported in the WHO regions of the Americas and the Western Pacific region, but not in the African or Southeast Asian region, despite recent ZIKV circulation [292]. By study design, the association between ZIKV infection and GBS has been found at individual and population level and with different study designs. By population, ZIKV infection has been linked to GBS in ZIKV endemic regions [189, 190, 192, 274–277, 279–281, 283, 286–291, 293] and travellers from non-affected countries who were exposed in these endemic regions [191, 278, 282, 284, 285]. There was insufficient evidence to examine the consistency of evidence about ZIKV and ITP.

Conclusion

The body of evidence has grown during the search period for version 2 but only for dimensions that were already populated in version 1 for GBS. There is still a limited understanding of the biological pathways that potentially cause the occurrence of autoimmune disease following ZIKV infection. Additionally, prospective comparative epidemiological studies are still lacking. It remains unclear how co-factors such as age and previous exposure to flaviviruses influences the risk of developing GBS. The evidence supports a temporal association between ZIKV and ITP but there is an absence of evidence for other dimensions of causality.

Question	n v1	n v2	Conclusion
Temporality			
1.1a	9	17	Expansion of the evidence. Additional case reports and case series were identified that confirmed that ZIKV infection preceded adverse autoimmune outcomes [189, 191, 192, 274–278, 280–283, 285, 287–289].
1.1b	9	4	Expansion of the evidence that on the population level ZIKV precedes GBS or ITP [279, 283, 290, 291].
1.2	7	14	Expansion of evidence that the interval between exposure to ZIKV and occurrence of symptoms is typical for para- or post-infectious autoimmune-mediated disorders [189, 192, 274–278, 280–285, 287].
Biological plausibility			
2.1	3	0	No additional evidence was identified that ZIKV epitopes mimic host antigens (molecular mimicry).
2.2	1	0	No additional evidence was identified that ZIKV infection leads to an increased in detectable autoreactive immune cells or autoreactive antibodies.
2.3	0	0	There is no evidence on other biologically plausible mechanisms of ZIKV infection leading to GBS/ITP.
Strength of association			
3.1	1	0	No additional evidence was identified on the association between Zika infection and GBS/ITP at the individual level.
3.2	2	4	Expansion of evidence. GBS incidence increased in several regions, during the same time ZIKV was circulating [279, 283, 290, 291].
Exclusion of alternatives			
4.1	7	9	Confirmation of the evidence where other infections were assessed. However, often previous DENV infection was reported, and not excluded [189, 190, 192, 277, 280, 281, 283, 287, 290].
4.2	0	1	Expansion on the evidence where vaccines were excluded [189].
4.3	0	6	Expansion on the evidence where other systemic illnesses were excluded [189, 190, 192, 275, 284, 286].
4.4	0	2	Expansion on the evidence where medication, drugs or other chemicals was excluded [275, 284].
Cessation			
5.1	0	0	No relevant studies identified that intentional removal or prevention of ZIKV infection in individuals leads to a reduction in cases with GBS/ITP.
5.2	0	0	No relevant studies identified that intentional removal or prevention of ZIKV infection at population level leads to a reduction in cases with GBS/ITP.

5.3	6	2	Expansion. Additionally, in Venezuela and the Dominican Republic, it was shown that GBS cases decreased with a decrease in reported ZIKV cases [283, 290].
Dose-response			
6.1	0	0	No relevant studies identified that the risk and the clinical severity of GBS/ITP are associated with viral titres.
Animal experiments			
7.1	0	0	No relevant studies identified where the inoculation of animals with ZIKV leads to an autoimmune reaction resulting in peripheral neuropathy or thrombocytopenia.
7.2	0	0	No relevant studies identified that other animal experiments support the association of ZIKV infection and GBS/ITP.
Analogy			
8.1	NA	NA	No additional studies identified that other flaviviruses or arboviruses cause GBS/ITP.
8.2	NA	NA	No additional studies identified that other pathogens cause GBS/ITP.
8.3	NA	NA	No additional studies identified that explain which pathogen or host factors facilitate the development of GBS/ITP.
Specificity			
9.1	0	0	No relevant studies identified that pathological findings in cases with GBS/ITP are specific for ZIKV infection.
Consistency			
10.1	NA	NA	Confirmation that the association between ZIKV cases and cases with GBS is consistently found across different geographical regions.
10.2	NA	NA	Confirmation that the association between ZIKV cases and cases with GBS is consistently found across different populations/subpopulations.
10.3	NA	NA	No additional studies identified that the association between ZIKV cases and cases with GBS/ITP is consistently found across different ZIKV lineages/strains.
10.4	NA	NA	Confirmation that the association between ZIKV cases and cases with GBS is consistently found across different study designs.

Table 3.3: Summary of the evidence on the relation between ZIKV infection and adverse autoimmune outcomes. Evidence is displayed per dimension of the causality framework and per question. Zika virus (ZIKV); Dengue virus (DENV); Guillain-Barré syndrome (GBS); immune-mediated idiopathic thrombocytopaenia purpura (ITP).

3.4.3 Search results from January 19, 2017 to January 05, 2018

Automated search and de-duplication processes identified 2,410 publications about any aspect of ZIKV infection. The next update, v3, of this review will address causality dimensions in the realm of epidemiological studies; strength of association, dose-response relationship, specificity and consistency.

3.5 Discussion

3.5.1 Statement of principal findings

This systematic review confirms evidence of a causal association between ZIKV and adverse congenital outcomes and between ZIKV and GBS, although evidence about biological plausibility is still lacking. We assessed evidence about an association between ZIKV and ITP but found that this only addressed the dimension of temporality. The review is transitioning from classic systematic review methods to those of a living systematic review.

3.5.2 Strengths and limitations of the study

The strengths of this study are the systematic approach to the identification, selection and extraction of data following a causality framework that provides a structure for the consideration of heterogeneous sources of evidence and a large set of review questions. Automation of the review output allows rapid updating of tables of results. We have also developed methods to automate search and de-duplication of search results to make the transition to a living systematic review that will allow continual updating of results. The main limitation of the classic systematic review of such a complex topic is the high workload and time required to maintain it. Another limitation, resulting from the large number of review questions, is the time taken to resolve inter-reviewer differences in interpretation of eligibility criteria. This could have resulted in subjectivity over decisions about inclusion in the review. Although a second reviewer checked all extractions, changes in the review team could introduce inconsistency. As in version 1, we used case definitions as authors described them in individual publications. This potential source of information bias is likely to decrease over time as standardised case definitions and protocols are adopted [294]. As in the previous version, we did not systematically apply quality assessment tools to individual studies. Because much of the technical infrastructure was built as the evidence emerged, output was delayed. As much of the LSR methodology was novel, it took time to find a balance between speed and efficiency.

3.5.3 Strengths and weaknesses in relation to other studies, discussing particularly any differences in results

Our systematic review differs from most standard reviews because of the number of questions within the dimensions of the causality framework and the number of outcomes. Other recent examples of living systematic reviews only distinguish between two study types (RCT and non-RCT) [139] and are guided by only a small set of review questions [295, 296]. Our review conclusion, confirming evidence for a causal association between ZIKV and GBS differs from that of a review [297] of the findings of four case reports [58, 280, 298, 299] and one case-control study [176]. The authors found insufficient evidence to confirm the presence of an acute motor axonal neuropathy variant of GBS. They did not, however, suggest an

alternative explanation for the increase in incidence of GBS in the countries that experienced ZIKV outbreaks. The two versions of our review included 64 publications about ZIKV and GBS across ten dimensions of causality.

3.5.4 Meaning of the study: possible mechanisms and implications for basic researchers, clinicians or policymakers

The conclusions on the causal relation between ZIKV and adverse congenital outcomes and ZIKV and GBS did not change with this update. We found insufficient evidence about the association between ZIKV and ITP to state with certainty that there is a causal association. The total volume of evidence about the association between ZIKV and GBS is less than for the association with adverse congenital outcomes. There is, in particular a lack of published research to elucidate biological mechanisms for direct neuronal or autoimmune damage in GBS [48]. The descriptive data about the numbers and types of different studies over time illustrates how evidence about a new, or re-emerging, infection emerges over time. The evidence from many regions that were affected by the ZIKV outbreak remains limited to anecdotal evidence of adverse outcomes, in the form of case reports or case series. The slowing of ZIKV transmission in 2017 means that fewer people are being affected by ZIKV and its complications and fewer people are being enrolled into prospective studies. Further progress in epidemiological research will rely more heavily on research consortia who are contributing to joint analyses of data from existing studies.

3.5.5 Unanswered questions and future research

As the volume and complexity of the evidence in different causality dimensions accumulates, the need for expert input and interpretation of the findings of this systematic review increases. The focus of research on ZIKV and causal associations with different types of adverse outcomes is also changing. For congenital abnormalities resulting from ZIKV vertical transmission, epidemiological research should focus on the need for observational comparative studies of CZS and to quantify the strength of association and clarify associations with gestational age, symptomatology and viral load and potential co-factors such as previous dengue infection and flavivirus vaccination. WHO standardised study protocols provide suggestions for exclusion of alternative explanations and exploration of co-factors (Harmonization of ZIKV Research Protocols). For GBS, epidemiological studies are needed to quantify the association with ZIKV more precisely, but also to determine whether there are distinct phenotypes resulting from autoimmune mechanisms or direct neuronal involvement. For ITP, additional evidence across all causality dimensions is needed.

3.5.6 Planned updates of a living systematic review

Living systematic review methodology and techniques will continue to develop. Since a chain is only as strong as its weakest link, any processing step has the potential to slow down trough put speed of a living systematic review. Clearly defined protocols that define not only update frequencies but also agreed upon through put speed of different actors in the publishing process is vital. The next update of the systematic reviews will use living systematic review methods to assess the evidence for 2017 and early 2018 (version 3, Figure 3.2). The review will, for the first time, separate evidence from epidemiological study designs from *in vitro* and *in vivo* laboratory studies. We will narrow down the inclusion criteria based on study type. Epidemiological evidence will address the causality dimensions 'strength of association',

'dose-response', 'specificity' and 'consistency'. Several co-factors might play a role in the strength of association. Thus, we will continue to collect information on previous DENV infection, yellow fever vaccination status, socioeconomic status, gestational age and others factors that might play a role in the severity of the outcome. We will amend the protocol with a more focused search strategy and inclusion criteria (Supplementary File 3).

Systematic reviews of questions addressed by laboratory studies are less frequent than those addressing epidemiological research questions. There is still need to update understanding of the causality dimensions 'biological plausibility' and 'animal experiments', particularly to increase our understanding of biological pathways for ZIKV effects on the peripheral nervous system and the immune system. We encourage and welcome collaboration from scientists with expertise in these fields to update systematic reviews for these causality dimensions.

3.5.7 Conclusion

This systematic review confirms previous conclusions that ZIKV is a cause of congenital abnormalities, including microcephaly and is a trigger of GBS. Evidence suggests an association with idiopathic thrombocytopaenia purpura but is not conclusive. The transition to living systematic review techniques and methodology provides a proof of concept for the use of these methods to synthesise evidence about an emerging pathogen such as ZIKV, ultimately leading to integration in the whole public health information cycle [300]. With the infrastructure for living systematic review methods and open source access to the software and outputs, we aim to enhance outbreak preparedness and the study of emerging and re-emerging pathogens.

3.6 Acknowledgements

We thank the members of the WHO Zika Causality Working Group for their input on the conceptualisation of the causality framework and Anina Häfliger for her assistance on screening publications.

3.7 Supplementary material

Supplementary material is available online in the published version of the manuscript http://doi.org/10.12688/f1000research.13704.1.

- Supplementary Table 1 – Bradford Hill's "viewpoints" of causation
- Supplementary Table 2 – Evidence table adverse congenital outcomes version 2
- Supplementary Table 3 – Evidence table GBS/ITP version 2
- Supplementary File 1 – PRISMA Checklist
- Supplementary File 2 – LSR automation methodology
- Supplementary File 3 – Search strategy version 3

Chapter 4

Zika virus infection as a cause of congenital brain abnormalities and Guillain-Barré syndrome: A living systematic review

Michel J. Counotte[1]
Kaspar W. Meili[1]
Katayoun Taghavi[1]
Guilherme Calvet[2]
James Sejvar[3]
Nicola Low[1]

[1] Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland
[2] Acute Febrile Illnesses Laboratory, Evandro Chagas National Institute of Infectious Diseases, Oswaldo Cruz Foundation, Rio de Janeiro, Rio de Janeiro, Brazil.
[3] Division of High-Consequence Pathogens and Pathology, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA

This article is published in ***F1000 Research***: Zika virus infection as a cause of congenital brain abnormalities and Guillain-Barré syndrome: A living systematic review (2019) DOI:10.12688/f1000research.19918.1.

Contribution: I coordinated and participated in the screening and extraction of evidence. I performed the analysis and the risk of bias assessment, I made the figures and wrote the first draft of the manuscript, and incorporated all feedback until the manuscript was published. I maintain the review as living systematic review.

4.1 Abstract

Background: The Zika virus (ZIKV) caused a large outbreak in the Americas leading to the declaration of a Public Health Emergency of International Concern in February 2016. A causal relation between infection and adverse congenital outcomes such as microcephaly was declared by the World Health Organization (WHO) informed by a systematic review structured according to a framework of ten dimensions of causality, based on the work of Bradford Hill. Subsequently, the evidence has continued to accumulate, which we incorporate in regular updates of the original work, rendering it a living systematic review.

Methods: We present an update of our living systematic review on the causal relation between ZIKV infection and adverse congenital outcomes and between ZIKV and Guillain-Barré syndrome (GBS) for four dimensions of causality: strength of association, dose-response, specificity, and consistency. We assess the evidence published between January 18, 2017 and July 1, 2019.

Results: We found that the strength of association between ZIKV infection and adverse outcomes from case-control studies differs according to whether exposure to ZIKV is assessed in the mother (OR 3.8, 95% CI: 1.7-8.7, I^2=19.8%) or the foetus/infant (OR 37.4, 95% CI: 11.0-127.1, I^2=0%). In cohort studies, the risk of congenital abnormalities was 3.5 times higher after ZIKV infection (95% CI: 0.9-13.5, I^2=0%). The strength of association between ZIKV infection and GBS was higher in studies that enrolled controls from hospital (OR: 55.8, 95% CI: 17.2-181.7, I^2=0%) than in studies that enrolled controls at random from the same community or household (OR: 2.0, 95% CI: 0.8-5.4, I^2=74.6%). In case-control studies, selection of controls from hospitals could have biased the results.

Conclusions: The conclusions that ZIKV infection causes adverse congenital outcomes and GBS are reinforced with the evidence published between January 18, 2017 and July 1, 2019.

4.2 Introduction

The Zika virus (ZIKV), a mosquito-borne flavivirus, caused a large outbreak of infection in humans in the Americas between 2015-2017 (WHO Zika -Epidemiological Update). Since then, the circulation of ZIKV has decreased substantially in the Americas [301] but ZIKV transmission will likely continue at a lower level . Smaller outbreaks have been reported from countries in Africa and Asia, including Angola and India [302], and Singapore [303]. Regions with endemic circulation, such as Thailand [304], have the potential for new ZIKV outbreaks with adverse outcomes [36].

The World Health Organization (WHO) declared ZIKV as a cause of adverse congenital outcomes and Guillain-Barré syndrome (GBS) as early as September 2016 [305], informed by a systematic review of evidence structured according to a framework of ten dimensions of causality, based on Bradford Hill (Table 4.1) [29]. The accumulation of evidence on the adverse clinical outcomes of ZIKV has barely slowed down since the WHO declared the Public Health Emergency of International Concern (PHEIC) on February 1st, 2016, with approximately 250 research publications on ZIKV appearing every month (see Zika Open Access Project). We updated the systematic review to January 18, 2017 as a living systematic review by introducing automated search methods to produce a high quality, up to date, online summary of research [136] about ZIKV and its clinical consequences, for all the causality dimensions [306].

Since 2017, understanding about the pathogenesis of how ZIKV causes congenital

abnormalities has evolved [307, 308]. The quality of diagnostic methods, especially for acute ZIKV infection, has also improved [309–311]. More importantly, understanding of the limitations of diagnostic testing, and the need for interpretation in the context of other flavivirus infections, has developed. Important epidemiological questions about the associations between ZIKV infection and adverse congenital outcomes and GBS remain unanswered, however. Much of the early epidemiological evidence, which relied on surveillance data, was limited in use because of issues with the quality of the reporting and case definitions. The reported strength of association between ZIKV and adverse outcomes has varied in studies of different designs and in different settings. Evidence for a dose-response relationship with higher levels of exposure to ZIKV resulting in more severe outcomes, of clinical findings that are specific to ZIKV infection, or of adverse outcomes caused by different lineages of ZIKV was not found in the earlier systematic reviews.

The objectives of this study are to update epidemiological evidence about associations between ZIKV infection and adverse congenital outcomes and between ZIKV and GBS for four dimensions of causality: strength of association, dose-response, specificity, and consistency.

4.3 Methods

We performed a living systematic review, which we have described previously [306]. This review updates the findings of the previous reviews [29, 306] and will be maintained up to date, in accordance with the methods described below. Reporting of the results follows the Preferred Reporting Items of Systematic reviews and Meta-Analyses (PRISMA) statement (Supplementary File 1) [134].

4.3.1 Focus on epidemiological aspects of causality

This review and subsequent updates focuses on four dimensions of causality that are examined in epidemiological study designs: strength of association, dose-response relationship and specificity of effects and consistency of association (Table 4.1, Extended data - Supplementary File 2). Evidence for domains of causality that are typically investigated in *in vitro* and *in vivo* laboratory studies (Table 4.1) was not sought. In the absence of licensed vaccines or treatments for ZIKV infection, we did not search for evidence on the effects of experimental removal of ZIKV.

4.3.2 Eligibility criteria

We considered epidemiological studies that reported original data and assessed ZIKV as the exposure and congenital abnormalities or GBS as the outcomes. We based the exposure and outcome assessment on the definitions used in the publications. We applied the following specific inclusion criteria (Extended data, Supplementary File 2):

Strength of association: at the individual level, we selected studies that included participants both with and without exposure to ZIKV (Figure 4.1), such as cohort studies and case-control studies. At the population level, we included studies that assessed the outcome during the ZIKV outbreak and provided a comparison with pre or post-outbreak incidence of the outcome.

Dose-response relationship: we included studies that assessed the relation between the level of the viral titre or the presence or severity of the symptoms and the occurrence or severity of the outcome.

Review	Baseline [8]	Update 1 [10]	Update 2 [this review]
Period	<May 30, 2016	May 30, 2016-January 18, 2017	>January 18, 2017 -July 01, 2019
Search strategy	"ZIKV" or "Zika"	"ZIKV" or "Zika"	Focussed search strategy (Supplementary File 2)
Study design	Epidemiological studies; *in vivo*/*in vitro* studies; surveillance reports		Epidemiological studies
Dimensions of the causality framework based on Bradford Hill*	Temporality (cause precedes effect)		
	Biological plausibility of proposed biological mechanisms		
	Strength of association		Strength of association
	Exclusion of alternative explanations		
	Cessation (reversal of an effect by experimental removal of, or observed a decline in, the exposure)		
	Dose-response relationship		Dose-response relationship
	Experimental evidence from animal studies		
	Analogous cause-and-effect relationships found in other diseases		
	Specificity of the effect		Specificity of the effect
	Consistency of findings across different study types, populations and times		Consistency of findings across different study types, populations and times

Table 4.1: Comparison of the search strategy included study designs and causality dimensions addressed in the different review periods. *The causality framework is described elsewhere in detail [177].

Specificity of the outcome for ZIKV exposure: we included studies that assessed whether the pathological findings in cases with the outcome are specific for ZIKV infection.

Consistency: we looked at eligible studies to determine the consistency of the relationship between ZIKV exposure and the outcomes across populations, study designs, regions or strains.

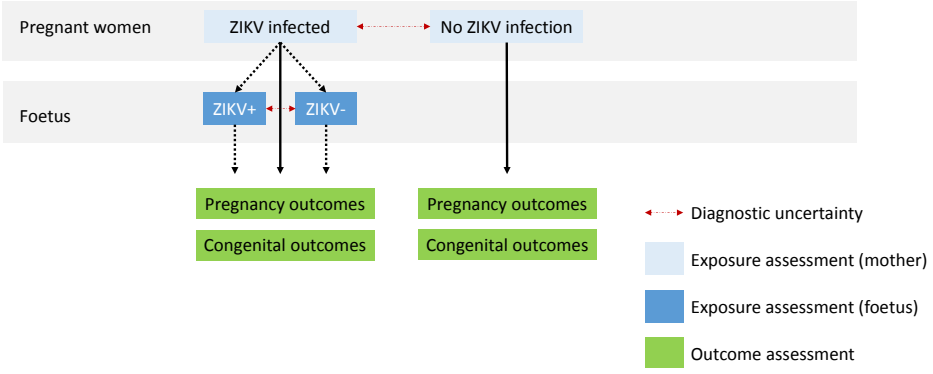


Figure 4.1: For congenital abnormalities due to ZIKV, exposure assessment in mother-infant pairs can be performed in the mother or the foetus or infant.

4.3.3 Search and information sources

We searched PubMed, Embase, LILACS and databases and websites of defined health agencies (Extended data, Supplementary File 2). We included search terms for the exposure, the outcome and specific study designs. We also performed searches of the reference lists of included publications. A detailed search strategy is presented in Supplementary File 2. For this review, the search covered the period from January 19, 2017 to July 1, 2019.

4.3.4 Study selection and extraction

One reviewer screened titles and abstracts of retrieved publications. If retained, the same reviewer screened the full text for inclusion. A second reviewer verified decisions. One reviewer extracted data from included publications into piloted extraction forms in REDCap (version 8.1.8 LTS, Research Electronic Data Capture) [312]. A second reviewer verified data entry. Conflicts were resolved by consulting a third reviewer.

4.3.5 Synthesis of evidence

First, we summarised findings for each dimension of causality and for each outcome descriptively. Where available, we calculated unadjusted odds ratios (OR) or risk ratios (RR) and their 95% confidence interval (CI) from published data for unmatched study designs. For matched study designs, we used the effect measure and 95% CI presented by the authors. For publications that presented results for multiple measures of exposure and/or outcome, we compared these results. We applied the standard continuity correction of 0.5 for zero values in any cell in the two-by-two table [313]. We used the I^2 statistic to describe the percentage of variation across studies that is due to heterogeneity for reasons other than chance [147]. Quantitative synthesis was performed using R 3.5.1 [314]. We conducted random effects meta-analyses using the R package metafor (version 2.0-0) [313]. Finally, we compared descriptive and quantitative findings from this review period with previous versions of the review [29, 306].

4.3.6 Searching and screening frequency

Daily searches of PubMed, Embase and LILACS are automated and monthly searches are performed manually for other information sources in the first week of the month (Extended data, Supplementary File 2), with screening of all retrieved publications on the same day. The search strategy consisted of a combination of free terms and MESH terms that identified the exposure and outcomes (Extended data, Supplementary File 2). Searches from multiple sources were combined and automatically deduplicated by an algorithm that was tested against manual deduplication. Unique records enter a central database, and reviewers are notified of new content.

4.3.7 Frequency of results update

The tables and figures presented in this paper will be updated every six months as a new version of this publication. As soon as new studies are included, their basic study characteristics are extracted and provided online https://zika.ispm.unibe.ch/assets/data/pub/causalityMap/.

4.3.8 Duration of maintenance of the living systematic review

We will keep the living systematic review up to date for as long as new relevant data are published and at least until October 31, 2021, the end date of the project funding.

4.3.9 Risk of bias/certainty of evidence assessment

To assess the risk of bias of cohort studies and case-control studies, we compiled a list of questions in the domains of selection bias, information bias, and confounding,

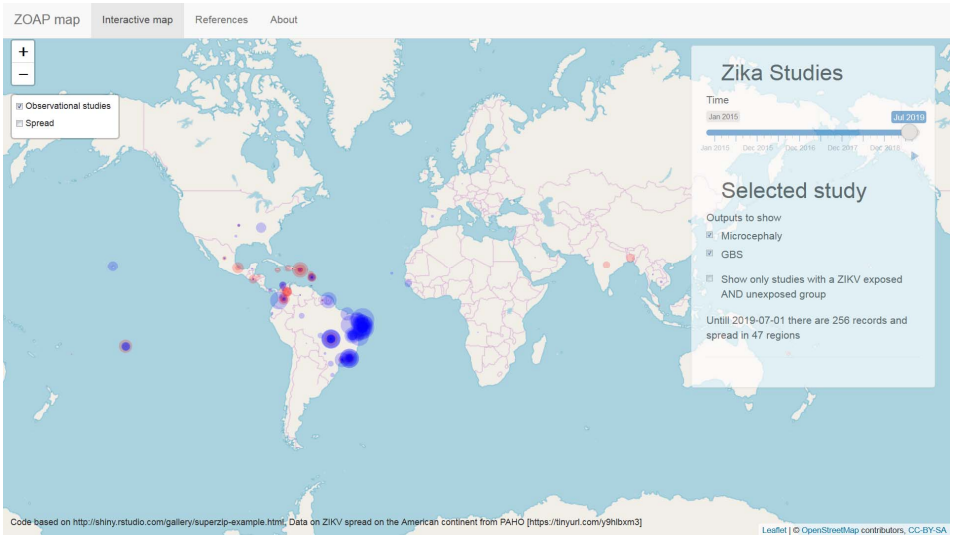


Figure 4.2: Map of the epidemiological studies that report on adverse congenital outcomes (blue) or Guillain-Barré syndrome (red) associated with Zika virus exposure. The size of the points correspond with the number of exposed individuals with the adverse outcome, according to the definitions used in the publications.

based on the quality appraisal checklist of the United Kingdom National Institute for Health and Care Excellence (NICE) [https://www.nice.org.uk/process/pmg4/chapter/-g-quality-appraisal-checklist-quantitative-studies-reporting-correlations-and] and literature [315]. Two independent reviewers conducted the quality assessment. Disagreements were resolved by a third reviewer.

4.4 Results

4.4.1 Search results from January 19, 2017 to July 1, 2019 (Update 2)

From January 19, 2017 to July 1, 2019 we screened 1941 publications, of which we included 638 based on title and abstract. After reviewing the full text, 249 publications were included (Table 2, Figure 4.2 Of these publications, 195 reported on congenital abnormalities linked to ZIKV [50, 171, 316–507] and 59 on GBS [63, 303, 339, 360, 392, 414, 442, 508–559]. Five outbreak reports, described both outcomes [339, 360, 392, 414, 442].

4.4.2 Adverse congenital outcomes

We included 39 case reports [323, 330, 332, 345, 351, 357, 376, 378, 379, 385, 393, 395, 396, 401, 404, 405, 410, 420, 440–442, 446, 447, 449, 452, 461, 462, 464, 465, 468, 482, 488, 489, 494, 499, 501, 503, 507], 62 case series [50, 316, 320–322, 324–326, 329, 335, 339, 340, 342–344, 346, 348, 352, 353, 355, 356, 359, 362, 363, 367, 368, 371, 387, 390, 399, 400, 403, 409, 412, 415, 416, 424, 427, 429, 431, 435–437, 443–445, 455, 457, 458, 460, 466, 467, 469, 473, 475, 486, 491, 492, 496–498, 502], 10 case-control studies [354, 398, 402, 421, 422, 430, 459, 481, 490, 504], 35 cohort studies [317, 318, 327, 333, 334, 336, 337, 347, 350, 364, 366, 374, 375, 380, 386, 394, 411, 423, 426, 433, 434, 438, 448, 451, 453, 454, 456, 463, 474, 480, 485, 487, 493, 500, 506], 19

Table 4.2: Included publications in the baseline review, update 1 and update 2 (this version), by outcome and epidemiological study design.

Outcome	**Adverse congenital outcomes, number of publications**			**GBS, number of publications**		
Review period/version	Baseline*	Update1†	Update2	Baseline*	Update1†	Update2
Study design						
Case report	9	13	39	9	5	17
Case series	22	12	62	5	11	22
Case-control study	0	2	10	1	1	7
Cohort study	1	8	35	0	0	0
Cross-sectional study	2	1	19	0	1	3
Controlled trials	0	0	0	0	0	0
Ecological study/outbreak report	5	4	27	19	7	9
Modelling study	2	0	3	0	0	1
Total:	**41**	**40**	**195**	**34**	**25**	**59**

* Baseline review, earliest date of each information source to May 30, 2016 [29]; † Update 1, May 30, 2016 to January 18, 2017 [306].

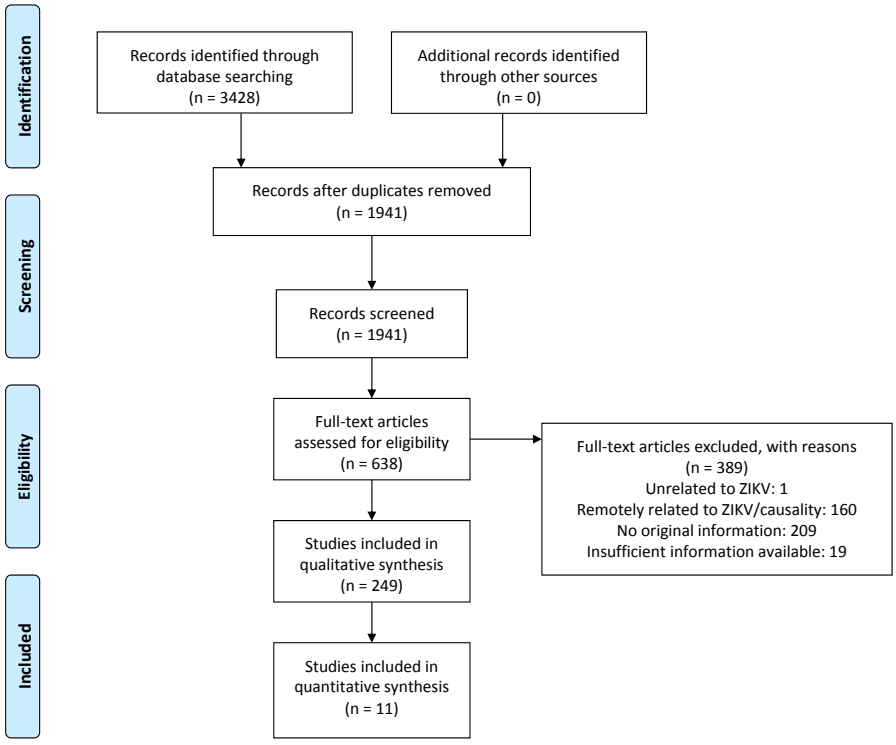


Figure 4.3: PRISMA flow-chart publications retrieved, screened and included between January 18, 2017 and July 1, 2019. Adapted from: Moher et al. (2009) [134].

cross-sectional studies [171, 319, 341, 349, 358, 370, 372, 377, 383, 391, 397, 408, 417–419, 428, 432, 471, 505], seven ecological studies [331, 338, 389, 407, 476, 477, 495], three modelling studies [328, 382, 384] and 20 outbreak reports [360, 361, 365, 369, 373, 381, 388, 392, 406, 413, 414, 425, 439, 450, 470, 472, 478, 479, 483, 484] that report on congenital abnormalities linked to ZIKV.

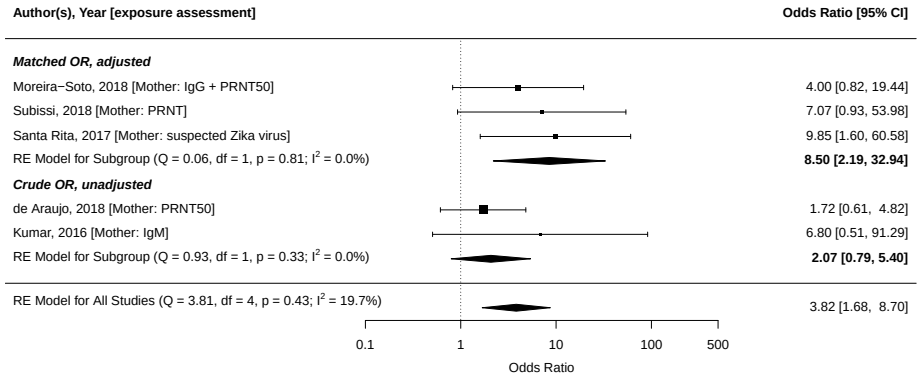
Causality dimensions

Strength of association

Individual level: In this review period, five case-control studies reported on strength of association, four in Brazil (n=670 participants) [354, 398, 421, 459] and one in French Polynesia (n=123 participants) [481]. The studies assess adverse pregnancy outcomes including infants born with microcephaly, according to exposure to ZIKV for cases. Of these, all studies matched controls, based on gestational age and/or region. During the review period up to January 18, 2017, we included one case-control study [199], which we replaced with a publication reporting the final results of the study [354]. The meta-analyses incorporate estimates from studies identified in all review periods. Assessment of exposure status varied between the studies (Extended data, Supplementary File 3). In five case-control studies, exposure to ZIKV was assessed in the mother, based on clinical symptoms of 'suspected Zika virus infection' [459], or presence of maternal antibodies measured by IgM (Kumar et al. (2016) [206]), PRNT (de Araujo et al. (2018) [354], Subissi et al. (2018) [481]), or both PRNT and IgG (Moreira-Soto et al. (2018)) maternal antibody [422]. In meta-analysis, we found that the odds of adverse congenital outcomes (microcephaly or congenital abnormalities) were 3.8 times higher in ZIKV-infected mothers (95% CI: 1.7-8.7, tau^2=0.18, I^2=19.8%, Figure 4.4). Moreira-Soto et al. (2018) found that in Bahia, Brazil, Chikungunya infection was also associated with being a case [422]. In two matched case-control studies, exposure to ZIKV was assessed in infants; Araujo et al. found a 73.1 (95% CI 13·0–Inf) times higher odds was reported for microcephaly when ZIKV infection was assessed by reverse transcription polymerase chain reaction (RT-PCR) in the neonate [354]. Krow-Lucal et al. (2018) found an OR of 21.9 (95% CI: 7.0-109.3) based on evidence of recent Zika infection assessed using IgM followed by PRNT in infants in Paraiba, Brazil [398]. When exposure was assessed at the infant-level, the combined odds of adverse congenital outcomes was 37.4 times higher (95% CI: 11.0-127.1, tau^2=0, I^2=0%, Figure 4.4).

In this review period, one cohort study reported on strength of association, in 610 pregnant women returning from ZIKV-affected areas in Central and South America to the USA [318]. Maternal ZIKV exposure was measured using RT-PCR or IgM followed by plaque reduction neutralisation test (PRNT). Among the 28 infants born to ZIKV-infected mothers, none were diagnosed with microcephaly and, one was born with a major malformation. In the ZIKV-unexposed group, eight out of 306 had major malformations. A complete overview of different outcomes assessed is presented in the extended data, Supplementary File 3. During the review period up to January 18, 2017, we included two cohort studies, one in women with rash and fever (Brasil et al. (2016)) and one in unselected pregnant women (Pomar et al. (2017)) [196, 212]. In meta-analysis of all three studies, we found that the risk of microcephaly was 3.5 times higher in ZIKV-infected mothers of babies (95% CI: 0.90-13.51, tau^2=0, I^2=0%, Figure 4.5).

Population level: At a population level, data from Mexico collected at different altitudes during the ZIKV outbreak, showed that the risk of microcephaly was increased in regions at altitudes below 2200m, in which ZIKV can circulate [384]. Hay et al. (2018) reanalysed surveillance data from Colombia and northeast Brazil and concluded that time-dependent



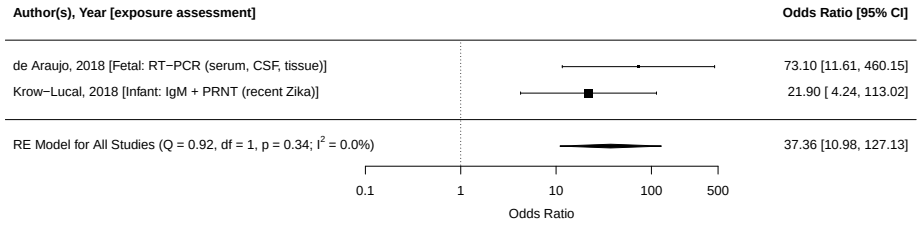


Figure 4.4: Forest plot and meta-analysis of case-control studies reporting on ZIKV infection assessed in mothers (A) and in infants (B) and adverse congenital outcomes (microcephaly, congenital malformations, central nervous system abnormalities). The odds ratio from the five case-control studies that assess exposure in mothers combined is 3.8 (95% CI: 1.7-8.7, tau^2=2.37, I^2=19.8%); the odds ratio for the studies that assess exposure in infants is 37.4 (95% CI: 11.0-127.1, tau^2=0, I^2=0%). The odds ratios are plotted on the log scale. Abbreviations: CSF, cerebrospinal fluid, PRNT, plaque reduction neutralisation test; RE, random effects; RT-PCR, reverse transcription polymerase chain reaction.

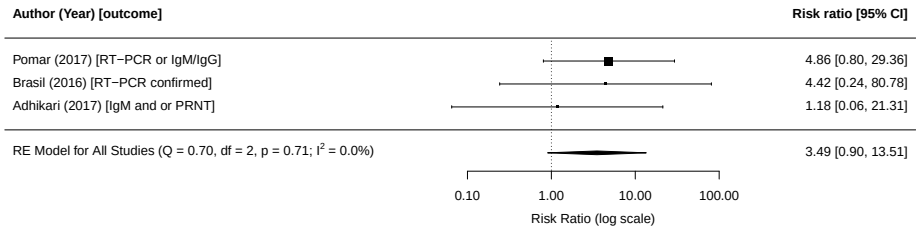


Figure 4.5: Forest plot and meta-analysis of cohort studies reporting on ZIKV infection and adverse congenital outcomes. The risk ratio from the random effects model is 3.5 (95% CI: 0.9-13.5, tau^2=0, I^2=0%). The risk ratios are provided on the log scale. Abbreviations: PRNT, plaque reduction neutralisation test; RE, random effects; RT-PCR, Reverse transcription polymerase chain reaction.

reporting changes might have caused apparent inconsistencies in the proportion of congenital abnormalities as a result of maternal ZIKV infection [382].

Dose response

Halai et al. (2017) [118] examined the severity of congenital outcomes according to measures of the severity of maternal ZIKV infection in a subset of mothers in the cohort presented by Brasil et al. (2016) [196]. They evaluated ZIKV load, assessed by RT-PCR using the cycle threshold (CT) as a measure of number of RNA copies, and a severity score of symptoms in 131 pregnant women. They concluded that neither higher viral load nor more severe symptoms was associated with more severe congenital abnormalities [380]. Moreira-Soto et al. found higher maternal antibody titers in microcephaly cases compared with controls [422]. In previous review periods, Honein et al. (2016) compared outcomes in neonates born to symptomatic and asymptomatic infected pregnant women returning to the USA with possible ZIKV infection and found no differences [204].

Specificity

Although some outcomes, such as lingual phenotype [372] or neurogenic bladder [560], have been hypothesised as a specific phenotype for congenital ZIKV infection, no additional evidence was identified that certain congenital adverse findings are specific for congenital ZIKV infection.

Consistency

Geographical region: All four WHO geographic regions (the Africa region [AFRO], the American region [AMRO], the South-East Asian region [SEARO] and the Western Pacific region [WPRO]) with past or active ZIKV transmission have now reported congenital abnormalities due to ZIKV infection. During this review period, the first congenital abnormality due to infection with the Asian lineage of the virus on the African mainland occurred in a traveller returning from Angola [464]. Possible cases of congenital abnormalities have occurred in Guinea-Bissau [457]. In the most recent WHO situation report from March 2017, two cases of

microcephaly are documented in Thailand and one in Vietnam, which were also described in detail in other works [420, 498, 499]. We identified another publication on congenital abnormalities due to endemic ZIKV in Cambodia [346]. The occurrence of congenital adverse outcomes in AFRO, SEARO, and WPRO seems sporadic, despite the endemic circulation of ZIKV. As noted above, the observed complication rate varied strongly between regions. Extended data, Supplementary File 3 provides a full overview of the published studies on congenital abnormalities per region and country.

Traveller/non-traveller populations: In this update, we found further evidence that congenital abnormalities occurred in infants born to women travellers returning from ZIKV-affected areas and women remaining in those areas. In total, 25 publications report on 272 congenital abnormalities due to ZIKV infection in travellers [323, 329, 334, 366, 378, 387, 395, 400, 404, 405, 416, 424, 446, 448, 451–453, 465, 468, 469, 471, 482, 497, 501, 503], with 109 publications reporting congenital abnormalities due to ZIKV in 2652 non travellers [50, 171, 316, 320, 321, 324–327, 330, 332, 333, 335–337, 339, 340, 342–346, 348, 349, 351–357, 362, 363, 367, 368, 370–372, 374–377, 386, 391, 393, 394, 398, 399, 402, 403, 408–411, 415, 418, 420–423, 426–430, 433–438, 440–442, 444, 445, 447, 454, 456–459, 461–464, 466, 467, 473, 475, 481, 485, 487–494, 496, 499, 500, 502, 504–507]

In this review period, evidence emerged that transmission through sexual contact with infected travellers also resulted in foetal infection [396, 501].

Study designs: The association between ZIKV infection and congenital abnormalities was consistent across different study designs (Table 2).

Lineages: We found no new evidence of consistency across different lineages from observational studies. The currently observed adverse congenital outcomes are linked to the ZIKV of the Asian lineage.

Risk of bias assessment

In all case-control studies, uncertainty about the exposure status due to imperfect tests could result in a bias towards the null. Some studies might suffer from recall bias where exposure was assessed by retrospectively asking about symptoms [459, 481]. For the cohort studies [196, 318], the enrolment criteria were based on symptomatology. As a result, even in the absence of evidence of ZIKV, the unexposed groups might have had conditions that were unfavourable to their pregnancy. We expect this to bias the results towards the null or underestimate the true effect. Owing to imperfect diagnostic techniques, both false positives (IgM, cross reactivity) and false negatives (due to the limited detection window for RT-PCR) might occur, potentially resulting in bias; the direction of this bias would often be towards the null. None of the studies controlled for potential confounding. Extended data, Supplementary File 4 provides the full risk of bias assessment of the studies included in the meta-analysis.

4.4.3 GBS

During this review period, we included 17 case reports [442, 512, 518, 524, 528, 529, 531, 532, 535, 537, 540, 542, 545, 548, 551, 557, 558], 22 case series [339, 508–511, 513, 514, 516, 519, 521, 522, 525, 526, 536, 539, 541, 543, 546, 549, 553, 556, 559], seven case-control studies [303, 523, 527, 533, 544, 547, 550], one ecological study [530], one modelling study [63] and eight outbreak reports [360, 392, 414, 515, 534, 552, 554, 555] that reported on ZIKV infection and GBS.

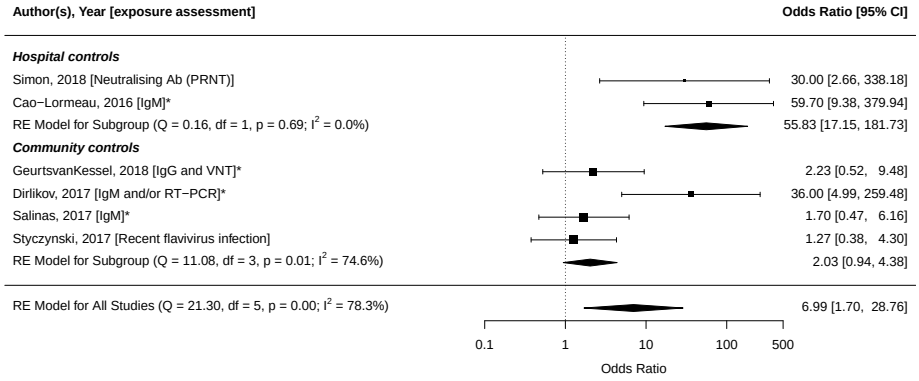


Figure 4.6: Forest plot of six included case-control studies and their exposure assessment. Odds ratios (ORs) are shown on the log-scale. The meta-analysis is stratified by the selection of controls: Hospital controls, or community/household controls. Most similar exposure assessment measures are compared (IgM [176, 527, 544, 547], recent flavivirus infection [550], or IgM and/or RT-PCR [523]). OR: 7.0 [95% CI: 1.7-28.8, tau^2=2.78, I^2=78.3%]. ORs from studies marked with an asterisk (*) are matched ORs, unmarked studies provided crude ORs.

Causality dimensions

Strength of association

Individual level. The number of studies reporting on the strength of association between ZIKV infection and GBS at an individual level increased substantially. We identified five case-control studies [523, 527, 544, 547, 550] published since the previous update, which included one case-control study from French Polynesia [176]. All studies were matched for age and place of residence. In the studies from Brazil, Colombia, Puerto Rico and New Caledonia, temporal clustering of cases in association with ZIKV circulation was documented [523, 544, 547, 550]. In Bangladesh, ZIKV transmission was endemic [527]. Exposure assessment was based on serology [544, 550] or a combination of RT-PCR and serology [523, 527, 547]. Extended data, Supplementary File 3 shows the variability in ORs according to criteria for ZIKV exposure assessment, based on unmatched crude data extracted from each case-control study. Figure 4.6 shows the association between GBS and ZIKV infection, using the diagnostic criteria that were most similar across studies. Heterogeneity was considerable (I^2=78.3%), but was reduced slightly after stratification based on the method of selection of controls. The summary OR was higher in studies that enrolled controls from hospital (OR: 55.8, 95% CI: 17.2-181.7, tau^2=0, I^2=0%) [176, 547] than in studies that enrolled controls at random from within the same community [523, 544, 550] or from the same household [527] (OR: 2.0, 95% CI: 0.8-5.4, tau^2=0.46, I^2=74.6%). Amongst studies with community controls, ORs were lower when enrolment and assessment took place several months after onset of symptoms [544, 550] than in studies with contemporaneous enrolment [523, 527]. To further illustrate the heterogeneity in exposure assessment between and within the studies, we provide additional aggregations of the data in Extended data, Supplementary File 3.

Population level. At a population level, Mier-Y-Teran-Romero et al. (2018) showed that the estimated incidence of GBS ranged between 1.4 (0.4–2.5) and 2.2 (0.8–5.0) per 10,000

ZIKV infections comparing surveillance/reported cases from Brazil, Colombia, Dominican Republic, El Salvador, French, Honduras, Puerto Rico, Suriname, Venezuela, and Micronesia. The across-location minimum and maximum estimates were used to estimate an average risk of having GBS and being reported after ZIKV infection across locations of approximately 2.0 GBS cases per 10,000 infections (95% credible interval 0.5–4.5 per 10,000 ZIKV infections) [63].

Dose response

In a case-control study, Lynch et al. (2018) found higher titres of neutralising antibodies in ZIKV-infected GBS cases than in patients with symptomatic ZIKV infection but without GBS [533].

Specificity

Dirlikov et al. (2018) compared Puerto Rican GBS cases reported through public health surveillance that were preceded by ZIKV and cases that were not preceded by ZIKV infection [522]. Clinical features involving cranial nerves were observed more frequently in ZIKV-related cases and, at a six-month follow-up visit, residual cranial neuropathy was noted more often in this group. However, clinical symptoms did not allow a distinction to be made between ZIKV and non-ZIKV related GBS.

Consistency

Geographical region: During this review period, GBS likely due to ZIKV infection was reported in Asia; including Thailand, Bangladesh, Singapore and India [303, 332, 511, 527]. Publication in the WHO Region of the Americas followed the pattern as observed before and no GBS linked to ZIKV infection was reported in Africa. Extended data, Supplementary File 3 provides a full overview of the published studies on congenital abnormalities by region and country. In a reanalysis of surveillance data from the Region of the Americas, Ikejezie et al. (2016) found consistent time trends between GBS incidence and ZIKV incidence [530].

Traveller/non-traveller populations: In studies included in this update, we found additional evidence of GBS in both travellers and non-travellers with ZIKV infection. Ten publications report on 11 travellers [512, 514, 528, 531, 532, 537, 542, 551, 557, 558], while 34 publications report GBS or ITP due to ZIKV in 402 non travellers [303, 339, 442, 508, 513, 516–527, 529, 533, 535, 539–541, 543–550, 553, 556, 559].

Study designs: Across the different study designs, the relation between GBS and ZIKV is consistently shown. Table 2 and Extended data, Supplementary File 3 provide an overview of the included study designs.

Lineages: We still lack evidence on the consistency of the relation between GBS and ZIKV across different lineages from observational studies. The observed cases of GBS were linked to ZIKV of the Asian lineage.

4.4.4 Risk of bias assessment

Potential selection bias in case-control studies was introduced by the selection of controls from hospitals rather than from the communities in which the cases arose [176, 547]. Uncertainty about the exposure status due to imperfect tests would tend to result in a bias

towards the null. Two case-control studies did not conduct a matched analysis although controls were matched, and no study controlled for potential confounding by factors other than those used for matching. Exclusion criteria and participation rate, especially of the controls, were poorly reported. Extended data, Supplementary File 4 provides the full risk of bias assessment of the studies included in the meta-analysis.

4.5 Discussion

In this living systematic review, we summarised the evidence from 231 observational studies in humans on four dimensions of the causal relationship between ZIKV infection and adverse congenital outcomes and GBS, published between January 18, 2017 and July 1, 2019.

4.5.1 Strengths and limitations

The strengths of this living systematic review are that, first, we automated much of the workflow [306]; we search both international and regional databases daily and we screen papers for eligibility as they become available, so publication bias is unlikely. Second, we have quantified the strength of association between ZIKV infection and congenital abnormalities and GBS and investigated heterogeneity of outcome and exposure assessment within and between studies. Third, for congenital outcomes, we included studies with both microcephaly and other possible adverse outcomes, which acknowledges the spectrum of congenital adverse outcomes caused by ZIKV. This work also has several limitations. First, we have not assessed the dimensions of the causality framework that involve laboratory studies, so we have not updated the pathobiology of ZIKV complications, which was addressed in the baseline review [29] and the first update to January 2017 [306]. Limiting the review to epidemiological domains has allowed more detailed analyses of these studies and we hope that scientists with expertise in laboratory science will continue to review advances in these domains. Second, the rate of publications on ZIKV remains high so, despite the reduced scope and automation, maintenance of the review is time-consuming and data extraction cannot be automated. Third, this review could suffer from continuity bias, which is important for the conduct and interpretation of living systematic reviews and results from changes in the author team. Careful adherence to the protocol will reduce this risk.

4.5.2 Interpretation of the findings

ZIKV and congenital abnormalities: Since the earlier versions of the review [29, 306], evidence on the causal relationship between ZIKV infection and congenital abnormalities has expanded. Unfortunately, the total number of cases investigated in the published cohort or case-control studies remains small. In case-control studies in which infants with microcephaly or other congenital abnormalities are compared with unaffected infants, the strength of association differs according to whether exposure to ZIKV is assessed in in the mother (OR 3.8, 95% CI: 1.7-8.7, tau^2=0.18, I^2=19.8%) or the foetus/infant (OR 37.4, 95% CI: 11.0-127.1, tau^2=0, I^2=0%). This large difference in effect size can be explained by the fact that not all maternal ZIKV infections result in foetal infection. In cohort studies, the risk of congenital abnormalities was 3.5 times higher (95% CI: 0.9-13.5, I^2=0%, tau^2=0) in mothers with evidence of ZIKV infection than without, which is similar to the OR for maternal exposure to ZIKV estimated from case-control studies. Further research is needed to understand the drivers of mother to child transmission. Higher maternal antibody titres were correlated

with a higher incidence of adverse congenital outcomes in one case-control study [422]. But, amongst ZIKV-infected mothers followed prospectively, severity of ZIKV infection was not associated with more severe congenital abnormalities [380]. Convincing evidence on a dose-response relation is therefore still lacking.

ZIKV and GBS: Evidence about the causal relation between ZIKV infection and GBS has grown since our last review [306]. The body of evidence is still smaller than that for congenital abnormalities, possibly because GBS is a rare complication, estimated to occur in 0.24 per 1000 ZIKV infections [176]. In this review, the strength of association between GBS and ZIKV infection, estimated in case-control studies, tended to be lower than observed in the first case-control study reported by Cao-Lormeau (2016) in French Polynesia [176]. It is possible that finding by Cao-Lormeau et al. was a 'random high', a chance finding [561]. Simon et al., however, found a similarly strong association in a case-control study in New Caledonia [547]; in both these studies, controls were patients in the same hospital. Although matched for place of residence, it is possible that they were less likely to have been exposed to ZIKV than the cases, resulting in an overestimation of the OR. In case-control studies in which controls were enrolled from the same communities as the cases, estimated ORs were lower, presumably because exposure to ZIKV amongst community-enrolled controls is less biased than amongst hospital controls [562]. Under-ascertainment of ZIKV infection in case-control studies in which enrolment occurred several months after the onset of symptoms [544, 550] is also likely to have reduced the observed strength of association. There is also possible evidence of a dose-response relationship, with higher levels of neutralising antibodies to both ZIKV and dengue in people with GBS [533]. However, the level of antibody titre might not be an appropriate measure of viral titre, and merely a reflection of the intensity of the immune response. Taking into account the entire body of evidence, inference to the best explanation [563] supports the conclusion that ZIKV is a cause of GBS. The prospect of more precise and robust estimates of the strength of association between ZIKV and GBS is low because outbreaks need to be sufficiently large to enrol enough people with GBS. In the large populations that were exposed during the 2015-2017 outbreak, herd immunity will limit future ZIKV outbreaks.

4.5.3 Implications for future research

The sample sizes of studies published to date are smaller than those recommended by WHO to obtain precise estimates of associations between ZIKV and adverse outcomes [Harmonization of ZIKV Research Protocols to Address Key Public Health]. Given the absence of large new outbreaks of ZIKV infection in 2017-2019, there is a need for consortia of researchers to analyse their data in meta-analyses based on individual participant data [Individual Participant Data Meta-analysis of Zika-virus related cohorts of pregnant women (ZIKV IPD-MA)]. Future collaborative efforts will help to quantify the absolute risks of different adverse congenital outcomes and allow investigation of heterogeneity between studies [204, 380, 386].

This review highlights additional research gaps. First, we did not assess the complication rates within the infected group in studies without an unexposed comparison group; the adverse outcomes are not pathognomonic for ZIKV infection, making an appropriate comparison group necessary. Even though there are no individual features of ZIKV infection that are completely specific, the growing number of publications on ZIKV will allow better ascertainment of the features of a congenital Zika syndrome [60]. In this review, we did not take into account the performance of the diagnostic tests in assessing the strength

of association. Future research should include robust validation studies, and improved understanding of contextual factors in the performance of diagnostic tests, i.e. the influence of previous circulation of other flaviviruses, the prevalence of ZIKV and the test used.

This living systematic review will continue to follow studies of adverse outcomes originating from ZIKV circulation in the Americas, but research in regions with endemic circulation of ZIKV is expected to increase. Such studies will clarify whether ZIKV circulation in Africa and Asia also results in adverse outcomes, as suggested by the case-control study of GBS from Bangladesh [527]. Increased awareness might improve the evidence-base in these regions, where misperceptions about the potential risks of ZIKV-associated disease with different virus lineages has been reported [564]. An important outstanding question remains whether the absence of reported cases of congenital abnormalities or GBS in these regions represent a true absence of complications or is this due to weaker surveillance systems or reporting [565]. The conclusions that ZIKV infection causes adverse congenital outcomes and GBS are reinforced with the evidence published between January 18, 2017 and July 1, 2019.

4.6 Supplementary Material

4.6.1 Underlying data

All data underlying the results are available as part of the article and no additional source data are required.

4.6.2 Extended data

Extended data can be found online at the Harvard dataverse: Living systematic review on adverse outcomes of Zika - Supplementary Material. 10.7910/DVN/S7USUI and Living systematic review on adverse outcomes of Zika - Figures and Table. 10.7910/DVN/DLP5AN. Supplementary material is available online in the published version of the manuscript http://doi.org/10.12688/f1000research.19918.1.

This project contains the following extended data:

- SupplementaryFile1Prisma.docx (PRISMA checklist)
- SupplementaryFile2Methods.docx (Supplementary file 2, additional information to the Methods)
- SupplementaryFile3Results.docx (Supplementary file 3, additional information to the Results)
- SupplementaryFile4ROB.tab (Risk of bias assessment)
- Fig1.pdf (Most recent version of Figure 1)
- Fig2.pdf (Most recent version of Figure 3, PRISMA flowchart)
- Fig3A.pdf (Most recent version of Figure 4A)
- Fig3B.pdf (Most recent version of Figure 4B)
- Fig4.pdf (Most recent version of Figure 5)

- Fig5.pdf (Most recent version of Figure 6)
- Table2.pdf (Most recent version of Table 2)

4.6.3 Reporting guidelines

PRISMA checklist and flow diagram for 'Zika virus infection as a cause of congenital brain abnormalities and Guillain-Barré syndrome: A living systematic review', 10.7910/DVN/S7USUI and Figure 4.3.

4.6.4 Grant information

This work was supported by the Swiss National Science Foundation [320030_170069], end date: 31/10/2021

Chapter 5

Sexual transmission of Zika virus and other flaviviruses: a living systematic review

Michel Jacques Counotte[1]
Caron Rahn Kim[2]
Jingying Wang[1]
Kyle Bernstein[3]
Carolyn Deal[4]
Nathalie Jeanne Nicole Broutet[2]
Nicola Low[1]

[1] Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland
[2] Department of Reproductive Health and Research, World Health Organization, Geneva, Switzerland
[3] Division of Sexually Transmitted Disease Prevention, Centers for Disease Control and Prevention, Atlanta, GA, USA
[4] National Institute of Allergy and Infectious Diseases, Rockville, MD, USA

This article is published in ***PLOS Medicine***: Sexual transmission of Zika virus and other flaviviruses: a living systematic review (2018) DOI: 10.1371/journal.pmed.1002611.

Contribution: I drafted the first version of the sexual transmission framework, I came up with the study idea, I coordinated the review team, I performed the analysis and the risk of bias assessment, I made the figures and wrote the first draft of the manuscript, and incorporated all feedback until the manuscript was published. I maintain the review as living systematic review.

5.1 Abstract

Background: Health authorities in the United States and Europe report an increasing number of travel-associated cases of sexual transmission of Zika virus (ZIKV) following the 2016 ZIKV outbreak. This, and other scientific evidence, suggests for the first time that ZIKV is sexually transmissible in addition to its primary mosquito-borne route. The objective of this systematic review and evidence synthesis was to help develop a more lucid epidemiology of ZIKV.

Methods and Findings: We performed a living (i.e., continually updated) systematic review of evidence published up to 15 April 2018 about sexual transmission of ZIKV and other arthropod-borne flaviviruses in humans and other animals. We defined seven key elements of ZIKV sexual transmission for which we extracted data: 1) rectal and vaginal susceptibility to infection, 2) incubation period following sexual transmission, 3) serial interval between the onset of symptoms in a primary and secondary case, 4) duration of infectiousness, 5) reproduction number, 6) probability of transmission per sex act, and 7) transmission rate. We identified 1217 unique publications and included 128, of which 77 presented data on humans and 51 presented data on animals. Laboratory experiments confirm that rectal and vaginal mucosae are susceptible to infection with ZIKV and that the testis serves as reservoir for the virus in animal models. Sexual transmission was reported in 36 human couples, of which 34/36 underwent male to female sexual transmission. The median serial symptom onset interval in 15 couples was 12 days (interquartile range: 10-14.5); the maximum was 44 days. We found evidence from two prospective cohorts that ZIKV RNA is present in human semen with a median duration of 34 days (95% CI: 28-41 days) and 35 days (no CI given) (low certainty of evidence, according to GRADE). Aggregated data about detection of ZIKV RNA from 37 case reports and case series indicate a median duration of 40 days (95% CI: 30-49 days) and maximum duration of 370 days in semen. In human vaginal fluid, median duration was 14 days (95% CI: 7-20 days) and maximum duration was 37 days (very low certainty). Infectious virus in human semen was detected for a median duration of 12 days (95% CI: 1-21 days) and maximum of 69 days. Modelling studies indicate that the reproduction number is below one (very low certainty). Evidence was lacking to estimate the incubation period or the transmission rate. Evidence on sexual transmission of other flaviviruses was scarce. The certainty of the evidence is limited because of uncontrolled residual bias.

Conclusion: The living systematic review and sexual transmission framework allowed us to assess evidence about the risk of sexual transmission of ZIKV. ZIKV is more likely transmitted from men to women than from women to men. For other flaviviruses evidence of sexual transmissibility is still absent. Taking into account all available data about the duration of detection of ZIKV in culture and from the serial interval, our findings suggest that the infectious period for sexual transmission of ZIKV is shorter than estimates from the earliest post-outbreak studies, which were based on RT-PCR alone.

5.2 Author summary

Why Was This Study Done?

- Sexual transmission of Zika virus (ZIKV) is now documented but the risks of transmission are not well understood.
- It is not known whether other flaviviruses can be transmitted through sexual intercourse.

What Did the Researchers Do and Find?

- We developed a sexual transmission framework for ZIKV infection that identified seven key elements related to ZIKV sexual transmission, and we conducted a living systematic review through April 15th 2018 of available evidence about each element.
- We found that, in known cases, sexual transmission of ZIKV is much more common from men to women than from women to men. For sexual transmission of ZIKV, the median serial interval—the time between onset of symptoms between two sexual partners—is 12 days.
- The median duration of ZIKV RNA persistence in semen is longer (34 days) than in the female genital tract (12 days). ZIKV can be detected for longer periods using reverse transcription polymerase chain reaction compared to viral culture.
- We found no evidence of sexual transmission for any other arthropod-borne flaviviruses.

What Do These Findings Mean?

- Studies about the duration of detection of ZIKV in bodily fluids and the serial interval suggest that the period ZIKV can transmit through sexual contact might be shorter than was anticipated from the earliest studies in 2016.

5.3 Introduction

Zika virus (ZIKV) can be transmitted between humans through sexual contact, although it is most commonly transmitted by infected Aedes spp. mosquitoes [566]. Sexual transmission of ZIKV has important implications for public health, for people living in endemic regions and for sexual partners of travellers returning to non-endemic regions from endemic regions, because ZIKV infection during pregnancy can cause congenital infection of the foetus and because ZIKV infection can trigger the immune-mediated neurological condition Guillain-Barré syndrome. ZIKV is an RNA flavivirus. Flaviviruses are a genus of viruses from the Flaviviridae family of which the majority are transmitted to vertebrates by infected mosquito or tick vectors [567].

Scientists working in Senegal in 2008 were the first to report presumed sexual transmission of ZIKV in a case report that documented their own symptoms and serological findings [44]. One scientist developed symptoms after returning to the USA and his wife, who had not travelled outside the USA, became unwell four days later. The large ZIKV outbreak (2015-2017) in the Americas resulted in additional reports of travel-associated ZIKV sexual transmission in the United States and Europe, which Moreira and colleagues synthesised in a narrative review of the literature up to December 2016 [568]. *In vivo* and *in vitro* experimental studies have provided evidence of the biological plausibility of this route of infection.

While possible sexual transmission has been established, there are many unanswered questions about the transmissibility of ZIKV through sexual intercourse. For mosquito-borne ZIKV infection, the incubation period and duration of viral shedding in serum have been estimated, allowing implications for blood donation to be assessed [55]. Additional information about parameters related to person-to-person transmission of ZIKV has not been systematically collated or quantified, although several narrative reviews have been published

[43, 569]. Evidence about sexual transmission of other arthropod-borne flaviviruses in humans, including West Nile virus (WNV), Yellow fever virus (YFV), Japanese encephalitis virus (JEV), and Dengue virus (DENV) [570], has not been synthesised but WNV and YFV has been detected in human semen [571, 572]. The primary objective of this review was to systematically review evidence about defined aspects of the sexual transmission of ZIKV. Secondary objectives were to systematically review evidence about the sexual transmissibility of other arthropod borne flaviviruses; and to establish these reviews using a living systematic review approach [136].

5.4 Methods

5.4.1 Sexual transmission framework

In March 2017, we developed a sexual transmission framework for ZIKV [47], based on standard concepts about person-to-person transmission of infection [573]. The framework includes key events in the course of an infection in an individual and transmission to a sexual partner, some of which can be measured and others that can only be determined indirectly or through modelling. Figure 5.1 shows these events and the relationships between the following elements: 1) susceptibility to infection, 2) incubation period after sexual transmission, 3) serial interval, 4) duration of infectiousness, 5) reproductive number, 6) probability of transmission per sex act, and 7) transmission rate. The framework does not include transmission from and to mosquitoes, which would be needed to estimate the proportion of all ZIKV infections due to sexual transmission. The sexual transmission framework defined the outcomes and informed the structure of the review.

5.4.2 Living systematic review

We performed this review as a living systematic review [136] because research into many aspects of ZIKV is a new and fast moving field. Several studies are ongoing [574] and have published interim results [49] and updated results could affect public health decisions. The protocol for this review was registered on May 19, 2017 in the database PROSPERO (CRD42017060338) [573]. We summarise the details that make the review a living systematic review in S2 Text. Future updates will be reported quarterly online (http://zika.ispm.unibe.ch/stf/) and in the online comments section of this publication. Reporting is in accordance to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (S1 PRISMA Checklist).

Search strategy

The search included the electronic databases: Pubmed, Embase, BioRxiv, Arxiv, PeerJ, Lilacs and online repositories from the United States Centers for Disease Control and Prevention, the European Centre for Disease Prevention and Control, the Pan American Health Organization, and the World Health Organization from the earliest date of each database and without language restrictions. The searches included medical subject headings (MeSH) and keywords for ZIKV and flaviviruses together with viral persistence and sexual transmission (S2 Text). An automated search is run every day, with results deduplicated and imported into REDCap (Research Electronic Data Capture, Vanderbilt University, OK). We checked reference lists of included studies to identify additional relevant studies. For this report, we identified studies published before and up to April 15, 2018.

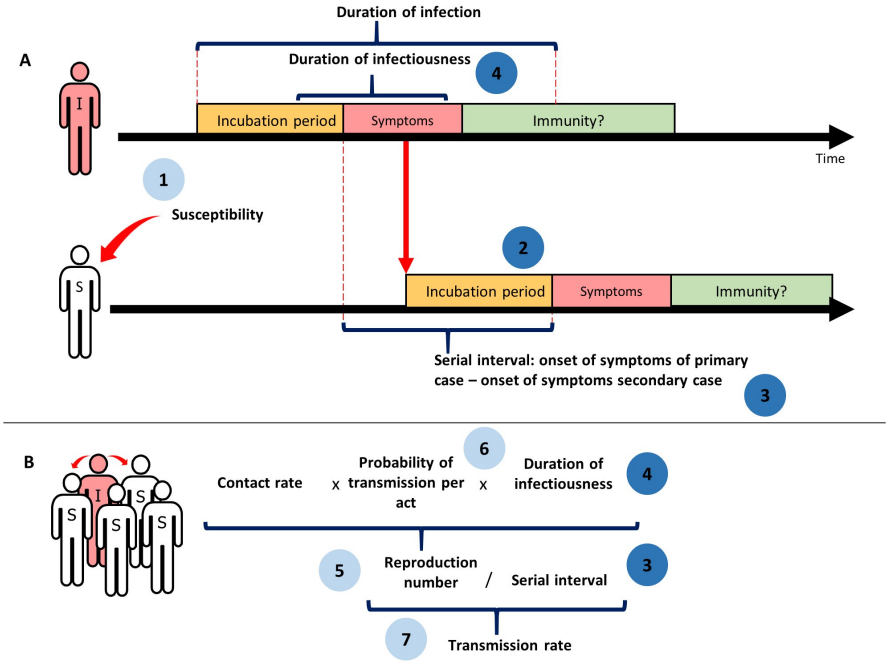


Figure 5.1: A schematic representation of the sexual transmission of ZIKV and its key parameters. Numbered circles show the seven key elements. Dark blue circles are elements for which evidence is based on empirical research. Light blue circles denote parameters derived from mathematical modelling studies and *in vivo* studies. Panel A shows the transmission between two individuals. The blue horizontal arrows show the time course of the disease for the primary infected individual who is infected and the secondary individual who starts as susceptible (element 1). The vertical solid red arrow represents a ZIKV transmission event, after which there is an incubation period (element 2) before symptoms develop. Element 3 is the serial interval, the period between the start of symptoms in the primary and the secondary individual. Element 4 is the duration of infectiousness. After the infection, individuals can become immune. Panel B shows the relation between different parameters at population level. The reproduction number (element 5) is the result of the contact rate, the probability of transmission per act (element 6) and the duration of infectiousness (4). The transmission rate (element 7) can be estimated using the reproduction number (5) and the serial interval (3).

Outcomes	Eligible study designs	Detailed eligibility criteria
Primary outcomes:		
2. Incubation period following sexual transmission	Observational epidemiological studies in humans (case report, case series, cohort studies, surveillance/outbreak reports).	Observational studies that report incubation period due to sexual transmission.
3. Serial interval		Observational studies that describe sexual transmission in humans where serial interval (time between onset of symptoms between sexual partners) is reported.
4. Duration of infectiousness		Observational studies that report duration of detection of virus in semen, cervical and vaginal secretions and saliva; diagnostic methods included reverse transcription polymerase chain reaction (RT-PCR) or viral culture.
Secondary outcomes:		
1. Susceptibility	*In vivo/in vitro* studies	*In vivo/in vitro* studies that report on the female genital tract, the male genital tract, presence of virus in saliva or sexual transmission of virus
5. Reproduction number due to sexual transmission 6. Probability of transmission per sex act 7. Transmission rate	Mathematical modelling studies	Modelling studies that report on the parameters of interest

Table 5.1: Eligibility criteria for each outcome.

5

Eligibility criteria

We included observational studies, *in vitro* and *in vivo* studies and mathematical modelling studies that directly addressed any of the elements of the sexual transmission framework in either humans and animals for ZIKV or other arthropod-borne flavivirus. We included observational studies that reported one or more cases of sexual transmission, one or more measurements of presence of virus in bodily fluids, or both. As bodily fluids we included semen, cervical and vaginal secretions and saliva; diagnostic methods included reverse transcription polymerase chain reaction (RT-PCR) or viral culture. We did not include reviews, editorials or commentaries that did not report original data. Table 5.1 provides an overview of the eligibility criteria for each outcome. Primary outcomes can be directly estimated from observational studies and secondary outcomes are calculated or inferred from indirect evidence.

Study selection and data extraction

One reviewer screened titles and abstracts of retrieved papers. If retained in the first step, we screened the full text of the paper. One reviewer extracted data into piloted extraction forms in REDCap [312]. A second reviewer verified exclusion decisions and data entry.

Synthesis of the evidence

We provide descriptive summaries of findings about the elements of the ZIKV sexual transmission framework for basic research studies (element 1), observational epidemiological studies (elements 2-4) and mathematical modelling studies (elements 5-7). In addition, we used data from included studies to calculate estimates for the serial interval, the period between the start of symptoms in the primary and the secondary individual, and the duration

of the detection of ZIKV. We report the median serial interval and its interquartile range. To estimate the duration of detection of ZIKV positivity, we conducted interval censored survival analysis and fitted Weibull distributions using the 'straweib' package [575, 576] in R (version 3.4.1), based on previous studies [49, 575, 576]. We assumed that all infected patients were RT-PCR or viral culture positive at symptom onset. We report median estimated durations and corresponding 95% confidence intervals. Additional information about the methods is provided in S3 Text. For other flaviviruses we summarise findings from all study types descriptively.

Certainty assessment of the evidence

We assessed the methodology of included studies using specific checklists for each study type. For observational studies we used National Institutes of Health (NIH) Quality Assessment Tool for Case Series Studies [577] and United Kingdom National Institute of Health and Clinical Excellence (NICE) checklists for case-control studies and cohort studies [578]. For *in vivo* studies we used the SYstematic Review Center for Laboratory animal Experimentation's (SYRCLE) risk of bias tool for animal studies [579] and for mathematical modelling studies the International Society for Pharmacoeconomics and Outcomes Research (ISPOR) Questionnaire to Assess Relevance and Credibility of Modeling Studies [580]. We performed the assessment by a consensus-driven approach among multiple reviewers. We appraised the certainty of the key parameters according to the Grading of Research Assessment Development and Evaluation (GRADE) tool [581–583] (S6 Table). In accordance with GRADE, assessments of the overall certainty of evidence from observational studies started at low certainty. We downgraded the level of certainty for small sample size and evidence from case reports or case series. We assessed outcomes of mathematical modelling studies as high, medium, low or very low certainty.

5.5 Results

We identified 1227 unique citations and excluded 901 by title and abstract screening (Figure 5.2). Of the remaining 326 potential eligible citations with relevant abstracts, 128 publications were eligible for inclusion. Table 5.2 summarises characteristics of the included studies.

5.5.1 Basic research studies

We included 41 *in vivo* and *in vitro* studies of ZIKV [266, 584–616] (Table 5.2) Of these 41 studies, six were *in vitro* studies and 35 were studies in *in vivo* animal models; 12 in non-human primates (NHP) such as Cynomolgus macaques (*Macaca fascicularis*), Rhesus macaques (*Macaca mulatta*) and Common marmosets (*Callithrix jacchus*) and 23 in mice. In one study, both guinea pigs and NHP were used [589]. These studies provide insight in the underlying biological mechanisms of susceptibility to ZIKV infection through sexual transmission and substantiate the biological plausibility of this transmission route.

Susceptibility (1)

In mouse and NHP models the vaginal and rectal mucosae were shown to be susceptible to infection with ZIKV [266, 592, 595, 598, 602, 614]. When ZIKV infected male mice were mated with uninfected female mice the female mice became infected [591, 592, 615]. Female to male transmission of ZIKV in mice was unsuccessful [592]. In Rhesus macaques, systemic infection

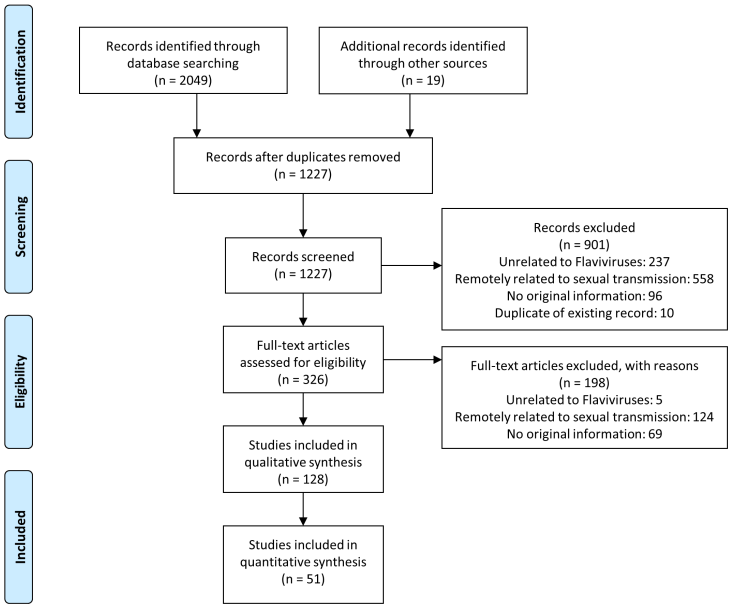


Figure 5.2: Flow diagram of reviewed studies. Numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage.

Category	Publications on ZIKV	Publications on other Flaviviruses
Epidemiological studies		
Case reports	44	7
Case series	18	1
Cohort studies	4	-
Outbreak or surveillance reports	1	-
Mathematical modelling studies	2	-
Basic research studies		
In vivo studies	35	7
In vitro studies	6	1
Review studies	-	2
Total publications:	110	18
Reporting on sexual transmission between two partners	24[a]	1
Publications used for quantitative analysis	51	-
Reporting serial interval	12[a]	-
Reporting at least one measurement in bodily fluids of interest using RT-PCR or viral culture	48[a]	-

Table 5.2: Overview of study designs of included studies. [a]Overlap in publications; one publication can report on multiple outcomes (e.g. reporting on serial interval and/or persistence and/or sexual transmission).

through oropharyngeal mucosal inoculation with ZIKV was only successful after inoculation with a very high dose of virus, suggesting a very low risk of oral mucosal transmission [604]. Four Rhesus macaques became viraemic after intranasal or intragastric inoculation with ZIKV [589]. In guinea pigs, direct transmission between animals infected subcutaneously with ZIKV and co-housed uninfected animals was seen [589].

In human prostate cells, testicular cells and mature spermatozoa are susceptible *in vitro* to ZIKV infection [585, 610, 612]. Human Sertoli cells can support high levels of ZIKV replication and persistence [617]. In multiple mouse models, using different strains of ZIKV, the testes seem to be a preferred site for viral replication, able to sustain high viral loads for a longer duration than other organs [592, 594, 597, 600, 602, 603, 607, 614]. In some of these models, ZIKV caused inflammation of the testes [592, 593, 601, 614], reduced testicular size and decreased levels of testosterone [609, 611, 614, 618]. The testes of experimentally infected NHP harboured high levels of ZIKV [599, 605]. High titres of ZIKV RNA were detectable in semen until day 28 in Rhesus and Cynomolgus macaques [605]. However, one group of Rhesus macaques had only low levels of viral RNA in the testes and no detectable virus in the prostate or epididymis. In common marmosets, ZIKV RNA in semen was sporadically detected [587].

The female genital tract of macaques was able to sustain ZIKV replication for shorter durations and with lower viral loads than the male genital tract [584, 590, 605]. Although cervical and endometrial cells were susceptible *in vitro* , virus was not detected in the female genital tract in mice or NHP for longer than 7 days after infection [584, 590]; in one study, the ovary sustained higher titres up to 14 days post infection. Intravaginal infection of mice led to systemic infection [266, 592, 596, 598, 608, 611, 613, 619] and to adverse congenital outcomes [592]. In pregnant female mice, sexual transmission led to more ZIKV dissemination to the female reproductive tract, compared to subcutaneous or intravaginal inoculation [619]. In NHP the incubation time following infection was longer for intravaginal infection compared with subcutaneous infection [586]. In mice, viral titres were lower in the salivary glands than the testes and ovaries. In NHP, viral RNA was detected in saliva up to 28-42 days [586, 588, 590, 599, 605] and ZIKV could be cultured at day 7 and day 14 [587, 605].

Risk of bias in *in vivo* studies

Most studies did not describe in detail the methods used to avoid bias. Detailed certainty assessment of the *in vivo* studies is provided in S6 Table.

5.5.2 Observational studies

ZIKV transmission between sexual partners

As of April 15, 2018, the US Centers for Disease Control and Prevention (CDC) reported that, of 5,672 cases of ZIKV infection, 52 were acquired through sexual transmission in the United States [45]. The European Centre for Disease Prevention and Control (ECDC) reported, as of 13 March 2017, 20 out of 1,737 cases of sexual transmission amongst those for which the route of transmission was known [46]. We included 67 reports about ZIKV sexual transmission, measurement of ZIKV infection status using RT-PCR or viral culture in samples of semen, vaginal fluid or saliva, or both [44, 49, 77, 514, 619–676]. Twenty-four of these studies reported on 36 couples in which a primary partner with ZIKV infection, who returned from a ZIKV endemic area, is suspected to have transmitted ZIKV to a secondary partner

[44, 514, 620, 621, 628, 629, 631, 632, 634–636, 638, 639, 643–645, 647, 667, 671, 672, 674] (Table 5.3). Thirty-four of 36 episodes of transmission were from man to woman (94%), one (3%) was from woman to man [632] and one (3%) was from man to man [634]. Penile-vaginal intercourse was reported as the most likely mode of transmission between men and women, but oral and anal intercourse were mentioned as possible transmission routes in some reports [44, 621, 629, 631, 632, 634, 638, 639, 647, 671, 672]. One study reported transmission of ZIKV from a vasectomised man to his female sexual partner [621]. Amongst primary infected individuals, 27/36 (75%) were symptomatic, 2/36 (6%) were asymptomatic and no symptom status was not reported for the remaining 7/36 (19%). ZIKV was detected by RT-PCR in blood, urine, saliva or semen in 14/36 (39%) of the primary partners and in 18/36 (50%) secondary partners. No diagnostic method was stated for 29/72 (40%) individuals. In 5/36 (14%) couples, the secondary partner had a history of travel to an endemic region [621, 635, 636, 639, 671].

Incubation period (2) and serial interval (3) We were not able to extract information on the incubation period following sexual exposure to ZIKV, since dates of exposure of the primary partner and dates of sexual intercourse with the secondary partner were rarely reported. Thirteen reports about 15 couples reported on dates of symptom onset for both partners. The median serial interval was 12 days (interquartile range: 10-14.5 days) [44, 621, 631, 632, 634–636, 638, 643, 645, 647, 671, 672] and the maximum was 44 days [671].

Duration of infectiousness (4) Duration of infectiousness was not measured directly in any included study. Observational studies measured the duration of detection of ZIKV in bodily fluids in case reports, case series and prospective cohort studies. S3 Text provides additional information. Case reports and case series We included 48 publications describing 180 individuals who underwent diagnostic testing by RT-PCR or viral culture on semen, vaginal fluid or saliva at one or more time points [77, 621–627, 629–631, 633–643, 645, 646, 648–656, 659–671, 673, 675]. In semen (data available from 37 case reports and case series from 119 individuals, Figure 5.3, S3 Text)[77, 621–623, 625, 626, 629–631, 633–636, 638–643, 645, 646, 648, 649, 651, 653–656, 661–663, 666–668, 670, 671, 675], the median duration of RT-PCR positivity was 39.6 days (95% CI: 29.9-49.0 days) and the maximum was 370 days [625]. The median duration based on viral culture was 9.5 days (95% CI: 1.2-20.3 days) (data from 22 men in 11 reports) and a maximum of 69 days [621]. The median duration of ZIKV positivity in any fluid from the female genital tract was 13.9 days (95% CI: 7.2-19.6 days) based on RT-PCR (data from 15 women in 7 reports) and a maximum of 37 days [668]. The median duration of ZIKV positivity of saliva was 7.3 days (95% CI: 4.2-10.8 days) based on RT-PCR (data from 76 individuals in 23 reports) and a maximum of 91 days [661]. There were too few data for analysis of viral culture specimens in female genital tract fluids and saliva. Prospective cohort studies: one cohort study enrolled 150 women and men with symptomatic ZIKV infection in Puerto Rico. ZIKV was detected by RT-PCR in 31/55 men, with a median duration of persistence of 34 days (95% CI: 28-41 days). ZIKV RNA was only detected in a few participants in saliva or vaginal fluids [49]. A second cohort study, amongst people returning from ZIKV endemic areas or infected in the United States, detected ZIKA RNA in semen of 60/184 symptomatic men [619]. The mean time to ZIKV RNA clearance was 54 days (95% CI: 53-55 days). The median duration was not reported, but plotted at approximately 35 days. Only three out of 19 of the semen samples provided within 30 days after symptom onset could be cultured; none of the 59 samples provided after 30 days could be cultured.

Characteristics	n (%)	References
Direction of transmission		
Male-female	34 (94%)	[44, 514, 620, 621, 628, 629, 631, 635, 636, 638, 639, 643–645, 647, 667, 671, 672, 674]
Female-male	1 (3%)	[632]
Male-male	1 (3%)	[634]
Symptomatic status		
Symptomatic	27 (75%)	[44, 620, 621, 628, 631, 632, 634–636, 638, 643, 645, 647, 667, 671, 672]
Asymptomatic	2 (6%)	[629, 639]
Not reported	7 (19%)	[514, 644, 674]
Secondary infected individual has travelled to endemic area		
Yes	5 (14%)	[621, 635, 636, 639, 671]
No	31 (86%)	[44, 514, 620, 628, 629, 631, 632, 634, 638, 643–645, 647, 667, 672, 674]
Serial interval reported		
Yes	15 (42%)	[44, 621, 631, 632, 634–636, 638, 643, 645, 647, 671, 672]
No	21 (58%)	[514, 620, 628, 629, 639, 644, 667, 674]
Diagnostic certainty primary infected individual		
Confirmed with RT-PCR	14 (39%)	[621, 628, 631, 632, 634–636, 638, 639, 643, 645, 671]
Confirmed with serology	4 (11%)	[44, 647, 672]
Suspected	3 (8%)	[629, 647]
Not reported	15 (42%)	[514, 620, 644, 667, 674]
Diagnostic certainty secondary infected individual		
Confirmed with RT-PCR	18 (50%)	[621, 628, 629, 631, 632, 634–636, 638, 639, 643, 645, 647, 671]
Confirmed with serology	4 (11%)	[44, 647, 672]
Suspected	0 (0%)	–
Not reported	14 (39%)	[514, 620, 644, 667, 674]

Table 5.3: Key characteristics of the couples (n=36) for which sexual transmission of ZIKV was suspected.

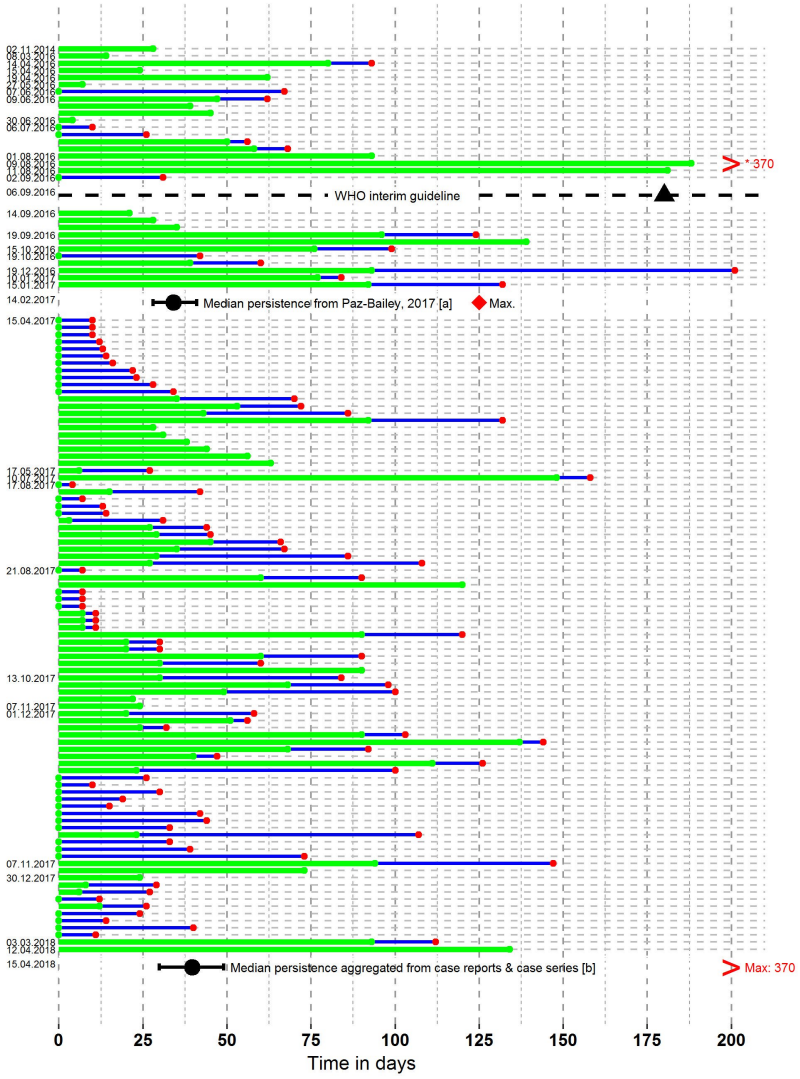


Figure 5.3: ZIKV detection in semen by RT-PCR. The X-axis indicates time in days from symptom onset. The labels on the Y-axis represent the date of publication of the studies and the date of analysis for the last line, in ascending order. Green lines represent the duration of RT-PCR positivity in individuals from case reports and case series (n=119), extending to the last positive RT-PCR measurement. Green dots at day 0 represent an assumption of RT-PCR positivity for patients with no sample taken at symptom onset. Blue lines represent the interval between the last positive measurement and the first subsequent negative measure (red dot). The black dotted line represents the publication of the WHO interim guidelines and the advised suggested duration of protected sexual intercourse (6 months, black triangle). The black dots and whisker bars represent median aggregated values and 95% confidence intervals for [a] a prospective cohort (n=55 men) [49], and [b] the aggregation of all available case reports and case series. Maximum values in these data sets are shown with a red diamond or a red greater than symbol for values outside the range of the image. Lines for which the date is not provided are from the same date as the line above. ZIKV, Zika virus; RT-PCR, reverse transcriptase PCR.

Risk of bias in observational studies

Studies varied widely in risk of bias and completeness of reporting (S6 Table). Many studies reporting on transmission events did not use reliable diagnostic methods in both partners, potentially leading to misclassification bias. The median duration of ZIKV persistence was higher in case reports and case series than in the prospective cohort study

Mathematical modelling studies

Reproduction number (5), transmission probability (6), transmission rate (7) We included two mathematical modelling studies, both of which used a deterministic structure [168, 169]. Gao et al. used surveillance data from Brazil, Colombia and El Salvador [168], Towers et al. used data from Colombia [169]. Both studies derived the reproduction number for ZIKV sexual transmission: 0.136 (95% CI: 0.009-0.521) [168] and 'likely below one' [169]. The two studies calculated the proportion of ZIKV infections resulting from sexual transmission as 3.04% (95% CI: 0.12-45.73%) [168] and 23% (95% CI: 1-47%) [169]. Neither study provides new information about the transmission probability per sex act or the transmission rate for sexual transmission of ZIKV.

Risk of bias of mathematical modelling studies

For both modelling studies, the data used to populate the model was not suitable to derive the outcome. Surveillance data on which these studies base their results, did not distinguish between vector transmitted ZIKV and sexually transmitted ZIKV. The results of these studies did not provide information about the size of the risk of sexual transmission. External validation for both models is lacking. Detailed certainty assessment is shown in S6 Table.

5.5.3 Sexual transmission framework parameters

Table 5.4 summarises findings for the outcomes of the sexual transmission framework and the GRADE assessment of the certainty of the evidence. S6 Table provides the GRADE evidence profile.

5.5.4 Other flaviviruses

We included 18 studies reporting on the sexual transmission potential of other arthropod-borne flaviviruses [52, 571, 677–691]. Ten of 18 studies (56%) were *in vitro* experiments or observations in animals and eight studies (44%) were case reports or case series. JEV was demonstrated to be transmissible from male to female pigs via semen [52, 677, 682, 683]. Persistence of virus was demonstrated for at least 17 days in boars [683]. JEV can be cultured from the seminal fluids of pigs [686]. In humans, we found one case report of male to female sexual transmission of WNV, although the secondary partner also lived in a mosquito-endemic area [685]. WNV was found post-mortem in the prostate and testis of a 43-year-old man on immunosuppressive therapy following a kidney transplant [678]. Intravaginal inoculation of WNV in mice led to local acute inflammation followed by systemic illness in a proportion of the animals [679]. The testes of six Japanese macaques (*Macaca fuscata*) showed low dengue virus (DENV) neutralizing antibodies titres [680]. Experimentally DENV infected pigtail macaques (Macaca nemestrina) showed dissemination of virus in the prostate gland and seminal vesicles [688]. DENV RNA could be detected in experimentally infected mice three days after infection [689]. Four case reports describe the presence of DENV in saliva

Parameter	Value	Sample size	References	GRADE
1. Susceptibility	Summary: based on animal models, rectal and vaginal mucosae are susceptible to infection. The testes form a reservoir for virus. Male-female transmission is more common than female-male transmission.	-	[266, 592, 594, 595, 597, 598, 600, 602, 603, 607, 614]	NA
2. Incubation period following sexual transmission	Could not be calculated	-		NA
3. Serial interval	Median: 12 days (interquartile range: 10-14.5 days)	15 couples	[44, 621, 631, 632, 634–636, 638, 643, 645, 647, 671, 672]	very low[1]
4. Duration of infectiousness				
Male genital tract RT-PCR (cohorts):	median: 34 days (95% CI: 28-41 days)	n=55	[49]	low[2]
	median: 35 days, mean: 54 days (95% CI: 53-55 days)	n=184	[619]	
Male genital tract RT-PCR (case reports and case series):	median: 39.6 days (95% CI: 29.9-49.0 days)	n=119		very low[2,3]
Male genital tract viral culture:	median: 9.5 days (95% CI: 1.2-20.3 days)	n=22	[77, 621, 622, 631, 640, 641, 651, 654, 661–663]	very low[1,2,3]
Female genital tract RT-PCR:	median: 13.9 days (95% CI: 7.2-19.6 days)	n=15	[659, 660, 664, 665, 668, 673]	very low[1,2,3]
Saliva RT-PCR:	median: 6.8days (95% CI: 4.3-9.6 days)	n=76	[623–625, 627, 631, 634, 637, 638, 640, 643, 649–652, 659–661, 663, 666, 668, 669, 675]	very low[1,2,3]
5. Reproduction number due to sexual transmission	<1	-	[168, 169]	very low[4]
6. Probability of transmission per sex act	Could not be calculated	-		NA
7. Transmission rate	(assumed)	-		NA
Proportion of cases due to sexual transmission	3.0% (95% CI: 0.1-45.7%); 23% (95% CI: 1-47%)	-	[168, 169]	very low[4]

Table 5.4: Summary of the evidence on sexual transmission of Zika virus as assessed using the sexual transmission framework. Estimates of the outcomes and publications that provide evidence for these different parameters of the sexual transmission framework are listed per outcome. Additionally, the certainty assessment using GRADE methodology is provided. NA, not applicable; RT-PCR, reverse transcriptase polymerase chain reaction. [1]Small sample size or small number of studies. [2]Indirect measure of duration of infectiousness [3]Risk of selection bias or selective reporting. [4]Serious indirectness and imprecision.

diagnosed by either RT-PCR or viral culture, for up to 7 days [687, 690, 691]. DENV RNA was demonstrated in the vaginal secretion of one patient up to 18 days after onset of symptoms [684]. Female mice that were mated to Tick-borne encephalitis virus infected male mice had worse reproductive outcomes than the ones mated to a group of non-infected males; in one female mouse the virus was detected [681]. YFV was demonstrated in the urine and semen of a patient using RT-PCR 21 days after onset of symptoms [571].

5.6 Discussion

This systematic review summarises published data related to sexual transmission of ZIKV and other arthropod-borne flaviviruses published on or before 15 April 2018. In animals, vaginal and rectal mucosa are susceptible to ZIKV, with the testis as a preferred site of replication. Male to female transmission was more frequent than female to male transmission in animal models and in humans. In humans, we estimated the serial interval for sexually transmitted infection to be 12 (interquartile range: 10-14.5) days. ZIKV was detectable in semen for a median of 34 (95% CI: 28-41) days by RT-PCR and 9.5 (95% CI: 1.2-20.3) days by viral culture. In mathematical modelling studies, the reproduction number for sexual transmission of ZIKV was below one. The overall certainty of the evidence was low. We found no evidence that other arthropod-borne flaviviruses can be sexually transmitted.

5.6.1 What the study adds to existing research

The ZIKV sexual transmission framework allowed us to synthesise evidence from both animal and human studies in a structured way, taking into account the risks of bias in the included studies. Susceptibility of tissues to ZIKV could only be assessed in animal models. There were consistent findings in animal models that help to explain the overrepresentation of reported cases of human male to female transmission, even though mice are not a natural host for ZIKV and *in vivo* studies often use immunocompromised animals. First, vaginal mucosa are more susceptible than urethral mucosa to infection [266, 592, 595, 598, 602, 614]. Second, high levels of ZIKV replication in the testes in mice and sustained detection of viral RNA and of virus in tissue culture in mice and NHP models is consistent with the longer duration of detection in men than women. Rectal mucosa is also susceptible to ZIKV so, although only observed once [634], unprotected anal intercourse is also a likely route of ZIKV transmission. The risk of bias of the included *in vivo* studies as assessed with the SYRCLE tool, was high. Most of these studies explored the suitability of animal models or investigated pathophysiological pathways and source of bias were rarely reported.

Our analysis shows that, when assessed from case reports and case series, the duration of detection of ZIKV in semen by RT-PCR is overestimated; all reports are of people with ZIKV detected and a small number of outliers influence the estimate. A prospective cohort study that enrolled people with symptomatic ZIKV infection consecutively estimated a shorter duration of persistence, but also showed that only half of the men and only one of 50 women had ZIKV detected in genital fluids [49]. Case reports and case series are early sources of information about a new disease but, by their nature, researchers report novel and unusual findings. Parameters and effect sizes estimated from aggregating data from these sources are likely to be overestimates, the so-called 'random high'; extreme values in a distribution that are observed by chance and are more likely to be reported because of they are noteworthy. As evidence accumulates in well-designed studies, the estimates decrease in size. Notably, the prospective cohort study in Puerto Rico found ZIKV in semen in only half of men with

symptomatic infection and only vaginal fluid in only one of 50 women. Similarly, ZIKV was found in semen in only 60/183 (33%) ZIKV infected men in the United States [619]. Persistence of viral RNA in body fluids is often used as a proxy for the duration of ZIKV infectiousness, although it remains unclear whether the presence of viral RNA corresponds with infectious virus. ZIKV RNA positivity persists for longer than detection of ZIKV in viral culture in both mice [592] and human semen samples. However, viral cultures might underestimate the duration of infectiousness if low pH or other specimen-dependent factors produce false negative results [627, 692]. The estimated serial interval was based on observations from only 15 couples, but was consistent with that of several respiratory infectious diseases [693]. The serial interval for sexual transmission was towards the lower end of estimates for mosquito borne transmission (10-23 days) [694].

Some elements of the infection process, such as the incubation period, transmissibility of ZIKV per sex act, and transmission rate could not be observed. In mathematical models published so far [168, 169, 695], the estimates were based on assumptions about transmissibility of mosquito-borne infection. Estimates from our review might provide more reliable data for use in future modelling studies. The potential for sustained sexual transmission of ZIKV appears low, based on the reproduction number estimated in mathematical modelling studies. The estimated reproduction number was higher for mosquito-borne transmission, 1.96 (95% CI: 0.45–6.23), than for sexual transmission [168], although this number is highly dependent on the geographical location [161]. This review did not find evidence supporting sexual transmission of other arthropod borne flaviviruses. The continual updating of the literature search identified a finding of YFV in urine and semen [571]. However, it remains to be clarified for many viruses if detection in semen means that there is a risk of sexual transmission [572].

5.6.2 Strengths and weaknesses of the study

The strengths of our living systematic review are the high coverage of the body of published literature, the structured overview and the re-analysis of individual patient data on persistence of ZIKV. The automation of search and deduplication processes makes it feasible to keep the review updated as new information becomes available. Updated analyses of the data from case reports show regression to the mean of the median estimate of the duration of RNA detection in semen (https://zika.ispm.unibe.ch/stf/). Future updates of this review will also allow for incorporation of techniques to synthesise mathematical modelling studies, such as multi-model ensembles. This study also has limitations. Screening and data extraction were not done by two independent reviewers because of time constraints but we believe that we reduced errors by having a second reviewer to check decisions and data extracted. The statistical methods used to estimate the duration of persistence of ZIKV in bodily fluids assume that all samples are positive for ZIKV at time zero [49], which might not be the case. Additionally, the sexual transmission framework might not include all factors that are required to investigate the risks of sexual transmission of ZIKV. The certainty of this body of evidence was assessed as being of low or very low because of bias in the observational study designs, and indirectness of evidence from animal studies. The certainty of the evidence base could increase if the design and reporting of both animal and human studies improve and if their findings are consistent with, and increase the precision of the evidence presented here.

5.6.3 Implications and next steps for researchers, clinicians and policymakers

The risk of sexual transmission of ZIKV is particularly relevant for women who are pregnant or planning a pregnancy, and people with high levels of sexual partner change such as some groups of men who have sex with men and women at high risk. An expert group has used the ZIKV sexual transmission framework to stimulate discussion about research priorities [47]. One important limitation to the generalisability of findings from our review is that the data that we analysed about sexual transmission of ZIKV in humans relied largely on information from travellers returning from endemic areas with symptomatic ZIKV infection and their sexual partners. This group probably differs from people in endemic regions in ways that could affect sexual transmission of ZIKV, such as previous exposure to other flaviviruses [696]. Additional studies in ZIKV endemic settings could enrol travellers who work in areas with mosquito-borne ZIKV transmission and who return to families living in areas, e.g. at high altitude, where the vector does not survive [47]. There are unanswered questions about the potential for sexual transmission of asymptomatic ZIKV infection, even though ZIKV is often asymptomatic [24, 697, 698], about clinical differences between ZIKV infections acquired through sexual and mosquito-borne routes, and about the long term consequences of ZIKV in the genital tract, such as its effects on the testis and on male infertility. Research about the potential for sexual transmission of other flaviviruses is needed, although these viruses often display different symptomatology or affinity for different species.

Clinicians and policymakers need information that helps to advise both opposite sex and same sex couples how to reduce the risk of sexual transmission of ZIKV. The relationship between detectable RNA in semen and infectiousness therefore needs to be further investigated in both laboratory and epidemiological studies. Current guidelines for travellers returning from endemic areas advise six months of protected intercourse [566]. As more information becomes available a revision of the duration of protection might be indicated.

5.6.4 Conclusions

This living systematic review gives an up to date synthesis of information about the sexual transmission of ZIKV with a structured framework. Planned regular updates will allow timely updating of relevant data from a rapidly expanding evidence base. We did not quantify the absolute risk of sexual transmission of ZIKV but appears small based on information about the proportion of people with symptomatic ZIKV who have ZIKV detected in genital secretions and the short median duration of detection of ZIKV in semen and vaginal fluid. Taking into account all available data about the duration of detection of ZIKV in culture and from the serial interval, our findings suggest that the infectious period for sexual transmission of ZIKV is shorter than estimates from the earliest post-outbreak studies, which were based on RT-PCR alone.

5.7 Supplementary material

Supplementary material is available online in the published version of the manuscript http://doi.org/10.1371/journal.pmed.1002611.

Chapter 6

Impact of age-specific immunity on the timing and burden of the next Zika virus outbreak

Michel Jacques Counotte[1]
Christian L. Althaus[1]
Nicola Low[1]
Julien Riou[1]

[1] Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland

This article is published in ***PLOS Neglected Tropical Diseases***: Impact of age-specific immunity on the timing and burden of the next Zika virus outbreak (2019) DOI: 10.1371/journal.pntd.0007978.

Contribution: I came up with the study idea, I contributed to the methodology, I wrote the model code, performed the analysis and made the figures. I wrote the first draft of the manuscript and incorporated all feedback until publication.

6.1 Abstract

The 2015–2017 epidemics of Zika virus (ZIKV) in the Americas caused widespread infection, followed by protective immunity. The timing and burden of the next Zika virus outbreak remains unclear. We used an agent-based model to simulate the dynamics of age-specific immunity to ZIKV, and predict the future age-specific risk using data from Managua, Nicaragua. We also investigated the potential impact of a ZIKV vaccine. Assuming lifelong immunity, the risk of a ZIKV outbreak will remain low until 2035 and rise above 50% in 2047. The imbalance in age-specific immunity implies that people in the 15–29 age range will be at highest risk of infection during the next ZIKV outbreak, increasing the expected number of congenital abnormalities. ZIKV vaccine development and licensure are urgent to attain the maximum benefit in reducing the population-level risk of infection and the risk of adverse congenital outcomes. This urgency increases if immunity is not lifelong.

6.2 Author summary

Zika virus (ZIKV) caused a major outbreak in the Americas between 2015–2017. It remains unclear if immunity after infection offers life-long protection at an individual level and how long herd immunity can protect a population against a new ZIKV outbreak. Data from Managua, Nicaragua showed an imbalance in protective immunity after ZIKV infection across different age-strata. We used this data to parameterize an individual based mathematical model to predict the future risk of a new ZIKV outbreak and to evaluate the effect of loss of immunity and the introduction of vaccination. We found that the 15–29 age range will be at highest risk of infection during the next ZIKV outbreak, increasing the expected number of congenital abnormalities. We show that vaccination could curb the risk of infection and could extend to herd immunity, but introduction within the next decade is crucial to provide the most benefit.

6.3 Introduction

Zika virus (ZIKV) is a flavivirus, which is transmitted primarily by mosquitoes of the genus *Aedes*. Before 2007, circulation of the virus only occurred sporadically in African and Asian countries [699, 700]. Between 2007 and 2013, ZIKV caused large-scale epidemics in the populations of Micronesia [24], French Polynesia [701] and other Pacific islands [699]. ZIKV probably became established in *Aedes aegypti* mosquitoes in the Americas between 2013-2014, [167, 702] and then spread rapidly across the continent. In 2015, doctors in Brazil started reporting clusters of infants born with microcephaly, a severe congenital abnormality, and of adults with Guillain-Barré syndrome, a paralyzing neurological condition, resulting in the declaration by the World Health Organization (WHO) of a Public Health Emergency of International Concern (PHEIC) [94]. WHO stated, in September 2016, that ZIKV in pregnancy was the most likely cause of the clusters of microcephaly, and other adverse congenital outcomes [29, 177]. The risk of an affected pregnancy appears highest during the first trimester, with estimates between 1.0 and 4.5% [186, 703]. By the beginning of 2018, over 220,000 confirmed cases of ZIKV infection had been reported from Latin America and the Caribbean [1], which is estimated to be only 1.02% ($\pm$ 0.93%) of the total number of cases, based on mathematical modelling studies [167].

Protective immunity conferred by infection, combined with high attack rates and herd immunity, can explain the ending of epidemics and the lack of early recurrence [156], as has

been seen with ZIKV [173]. The duration of protective immunity induced by ZIKV infection remains uncertain, since immunity to ZIKV infection was not studied extensively before the 2013 outbreaks. Evidence from seroprevalence studies in French Polynesia and Fiji found that levels of ZIKV neutralizing antibodies decrease with time [68]. If the fall in antibody levels means that people become susceptible to infection again, population level ZIKV immunity might be declining already. Even if protective immunity is lifelong, the risk of a new ZIKV outbreak will rise as susceptible newborns replace older individuals, lowering the overall proportion of the population that is immune. A modelling study, based on data from the 2013 epidemic in French Polynesia, estimated that ZIKV outbreaks are unlikely to occur for 12 to 20 years, assuming lifelong immunity [164].

A direct consequence of population renewal will be an unequal distribution of immunity by age group, with younger age groups at higher risk from a new epidemic than older people [173]. That effect will be amplified if ZIKV attack rates are lower in children than adults. Assessing the risk of ZIKV infection in women of reproductive age is essential because ZIKV infection in pregnancy, leading to adverse congenital outcomes, has such important implications for individuals, for public health and for investment in surveillance and mitigation strategies, including vector control, early warning systems, and vaccines [704, 705]. However, no vaccine is currently available against ZIKV. Phase I clinical trials of ZIKV candidate vaccines have shown levels of neutralizing antibody titers that were considered protective against reinfection [86, 87]. Some vaccines have already entered phase II trials [88], but some companies have stopped vaccine development [706].

Researchers in Managua, Nicaragua were the first to report the age-stratified seroprevalence of ZIKV antibodies in population-based surveys [182]. The first cases of autochthonous ZIKV infection in Nicaragua were reported in January, 2016, and an epidemic was observed between July and December of that year. Through case-based surveillance, the public health authorities of Nicaragua reported a total of 2,795 people with ZIKV detected by reverse transcriptase (RT) PCR over this period [1]. The number of symptomatic infections is likely much higher, owing to under-reporting. Furthermore, ZIKV infection is asymptomatic in 33 to 87% of cases [23], which are generally not identified by surveillance systems. Shortly after the end of the 2016 epidemic, Zambrana et al. analyzed sera from two large population-based surveys in Managua to measure the prevalence of IgG antibodies against ZIKV in 2- to 14-year olds (N=3,740) and 15- to 80-year olds (N=2,147) [182]. The authors reported ZIKV seroprevalence of 36.1% (95% confidence interval, CI: 34.5; 37.8%) among the 2-14 year age group and 56.4% (95% CI: 53.1; 59.6%) among the 15-80 year age group [182, 707]. The observed post-outbreak seroprevalence in adults is in line with findings from seroprevalence studies from French Polynesia, Brazil, and Bolivia [171, 697, 708].

In this study, we used published data from the 2016 ZIKV epidemic in Managua and developed an agent-based model (ABM) to predict the evolution of age-specific protective immunity to ZIKV infection in the population of Managua, Nicaragua during the period 2017–2097. We assessed: 1) the risk of a future ZIKV outbreak; 2) the consequences of a future ZIKV outbreak on women of reproductive age; 3) the influence of loss of immunity on future attack rates; and 4) how vaccination could prevent future ZIKV outbreaks.

6.4 Materials and methods

6.4.1 Modelling strategy

We assessed the consequences of future outbreaks of ZIKV infection in Managua, Nicaragua using a stochastic ABM. The model follows a basic susceptible-infected-recovered (SIR) framework and integrates processes related to ZIKV transmission, immunity, demography, adverse congenital outcomes and vaccination (Table 6.1). We parameterized the model based on published estimates or inferences from data about the 2016 ZIKV epidemic (Table 6.1, Supporting information S1). We considered different scenarios about the duration of immunity, the timing and scale of ZIKV reintroductions in the population, and the timing and scale of a hypothetical vaccination program targeted towards 15 year old girls.

Table 6.1: Parameterization of the agent-based model.

Parameter	Comment	Source
ZIKV epidemic parameters		
Transmission rate[a]	Inferred from the 2016 epidemic[b]	[182]
Recovery rate	Inferred from the 2016 epidemic[b]	[182]
ZIKV immunity		
Initial immunity[a]	Inferred from the 2016 epidemic[b]	[182]
Duration of immunity	Lifelong or decaying with time	5 scenarios[c]
Demography		
Initial age distribution	–	[709]
Birth rate	–	[709]
Death rate[a]	–	[710]
Ageing	Linear ageing at each time-step	–
ZIKV reintroduction		
Delay until reintroduction	1 to 80 years	80 scenarios[c]
Cases reintroduced	1, 5 or 10 cases	3 scenarios[c]
Risk of adverse congenital event		
Exposure	Proportion of women in the first semester of pregnancy	[709]
Risk of microcephaly	Upon infection during exposure time (3 levels of risk)	[186, 703]
Targeted vaccination		
Date of implementation	In 2021, 2025 or 2031	3 scenarios[c]
Effective coverage	Proportion of 15 year old girls vaccinated (0% to 80%)	5 scenarios[c]

[a] age-dependent parameters; [b] inferred from the 2016 epidemic by fitting a compartmental SIR model to these data, see Supporting information S1; [c] the different scenarios are discussed in the text in detail under the headings corresponding to the headings of this table.

6.4.2 Model structure

We simulated a population of 10,000 individuals for 80 years (2017–2097). We assigned agents' age and ZIKV infection status (susceptible S, infected I or immune R). Initial

conditions reflected the situation in Managua, Nicaragua in 2017, when there was no documentation of active transmission. In the outbreak-free period, we only considered demographic and immunity processes: births, deaths, ageing and, if applicable, loss of immunity and vaccination. Given the scarcity of these events at the individual level, we selected a long time-step of seven days and stochastically applied the transition probabilities at each time step for each agent. After a given time, ZIKV-infected cases were reintroduced in the population. Upon reintroduction, the time step was reduced to 0.1 days, and we evaluated the epidemic-related transition probabilities: Susceptible agents may become infected at a rate $\beta_a I/N$, where β_a is the age-dependent transmission rate and N the total population size. Infected individuals may recover with a rate γ. We ignored the influence of the vector population and assumed that the force of infection is directly proportional to the overall proportion of infected individuals. We allowed six months for the outbreak to finish after introduction. Simulations were conducted independently for each combination of scenarios and repeated 1,000 times. In the baseline scenario, we assumed no vaccination, no loss of immunity and a reintroduction of 10 infected individuals.

We implemented the model in 'Stan' version 2.18 [711] and we conducted analyses with R version 3.5.1 [314]. The Bayesian inference framework Stan permits the use of probability distributions over parameters instead of single values, allowing for the direct propagation of uncertainty. Stan models are compiled in C++, which improves the efficiency of simulations. The algorithm in Supporting information S1 describes the ABM in pseudo code. The model code and data are available from http://github.com/ZikaProject/SeroProject.

6.4.3 Parameterization

ZIKV epidemic parameters

We inferred the probability distributions for the age-specific transmission rate β_a and the recovery rate γ from data on the 2016 ZIKV epidemic in Managua, Nicaragua. We used surveillance data [182], which give weekly numbers of incident ZIKV infections, confirmed by RT-PCR (dataset A, n=1,165), and survey data on age-stratified ZIKV seroprevalence, measured among participants of pediatric and household cohort studies in Managua during weeks 5–32 of 2017 (dataset B, n=3,740 children and 1,074 adults) [182].

We conducted statistical inference using a deterministic, ordinary differential equation (ODE)-based version of the ABM with three compartments (S, I and R) and two age classes ($a \in \{1, 2\}$ corresponding to ages 0–14 and $\geq$15):

$$\frac{dS_a}{dt} = -\beta_a S_a \frac{\sum I_a}{N} \tag{6.1}$$

$$\frac{dI_a}{dt} = \beta_a S_a \frac{\sum I_a}{N} - \gamma I_a \tag{6.2}$$

$$\frac{dR_a}{dt} = \gamma I_a \tag{6.3}$$

We ignored demography in this model because it covers a short time span. We recorded the overall cumulative incidence of ZIKV cases using a dummy compartment:

$$\frac{dC}{dt} = \sum_a \beta_a S_a \frac{\sum I_a}{N} \tag{6.4}$$

in order to compute the weekly incidence on week t:

$$D_t = C(t) - C(t-1) \tag{6.5}$$

We fitted the model to weekly incidence data A using a normal likelihood after a square-root variance-stabilizing transformation [712]:

$$\Pr(A|\beta_a, \gamma, \rho, \sigma) = \prod_t \mathcal{N}(\sqrt{A}|\sqrt{\rho D}, \sigma) \tag{6.6}$$

where ρ is a reporting rate parameter and σ an error parameter. In addition, we also fitted the model to the number of individuals with anti-ZIKV antibodies at the end of the epidemic by age group B_a using a binomial likelihood:

$$\Pr(B|\beta_a, \gamma) = \prod_a \mathcal{B}(B_a|n_a, p_a) \tag{6.7}$$

where B_a the number of individuals with antibodies, n_a is the sample size in each age group, and $p_a = R_a(t_{end})/N_a(t_{end})$ the proportion of immune at the end of the epidemic. The full likelihood was obtained by multiplying Eq. 6.6 and Eq. 6.7. We chose weakly-informative priors for all parameters and fitted the model in Stan (Table 6.2). We describe the calculation of the basic reproduction number $\mathcal{R}_0$ in Supporting information S1. We used one thousand posterior samples for β_a and γ obtained by Hamiltonian Monte Carlo in the ABM model, ensuring the propagation of uncertainty of these parameters. In Supporting information S1 we provide a schematic representation of the models and the information flow. Parameter values can translate from deterministic to agent-based versions of an epidemic model if the time step is small [713], which was the reason for using a time step of 0.1 days.

Table 6.2: Parameter estimates inferred from incidence and sero-prevalence data on the 2016 ZIKV epidemic in Managua, Nicaragua.

Parameter	Interpretation	Prior	Posterior (median and 95% CrI)
β_1	Transmission for age group 0-14	Expon(0.1)	0.19 (0.16; 0.22)
β_2	Transmission for age group $\geq$15	Expon(0.1)	0.32 (0.30; 0.36)
1/γ	Duration of infectious period	Gamma$(1, 0.1)$	4.8 (4.3; 5.4)
ρ	Reporting rate	Beta$(1, 1)$	0.24% (0.21; 0.26)
$I(0)$	Initial number of infectious	Expon(0.1)	74 (40; 134)
$\mathcal{R}_0$	Basic reproduction number	–	1.58 (1.56; 1.59)

CrI: Credible interval.

ZIKV immunity

We used the deterministic model, described in the previous section, to infer the proportion of people with protective immunity within each age group at the end of the 2016 epidemic $\tilde{p}_a$. We used one thousand posterior samples of $\tilde{p}_a$ in the ABM to allow the propagation of uncertainty. Protective immunity to ZIKV after infection was lifelong in our first scenario, so the reduction of the overall proportion of immune individuals in the population decreased

only because of population renewal. Given the absence of evidence about the duration of immunity to ZIKV, we considered four scenarios assuming exponentially distributed durations of immunity with means of 15, 30, 60, 90, or 150 years. These values correspond to a proportion of initially immune agents that loses immunity after 10 years of 55%, 28%, 15%, 11% or 6%, respectively (Supporting information S1).

Demography

We based the initial age distribution of the population on data from the World Bank [714]. We used age-dependent death rates for 2016 from the World Health Organization [710]. For births, we computed a rate based on an average birth rate in Nicaragua of 2.2 births per woman, which was uniformly distributed over the female reproductive lifespan [709]. We defined the period of reproductive age between 15 and 49 years. The ageing process was linear, increasing the age of each agent by 7 days at each 7-day time step.

ZIKV reintroduction

We reintroduced ZIKV in the population after a delay of $d = \{1, \cdots, 80\}$ years in independent simulations. We chose this approach rather than continuous reintroductions to remove some of the stochasticity and assess more clearly the association between immunity decay and risk of an outbreak. As the probability of an extinction of the outbreak depends on the number of ZIKV cases reintroduced in the population, we considered three different values for the seed (1, 5 or 10 cases) and compared the results (Supporting information S1). Simulations using continuous reintroductions each year are presented in the Supporting information S1.

Risk of adverse congenital outcomes

The estimated number of microcephaly cases resulting from the reintroduction of ZIKV depended on the exposure, i.e. the number of pregnant women infected by ZIKV during their first trimester, to which we applied three different levels of risk, based on published estimates [186, 703]. We obtained the number of ZIKV infections among women aged 15–49 years from ABM simulations. As gender was not explicitly considered in the model, we assumed that women represented 50% of the population. We assumed a uniform distribution of births during the reproductive period, and considered that the first trimester constituted a third of ongoing pregnancies at a given time. We explored three different levels of risk of microcephaly in births to pregnant woman with ZIKV infection during the first trimester, as reported by Zhang et al. (2017), based on data from French Polynesia (0.95%, called low risk) and Brazil (2.19% and 4.52%, called intermediate and high risk, respectively) [167, 186, 703].

Vaccination

We examined the effects of a potential ZIKV vaccine, given to 15-year-old-girls. This vaccination strategy was used for rubella virus, which also causes congenital abnormalities, before the vaccine was included in the measles, mumps and rubella vaccine given in early childhood [715]. The main objective of vaccination would be the prevention of adverse congenital outcomes, including microcephaly. We simulated this intervention in the ABM, assuming vaccine implementation starting in 2021, 2025 or 2031. From that date, half of the agents reaching age 15, representing females, could transition to immune status R regardless of their initial status, with an effective vaccination coverage ranging from 20% to 80%.

6.4.4 Outcome analysis

From the simulations, we collected 1) the evolution of the age-specific ZIKV immunity in the population; 2) the attack rate resulting from the reintroduction of ZIKV at year d; 3) the age of newly infected individuals. We fitted a binary Gaussian mixture model to dichotomize the observed attack rates into either outbreaks or non-outbreaks. We defined the outbreak threshold as the 97.5% upper bound of the lower distribution. This corresponded to a threshold of 1%, so that attack rates $\geq$1% were considered as outbreaks. The age structure of newly infected individuals was used to compute relative risks of infection by age group.

6.4.5 Sensitivity analysis

We explored the effect of seasonality, of changes in vector density, of migration, and of an endemic circulation of ZIKV on our predictions regarding the attack rate and the proportion of introductions that result in an outbreak. Different scenarios, methods and assumptions are provided in Supporting information S2.

6.5 Results

6.5.1 2016 ZIKV epidemic

The fitted model (Fig. 6.1), resulted in a reporting rate of 0.24% (95% credible interval, CrI: 0.21; 0.26). The transmission rate in the 0—14 age group was 42% (95% CrI: 35; 48) lower than in the $\geq$15 age group. This corresponded to an overall basic reproduction number $\mathcal{R}_0$ of 1.58 (95% CrI: 1.56; 1.59). The predicted percentage of immune at the end of the epidemic was 36% (95% CrI: 34; 38) for the 0–14 age group and 53% (95% CrI: 50; 57) for the $\geq$15 age group.

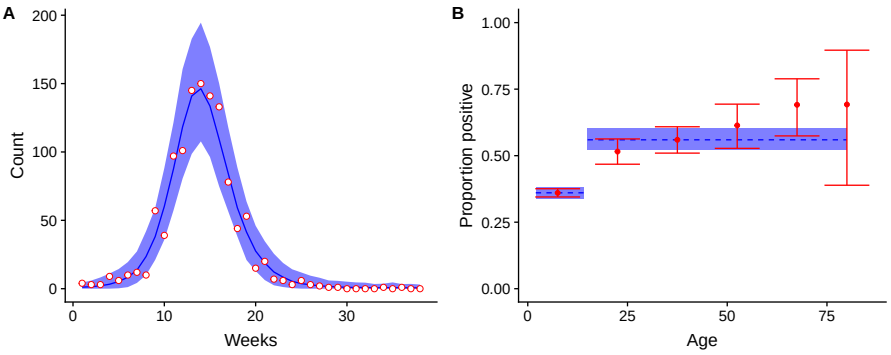


Figure 6.1: Model fit for the 2016 ZIKV epidemic. Model fit for (A) weekly incidence data and (B) post-epidemic sero-prevalence data from the 2016 ZIKV epidemic in Managua, Nicaragua. Data points are in red and the corresponding model fit (posterior median and 95% credible interval) is in blue.

6.5.2 Immunity and population

In our forward simulations, the expected population size increased by 42% between 2017 and 2097. Under the assumption that ZIKV infection results in lifelong protective immunity, population renewal will create an imbalance in the proportion immune in different age

groups. We expect the overall proportion of the population with protective immunity to have halved (from 48% to 24%) by 2051 and to be concentrated among the older age classes (Fig. 6.2A). The 0–14 year old age group will become entirely susceptible by 2031 and the 15–29 year old age group by 2046.

6.5.3 Future risk of ZIKV outbreak

Reintroductions of ZIKV in the population of Managua are unlikely to develop into sizeable outbreaks before 2035, 24 years after the 2016 epidemic, assuming lifelong immunity for individuals infected in 2016 (Fig. 6.2B). After this point, attack rates resulting from ZIKV reintroduction will rise steeply. By 2047, we predict that ZIKV reintroductions will have a 50% probability of resulting in outbreaks with attack rates greater than 1% (Fig. 6.2C). In 2047 the median attack rate of successful introductions is 3.6% (IQR: 2.0–6.2).

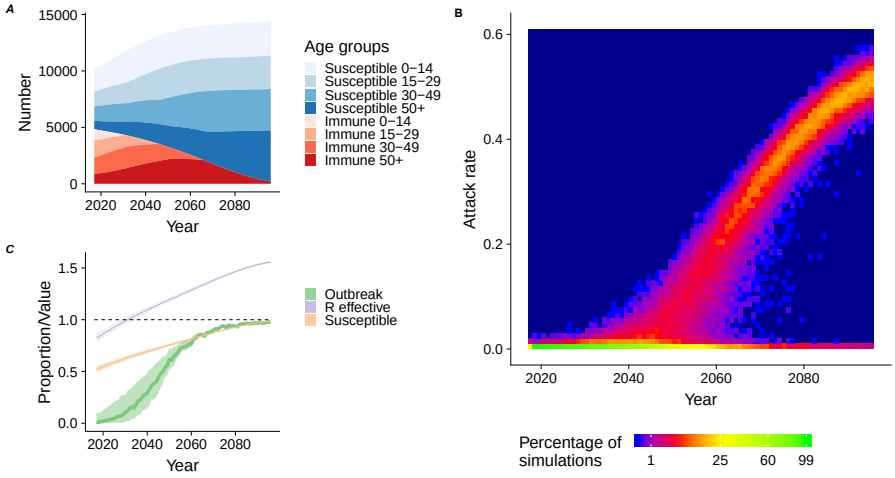


Figure 6.2: Future risk of ZIKV outbreaks. (A) The evolution of the immunity status per age group in a population of 10,000 agents for the next 80 years based on the demographic structure of Nicaragua. (B) Heat map of the distribution of the attack rates resulting from the reintroduction of ZIKV in the population at each year (1000 simulations for each year). (C) The evolution of the proportion of reintroductions resulting in outbreaks (with a threshold of 1%) with time (green), proportion of susceptible (orange), and effective reproduction number $\mathcal{R}_e$ (purple).

6.5.4 Risk of infection and microcephaly births in women of reproductive age

The differences between age groups in both immunity and transmission will result in a disproportionate burden of infection in the 15–29 year age class. The relative risk of infection in this age group ranges from 1.2 to 1.6, compared with the general population if an outbreak occurs during the period 2032–2075 (Fig. 6.3A). As most pregnancies occur in this age group, these women are also the most likely to experience a pregnancy with an adverse outcome. The increased risk of infection in this group implies that the number of adverse congenital outcomes resulting from a ZIKV outbreak during this period is likely to be higher than expected with a homogeneous distribution of immunity across ages. Assuming different

values for the added risk of microcephaly after a ZIKV infection during the first trimester, we expect the mean number of additional microcephaly cases due to ZIKV infection resulting from the reintroduction of the virus in Managua, Nicaragua to reach 1 to 5 cases per 100,000 population in 2060 (Fig. 6.3B).

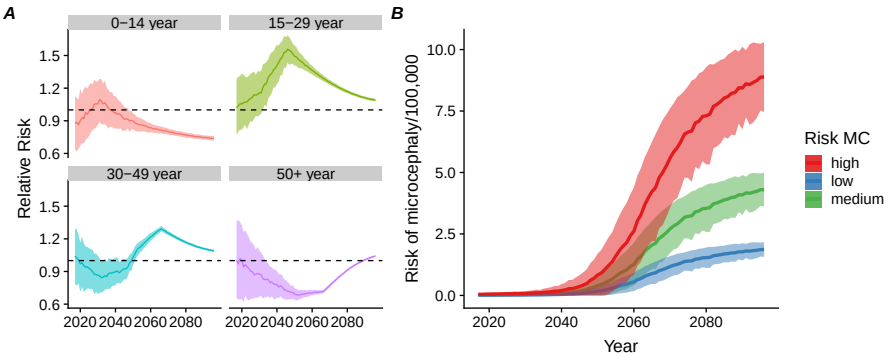


Figure 6.3: Risk of infection and microcephaly births in women of reproductive age. (A) Relative risk of ZIKV infection during a ZIKV outbreak per age group compared to the general population by year (median, interquartile range). (B) Expected number of additional microcephaly events associated with ZIKV infection during pregnancy per 100,000 total population according to three different risk scenarios.

6

6.5.5 Loss of immunity

If protective immunity to ZIKV is not lifelong, the time window before observing a rise in the attack rates resulting from ZIKV reintroduction will shorten (Fig. 6.4A). For instance, if 15% of the those who were infected in 2016 lose their immunity after 10 years (a mean duration of immunity of 60 years), the time until the risk of outbreak upon reintroduction reaches 50% would be 14 years earlier (2033) than with lifelong immunity (2047). If 55% lose their immunity after 10 years (a mean duration of immunity of 15 years), in 2024, 50% of the introductions result in an outbreak, and the attack rate in 2047 is 47%. Loss of immunity over time would reduce the relative risk in the 15–29 year old age group (Fig. 6.4B).

6.5.6 Targeted vaccination

The implementation of a vaccination program targeted towards 15 year old girls between 2021 and 2031 would reduce the risk of infection in women aged 15-29 years and would also indirectly reduce the overall risk of a ZIKV outbreak in the population (Fig. 6.5). If effective vaccine coverage is 60–80% amongst 15 year old girls, the prolongation of herd immunity could effectively mitigate the overall risk of a ZIKV outbreak in the population. The reduction in the number of microcephaly cases would then exceed what would be expected by considering only the direct protection granted by a vaccine to future mothers. A later implementation of the intervention would be less effective, as it becomes more difficult to maintain the herd immunity (Fig. 6.5B).

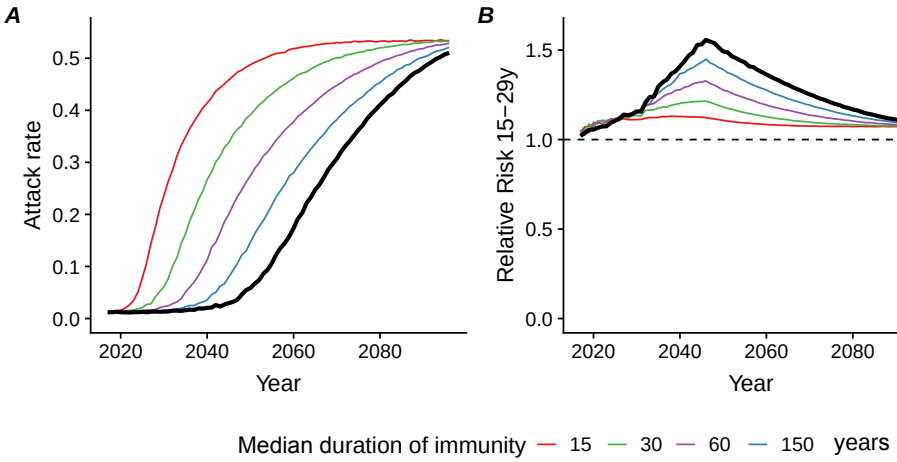


Figure 6.4: Loss of immunity. Consequences of alternative scenarios regarding the mean duration of protective immunity (15, 30, 60 and 150 years), compared with lifelong immunity (thick black line): (A) median attack rate of ZIKV among reintroductions resulting in outbreaks (with a threshold of 1%) and (B) relative risk of ZIKV infection during an outbreak in the 15–29 year age group compared with the general population.

6.5.7 Sensitivity analysis

We considered additional model features that may impact our predictions in a sensitivity analysis (Table 6.3 and Supporting information S2). Accounting for seasonality or for a future increase in vector abundance would result in higher transmission rates. This would lead to a shorter time window until a rise in the risk of ZIKV outbreak, and higher overall attack rates. A future diminution of vector abundance would have the opposite effects. Human migration from rural areas to Managua, Nicaragua would lead to a sharper decline of protective immunity in the population, also lowering the time window before the next ZIKV outbreak. Finally, a continuous endemic circulation of ZIKV in the region would increase the probability of an outbreak early on and lead to more stochasticity.

Table 6.3: Summary of the impact of features considered in the sensitivity analysis.

Scenario	Consequence on the model	Years until reintroductions outbreak (Year)
Baseline	–	31 years (2047)
Seasonality	Increased transmission rate (+12%)	21 years (2037)
Increased vector abundance	Increased transmission rate (+12%)	21 years (2037)
Decreased vector abundance	Decreased transmission rate (–12%)	44 years (2050)
Migration	Influx of susceptible individuals	23 years (2039)
Endemicity	Continuous reintroductions	23 years (2039)

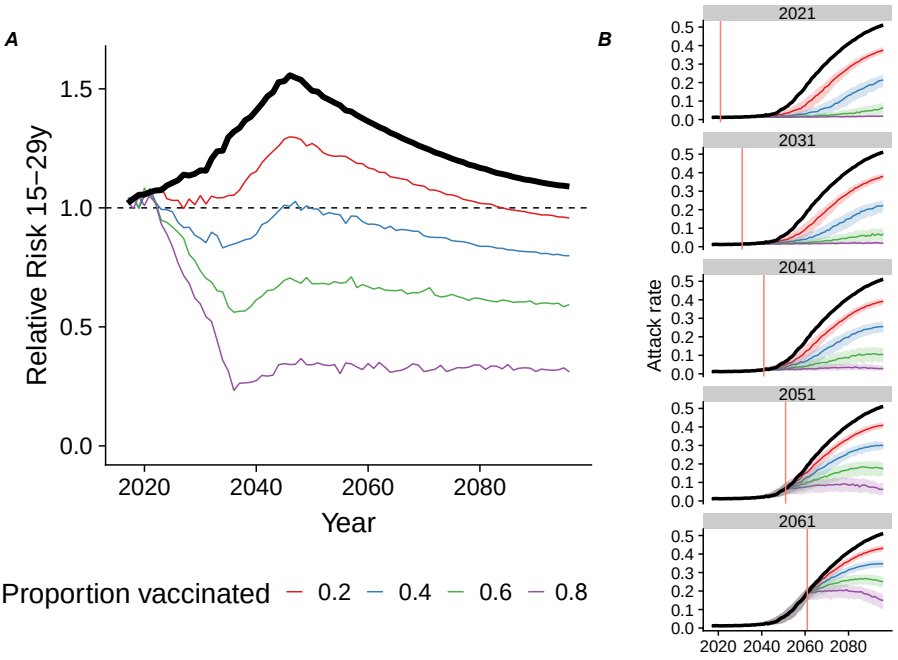


Figure 6.5: Targeted vaccination. Consequences of implementing a targeted vaccination program among 15-year-old-girls from 2021 onwards with various levels of effective vaccination coverage (from 20 to 80%) compared with no vaccination (thick black line). (A) relative risk of ZIKV infection during an outbreak in the 15–29 year age group compared with the general population and (B) attack rate of ZIKV among reintroductions resulting in outbreaks (median, interquartile range, with a threshold of 1%), when vaccination is introduced from 2021, 2031, 2041, 2051 or 2061 onwards (red vertical line).

6

6.6 Discussion

In this mathematical modelling study, we show that a new ZIKV outbreak in Nicaragua would affect proportionally more women in the young reproductive age range (15–29 years) than the general population, owing to the age-dependent infection pattern and population renewal. The risk of a new ZIKV outbreak in Nicaragua, after reintroduction, will remain low before 2035 because of herd immunity, then rise to 50% in 2047. If protective immunity to ZIKV decays with time, ZIKV recurrence could occur sooner. Timely introduction of targeted vaccination, focusing on females aged 15 years would both reduce the risk of adverse congenital outcomes and extend herd immunity, mitigating the overall risk of an outbreak and resulting in lower attack rates if an outbreak occurs.

6.6.1 Strengths and limitations of the study

A strength of our approach is that it allows for the propagation of uncertainty from the initial data into the risk assessment, by transferring the posterior distributions of the parameters from the deterministic model fitted to surveillance and seroprevalence data on the 2016 epidemic into the ABM used for simulations. Roche et al. showed that, when a sufficiently small time step was chosen, stochastic and deterministic models using the same parameter

values led to similar results [716]. Additionally, we benefited from the availability of high quality data from population-based surveys that included participants from age 2 to 80 years in Managua, Nicaragua. The age-stratified seroprevalence data allowed us to investigate the risk in different age groups and better assess the evolution of the age-specific immunity, which is crucial when studying adverse congenital events caused by ZIKV infection during pregnancy.

We chose a simple approach based on an SIR structure, similar to the model used by Netto et al. (2017), to focus on the dynamics of infection and immunity in the human population [171]. We did not model vector populations and behavior explicitly, as in some other studies [163, 164, 173]. This simplification limits the mechanistic interpretation of the epidemic parameters, but provides a phenomenological description of the transmission dynamics. We believe that this approach is appropriate because our main objective was to determine the risk of an outbreak after reintroduction of ZIKV, which is mostly influenced by the level of protective immunity in the human population. We acknowledge that the future occurrence of ZIKV in the area also depends on the presence of a competent vector. Our choice is supported by sensitivity analyses that show that more complex model structures (delayed SIR and Ross-MacDonald-type models) were not superior to a simple SIR structure in describing the 2016 ZIKV epidemic of Managua (Supporting information S1). Similarly, Pandey et al. (2013) showed that additional model complexity does not result in a better description of the dynamics of transmission of dengue virus (another Aedes-borne virus) in a human population compared with a SIR model [162]. In our model, the transmission rate (β_a) captures both human-mosquito and mosquito-human transmission; we assumed a constant transmission rate, as observed in the 2016 outbreak.

Despite having modeled the effect of migration on our predictions, uncertainty remains; factors such as the political instability in Nicaragua could drive migration and influence disease transmission, as we currently observe in Venezuela and bordering countries [717].

6.6.2 Interpretation in comparison with other studies

This study shows that the lower attack rate of ZIKV in children than in adults will hasten the emergence of a population that will be fully susceptible to infection, especially if immunity is not lifelong. The advantage of our approach is that we used the age-specific attack rates to model the processes of ageing in relation to protective immunity to ZIKV explicitly. Even with lifelong immunity, our model predicts that children aged 0–14 years will become entirely susceptible by 2031 and 15–29 year olds by 2046. In future outbreaks, the attack rate will then be highest amongst 15–29 year olds, including women who will be at risk of ZIKV infection in pregnancy. If immunity wanes, the time until the next ZIKV outbreak will be reduced and, in that case, the distribution of infection risk would be more equal across age groups (Fig. 6.4). Several authors have studied the time to a next ZIKV outbreak, but none studied the effect of the loss of immunity over time in relation to age. Assuming lifelong immunity, our estimates of the time until the risk increases are similar to the 12–20 years before re-emergence estimated for French Polynesia [164]. Netto et al. (2017) used an SEIR model to show that in Salvador, Brazil, the effective reproduction number was insufficient to cause a new outbreak during the "subsequent years" [171]. Lourenço et al. (2017) showed the same for the whole of Brazil: herd immunity should protect the population from a new outbreak in the coming years [172]. Ferguson et al. (2016) concluded that the age distribution of future ZIKV outbreaks will likely differ and that a new large epidemic will be delayed for "at least a decade" [173].

Other ZIKV vaccination studies confirm our findings. However, they do not show the effect in risk groups nor assume herd immunity from previous outbreaks as we did; Durham et al. (2018) showed that immunizing females aged 9 to 49 years with a 75% effective vaccine and a coverage of 90%, would reduce the incidence of prenatal infections by at least 94% [718]. Similarly, Bartsch et al. (2018) showed that women of childbearing age or young adults would be an ideal target group for vaccination [719]. Valega-Mackenzie et al. (2018) formulated a vaccination model for ZIKV transmission that included mosquito and sexual transmission [720]. They found that vaccination works if high coverage is achieved, both when sexual transmission or vector-borne transmission is most important.

6.6.3 Implications and future research

Our finding that people in the 15–29 year age range are more at risk of infection implies that we expect a higher number of congenital abnormalities due to ZIKV infection. Thus, vaccine development efforts should be increased. Our conclusions are drawn based on data from Managua, Nicaragua, but should be relevant to many regions in the Americas and the Pacific that have documented high post-epidemic levels of seropositivity [171, 697, 708]. In regions where ZIKV has not yet caused an epidemic but competent vectors are present, vaccination would be in place as well. Further age-stratified seroprevalence studies, using sensitive and specific tests and with longitudinal follow-up, are needed to improve our understanding of ZIKV antibody distribution in populations and to quantify the duration of immunity. This information will provide important information to improve mathematical modeling of ZIKV risk.

ZIKV vaccine development faces considerable hurdles. First, the evaluation of vaccine efficacy has stalled because the reduced circulation of ZIKV has reduced the visibility of ZIKV-associated disease [706]. Second, it remains unclear if neutralizing antibodies induced by vaccination are sufficient to protect women against vertical transmission and congenital abnormalities [90]. Third, it is not clear whether or how vaccine-induced antibodies against ZIKV will cross-react with other flaviviruses. To move vaccine development forward, we need to find regions where disease will occur to be able to conduct trials. This requires identifying populations that are at risk, and implementing surveillance there. These can either be regions where ZIKV is endemic, or where ZIKV outbreaks are likely to occur; throughout the Americas, there might be regions that did not experience an outbreak, but do have suitable conditions such as competent vectors. Conducting vaccine trials in disease outbreaks is complex, but there are tools to facilitate planning [721]. ZIKV in an endemic setting, such as in Africa and Asia, could prove a suitable setting as well. However, ZIKV circulation in endemic setting is not well described and the occurrence of adverse outcomes in this context is less documented [177]. Further research in understanding the transmission of the virus in an endemic context is therefore needed. Similarly, we need to increase the understanding of changes over time in vector abundance and population composition, since these influence the risk of new outbreaks.

6.6.4 Conclusion

Preparedness is vital; the time until the next outbreak gives us the opportunity to be prepared. The next sizeable ZIKV outbreak in Nicaragua will likely not occur before 2035 but the probability of outbreaks will increase. Young women of reproductive age will be at highest risk of infection during the next ZIKV outbreak. Vaccination targeted to young women

could curb the risk of a large outbreak and extend herd immunity. ZIKV vaccine development and licensure are urgent to attain the maximum benefit in reducing the population-level risk of infection and the risk of adverse congenital outcomes. The urgency of ZIKV vaccine development increases if immunity is not lifelong.

6.7 Acknowledgements

Calculations were performed on UBELIX (http://www.id.unibe.ch/hpc), the HPC cluster at the University of Bern.

6.8 Supporting information

S1 Text. Supplementary model description.

S2 Text. Sensitivity analysis.

Supporting information 1. Supplementary model description

Supplement to:

Impact of age-specific immunity on the timing and burden of the next Zika virus outbreak

Michel J. Counotte, Christian L. Althaus, Nicola Low and Julien Riou

Contents

S1.1 Model workflow

Figure 1 provides a workflow of the parameterization using an ODE model and subsection predictions using an ABM model.

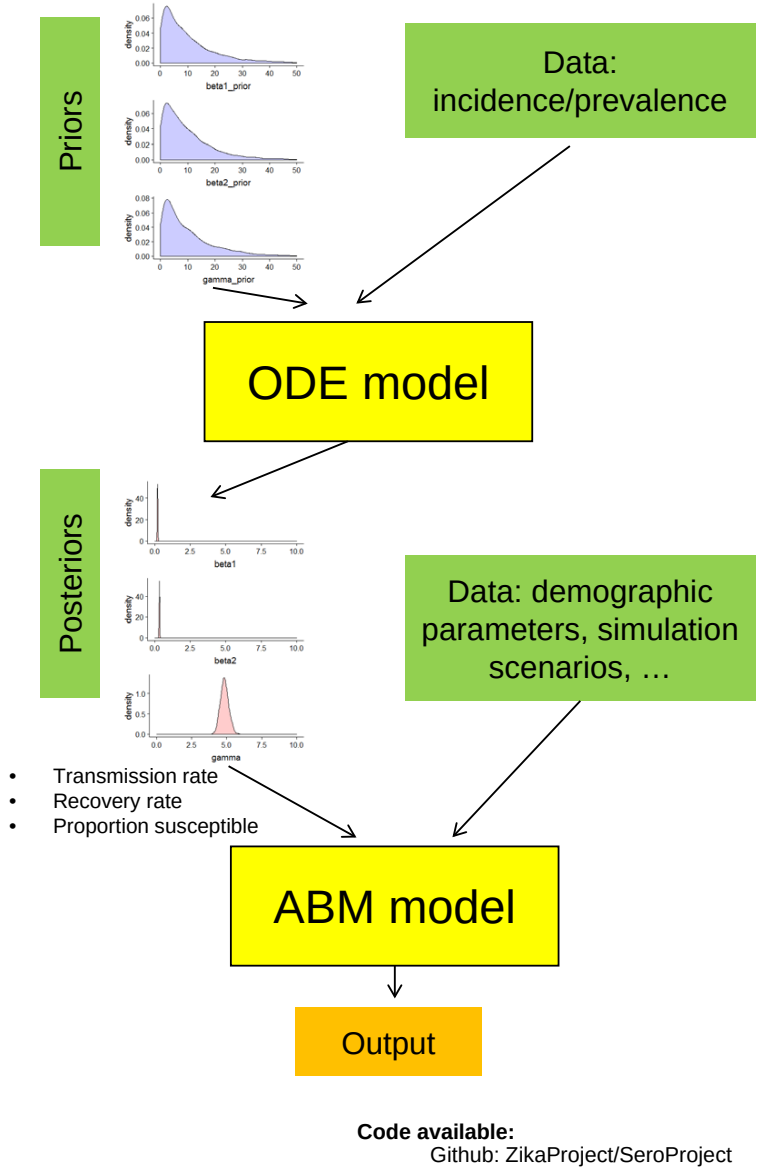


Figure 1: Graphic description of the model.

S1.2 Comparison of SIR model with SEIR model and the Pandey model.

We chose a simple SIR structure to model the transmission of ZIKV (Fig. 2). Other common choices include SEIR structures, including an incubation period, and a Pandey-type structure explicitly modelling the vector population as implemented in Champagne et al. (2016) [1]. We support this choice by conducting model selection using leave-one-out cross-validation (LOO-CV) [2]. The objective of LOO-CV is to estimate the leave-one-out information criterion (LOOIC), a measure of the pointwise out-of-sample prediction accuracy from a fitted Bayesian model. The estimation of the LOOIC relies on Pareto smoothed importance sampling (PSIS), a procedure for regularizing importance weights.

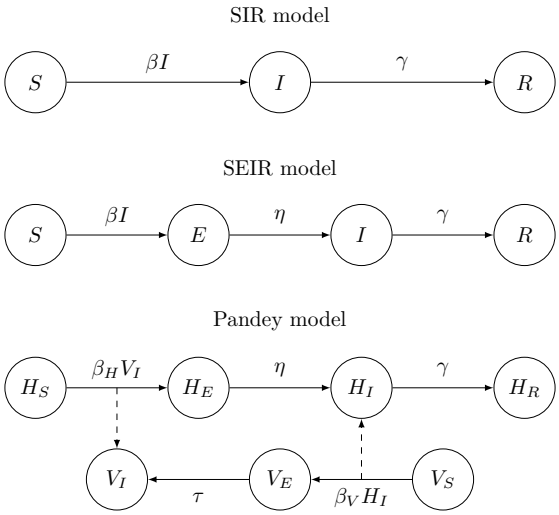


Figure 2: Description of the three compared models. These diagrams ignore the stratification in two age groups: 0-14 and ≥15.

We compared the fits of the SIR, SEIR and Pandey model to incidence and seroprevalence data from Managua, Nicaragua. The LOOIC for the SEIR model (93.9) was slightly lower than for the SIR model (95.2) but the the estimated pointwise difference in LOOIC (ΔLOOIC) of -1.3 was small compared to its standard error, indicating no evidence in support of a better fit. The ΔLOOIC between the SIR and Pandey models was also small and in favour of the SIR model. Overall, this model selection approach supported our choice of the SIR model.

Table 1: Model comparison

Model	LOOIC (SE)	ΔLOOIC (SE)
SIR	95.2 (8.7)	Ref.
SEIR	93.9 (8.9)	-1.3 (1.6)
Pandey	99.5 (8.3)	+4.3 (3.6)

S1.3 Prior predictive check

The choice of prior distribution is a crucial aspect of analyses conducted in a Bayesian framework. Prior predictive checks can be used to assess the adequacy of the choice of prior distributions [3]. The principle is to use the model to simulate artificial data from the chosen set of prior distribution. If the chosen set of priors can lead to any dataset that could plausibly be observed, then the priors can be qualified as "non-informative". Figure 3 shows that this is indeed the case here, as our choice of priors for β_1, β_2, γ, ρ

and $I(0)$ lead to a wide variety of possible epidemic data, from 0 to 4,000 cases reported weekly and from 0 to 100% post-epidemic seroprevalence.

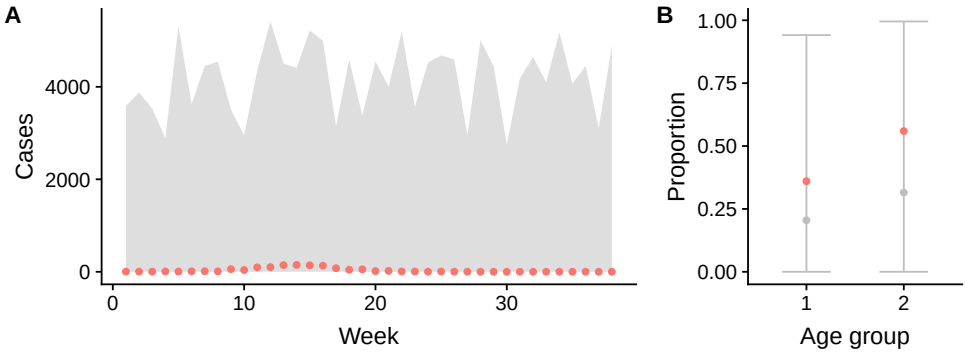


Figure 3: Prior predictive check for the model. (A) Weekly number of reported cases of Zika virus infection in Managua, Nicaragua (grey area shows the 95% range in the artificial data simulated from the prior distributions, red dots show actual data). (B) Post-epidemic seroprevalence (grey bar shows the 95% range in the artificial data, red dots are actual data).

S1.4 Comparison of "square root normal", "negative binomial" and "Poisson" approaches

Several approaches can be used to fit an ODE model to incidence data. The objective is to obtain the joint posterior distribution of the parameters β_1, β_2 and γ by considering the likelihood of the incidence data A. A straightforward choice for modelling count data is to use a Poisson distribution to link the output of the ODE system at time t D_t to weekly incidence data A_t:

$$\Pr(A|\beta_1, \beta_2, \gamma) = \prod_t \text{Poisson}(A_t|C_t) \tag{1}$$

A common problem using Poisson distributions is the presence of overdispersion. A direct solution is to use instead a negative binomial distribution, with an additional overdispersion parameter ϕ:

$$\Pr(A|\beta_1, \beta_2, \gamma) = \prod_t \text{Neg-Bin}(A_t|C_t, \phi) \tag{2}$$

However, the classical negative binomial distribution can struggle when data varies from 0 to large values, as it results in variance estimates that do not scale properly. A solution is to use a modified negative binomial distribution where the overdispersion parameter is scaled by the mean, so that:

$$\Pr(A|\beta_1, \beta_2, \gamma) = \prod_t \text{Neg-Bin}(A_t|C_t, C_t \times \phi) \tag{3}$$

An alternative is to use a normal distribution after a square-root transformation aimed at stabilizing the variance, as described in [4]:

$$\Pr(A|\beta_1, \beta_2, \gamma) = \prod_t \mathcal{N}(\sqrt{A_t}|\sqrt{C_t}, \sigma) \tag{4}$$

We decided to use this last solution in our model, but provide here a comparison of the model fit (Figure 4) and parameter estimates (Table 2) obtained with the other approaches. We show that all the approaches lead to very similar fits and parameter estimates, although the Poisson approaches leads to narrower credible intervals.

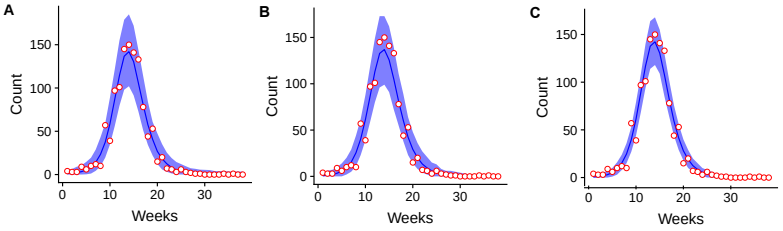


Figure 4: Comparison of fit with Sqrt (A), Poisson (B), and Modified negative binomial (C).

Table 2: Comparison of parameter estimates with Sqrt, negative binomial, and Poisson.

Approach	Square root normal	Poisson	Modified negative binomial
β_1	0.17 (0.15–0.20)	0.16 (0.14–0.18)	0.17 (0.15–0.19)
β_2	0.31 (0.28–0.34)	0.30 (0.27–0.32)	0.31 (0.29–0.33)
$1/\gamma$	5.24 (4.69–5.86)	5.58 (4.98–6.21)	5.35 (4.85–5.84)

S1.5 $\mathcal{R}_0$ calculation

We used the next generation matrix method described by Diekmann et al. (2010) to calculate $\mathcal{R}_0$ (eq. 5 - 7) [5]. β_1 is the transmission rate for the 0–14 age group; β_2 for the >15 group and γ is the common recovery rate.

We start by expressing the model with the infection matrix F and the migration matrix V:

$$F = \begin{pmatrix} \beta_1 & \beta_1 \\ \beta_2 & \beta_2 \end{pmatrix} \tag{5}$$

$$V = \begin{pmatrix} -\gamma & 0 \\ 0 & -\gamma \end{pmatrix} \tag{6}$$

$\mathcal{R}_0$ is defined as the square root of the largest eigenvalue of FV^{-1}:

$$\mathcal{R}_0 = \sqrt{\frac{\beta_1 + \beta_2}{\gamma}} \tag{7}$$

S1.6 Loss of immunity scenarios

We explored plausible scenarios of loss of immunity with mean durations of 15, 30, 60, 90, and 150 years (Fig. 5).

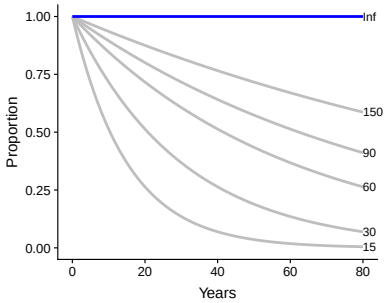


Figure 5: Different scenarios considered regarding the loss of immunity.

S1.7 ABM algorithm

Here, we provide pseudo code of the ABM (Algorithm 1).

Algorithm 1 ABM

procedure INITIALIZATION ▷ Add initial conditions S/R and sex per n individual
  for $n \leftarrow 1, popMax$ **do**
    $R[n] \leftarrow$ select random 1 or 0 with probability(age[n])
    $S[n] \leftarrow 1 - R[n]$
    $I[n] \leftarrow 0$
    sex$[n] \leftarrow$ select random 1 or 0 with probability 0.5
  end for
end procedure
procedure SIMULATION ▷ Simulation over $wkMax$ weeks
  for $wk \leftarrow 1, wkMax$ **do**
    for $n \leftarrow 1, popMax$ **do** ▷ Loop over $popMax$ individuals
      if individual is alive **then**
        procedure POPULATION DYNAMICS ▷ Pre-outbreak
          $Birth, Death, Ageing$
        end procedure
        procedure LOSS OF IMMUNITY ▷ Loss of immunity
          $[R \rightarrow S]$ with probability RateToProb(ξ)
        end procedure
        procedure VACCINATION ▷ Vaccination
          $[S \rightarrow R]$ with probability vaccinationProb, at $age[n]$
        end procedure
        procedure INFECTION, RECOVERY ▷ During outbreak
          $[S \rightarrow I]$ with probability RateToProb(β, $age[n]$)
          $[I \rightarrow R]$ with probability RateToProb(γ)
        end procedure
      end if
    end for
    procedure START OUTBREAK ▷ Introduction of infection
      if $wk = introductionWk$ **then**
        Change timestep: 7 days to 0.1 days
        Collect summary statistics pre-outbreak
        Introduce $introductionN$ infections
      end if
    end procedure
    total number alive ▷ Collect summary of week wk:
    total number infected
  end for
end procedure

S1.8 The number of infections introduced does influence the probability of an outbreak, but not the attack rate of successful outbreaks

The proportion of outbreaks (1% threshold) after introduction depends on the number of infections introduced; the attack rate of the successful outbreaks does not depend on the number of infections introduced (Fig. 6).

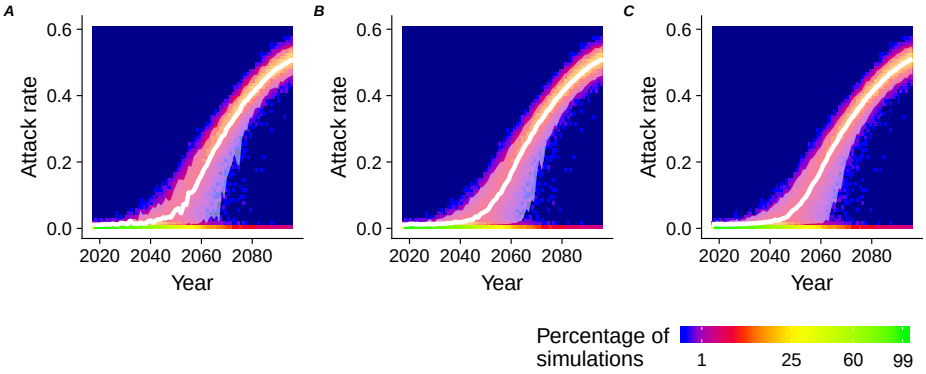


Figure 6: Attack rate over time for the introduction of (A) n=1, (B) n=5, (C) n=10 infections.

References

[1] Champagne C, Salthouse DG, Paul R, Cao-Lormeau VM, Roche B, Cazelles B. Structure in the variability of the basic reproductive number (R0) for Zika epidemics in the Pacific islands. Elife. 2016;5. doi:10.7554/eLife.19874.

[2] Vehtari A, Gelman A, Gabry J. Practical Bayesian model evaluation using leave-one-out cross-validation and WAIC. Statistics and Computing. 2017;27(5):1413–1432. doi:10.1007/s11222-016-9696-4.

[3] Gabry J, Simpson D, Vehtari A, Betancourt M, Gelman A. Visualization in Bayesian workflow. Journal of the Royal Statistical Society: Series A (Statistics in Society). 2019;182(2):389–402. doi:10.1111/rssa.12378.

[4] Guan Y. Variance stabilizing transformations of Poisson, binomial and negative binomial distributions. Stat Probab Lett. 2009;79(14):1621–1629. doi:10.1016/j.spl.2009.04.010.

[5] Diekmann O, Heesterbeek JAP, Roberts MG. The construction of next-generation matrices for compartmental epidemic models. J R Soc Interface. 2010;7(47):873–885. doi:10.1098/rsif.2009.0386.

Supporting information 2. Sensitivity analysis

Supplement to:

Impact of age-specific immunity on the timing and burden of the next Zika virus outbreak

Michel J. Counotte, Christian L. Althaus, Nicola Low and Julien Riou

Contents

S2.1 Introduction

The results regarding the future risk of ZIKV outbreak in Managua, Nicaragua presented in the main analysis rely upon several hypotheses and modelling choices. The potential effects of two main points of uncertainty, the rate of immunity loss and the introduction of targeted vaccination, were evaluated in the main text. Here, we assess the potential effects on our results of several additional features that were not considered in the main analysis.

S2.2 Seasonality

Variations in vector abundance according to the season may result in a variation in the transmission rate according to yearly cycles. We explored the effect of seasonality using the approached proposed by Netto et al. (2017) [1], that is based on a forcing cosine function f with a frequency of 52 weeks (equation 1). The amplitude α and the shift κ of the cosine function are estimated from data:

$$f(t, \alpha, \kappa) = 1 + \alpha \times \cos\left(\frac{6.283(t - \kappa)}{52}\right) \quad (1)$$

All things being equal, introducing seasonality will lead to an increase of the estimated transmission rate at certain times of the year, as in this case the decrease in incidence towards the end of the epidemic is not only caused by a lack of susceptibles, but also by the seasonal decrease in transmission. In forward simulations, this may lead to an earlier increase of the risk of outbreak if introductions happen at a favourable time. However, as the shift of the seasonal cycle κ is estimated from data, this model assumes that disease introduction in the population took place on the most optimal time. This could lead to an underestimation of the transmission rate if disease introduction occurred at a less optimal time in the season.

We compare our baseline model ignoring seasonality ("No seasonality", Fig. 1A-D) with a model including seasonal forcing with α and κ informed by data ("Flexible seasonal forcing", Fig. 1E-H) and

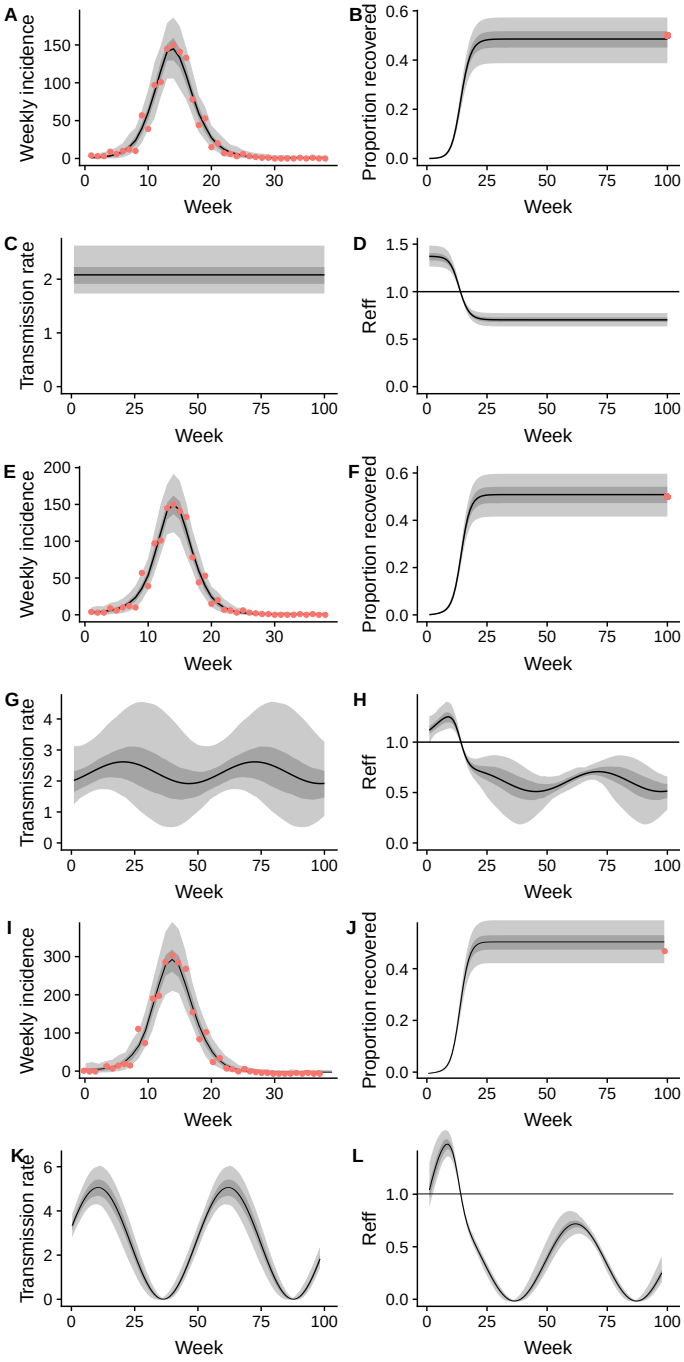


Figure 1: Model implemented without seasonality (A-D). Model implemented with flexible seasonal forcing: κ and α are estimated from data (E-H). Model implemented with full seasonal forcing: κ is estimated from data and α is fixed at 1 (I-L).

a model including seasonal forcing with α fixed to 1 ("Full seasonal forcing", Fig. 1I-L). Compared to no seasonality, including a flexible seasonal forcing leads to a peak estimate of $\mathcal{R}_0$ higher by 6%, and including full seasonal forcing higher by 12%.

We considered the impact of an augmentation of the transmission rate by 12% on the model predictions (Fig. 3, scenario n°2). Such an increase would result in a reduction of the time window before observing a rise in the risk of ZIKV outbreak (21 years until 50% of reintroductions result in outbreaks, compared with 31 years in the baseline model) and an increase of the attack rate at year 2047 to 15.3% (IQR: 11.2–18.4), compared with 3.6% (IQR: 2.0–6.2) in the baseline model.

S2.3 Varying vector densities

Changes in vector density may result in an increase or a decrease of the transmission rate. Vector densities may change over time according to human population densities and climate [2]. The complex interactions between climate, human demography and vector abundance make long term predictions of future vector abundance difficult. Therefore, we considered the impact of both an increase and a decrease of the transmission rate on our estimate of the future risk of ZIKV outbreak.

As reported in the previous section, an increase of the transmission rate by 12% would result in an earlier increase of the risk of outbreak and higher average attack rates (Fig. 3, scenario n°2). Conversely, a decrease of the transmission rate by 12% would extend the time window before observing a rise in the risk of outbreak (44 years until 50% of the simulated introductions result in outbreaks, Fig. 3, scenario n°3). The average attack rate in 2047 would be 1.6% (IQR: 1.3–2.5).

S2.4 Migration

Human migration may impact the future evolution of the proportion of susceptible individuals in Managua, Nicaragua. We considered the effect of urbanization, or an influx of rural inhabitants, which is a plausible scenario in this particular context [3]. Evidence suggests that rural populations have lower seroprevalence [4]. Urbanization might thus result in a quicker decline of protective immunity in the population than expected.

The International Institute for Applied Systems Analysis (IIASA) produces predictions of population and urbanization according to different Shared Socioeconomic Pathways (SSP) storylines. SSP storylines are different narrative scenarios of how trends change over time [5]. We considered a scenario where the projected urbanization in Nicaragua follows the SSP2 or "middle of the road" storyline [6]. This implies that the proportion of urbanization in Nicaragua will rise from 60 to 79% between 2015 and 2100 (Fig. 2). We considered an extreme situation where urbanization consists of an influx of fully susceptible individuals with a median age of 30. Under these conditions, the time until 50% of reintroductions result in an outbreak would decrease from 31 to 23 years and the attack rate in 2047 is 12.7% (IQR: 8.7–16.2) (Fig. 3, scenario n°1).

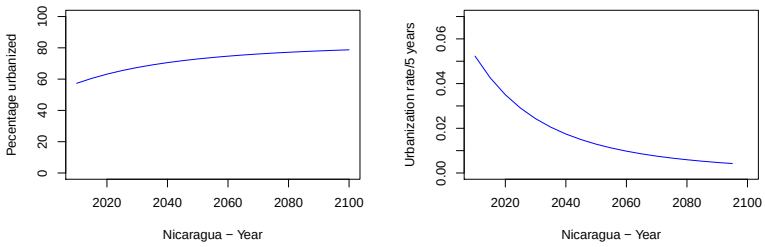


Figure 2: Proportion and rate of urbanization in Nicaragua according to the SSP2 scenario [6].

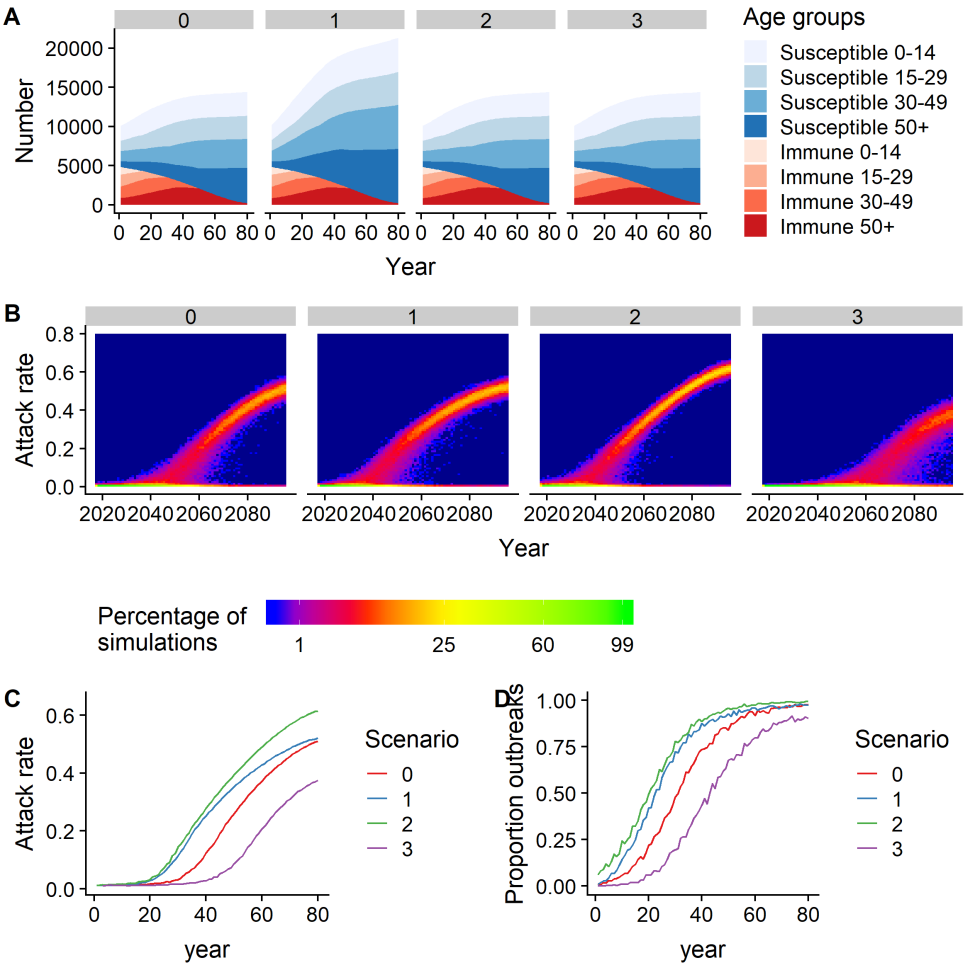


Figure 3: Comparison of the predicted evolution of protective immunity (A); the distribution (B) and the average (C) of the attack rates resulting from the reintroduction of ZIKV in the population each year; and the proportion of reintroductions resulting in an outbreak with attack rate ¿1% across four modelling scenarios: baseline scenario used in the main analysis (n°0), scenario including migration from rural areas (n°1), scenario corresponding to a transmission rate increased by 12% (n°2) or decreased by 12% (n°3).

S2.5 Endemic transmission

In the main analysis, we considered that ZIKV entirely disappeared from Managua, Nicaragua after the epidemic waves of 2015-2017. This assumption is coherent with the sharp decline in reported cases of ZIKV on the continent after 2017 and the limited evidence on ZIKV infection of new world monkeys (limiting the establishment of sylvatic endemic circulation cycles [7]). If low level circulation exists and ZIKV becomes endemic in the Americas, repeated reintroductions in Managua, Nicaragua would occur which would have implications on our projections.

We considered this scenario by modifying the rate of reintroduction of ZIKV in our simulations. In the main model, we consider a single introduction per simulation, corresponding to an epidemic setting where ZIKV does not circulate and has to be reintroduced from outside. To mimic endemic circulation, we consider continuous reintroductions of ZIKV into our population with a monthly probability of 1/12

(on average one introduction per year), in the presence and absence of seasonality (Fig. 5).

With these conditions, the time until 50% of the simulations result in an outbreak where at least 1% of the population is affected would be 23 years in absence of seasonality, and 22 years when seasonality was considered (Fig. 4). The median attack rate in 2047 would be 2.5% (IQR: 1.7–4.1) in the absence of seasonality and 5.1% (IQR: 2.3–13.9) when seasonality is considered. Continuous reintroductions would also result in a lot more variability due to stochasticity.

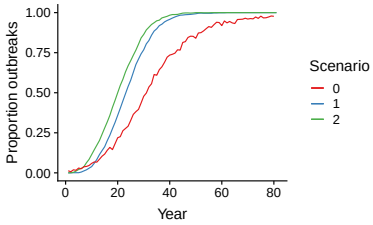


Figure 4: Comparison of the different ABM simulation scenarios taking into different levels of endemicity and seasonality and the effect on the proportion of outbreaks. Baseline scenario with one reintroduction per simulation (0), continuous reintroductions without seasonality (1), continuous reintroductions with seasonality (2).

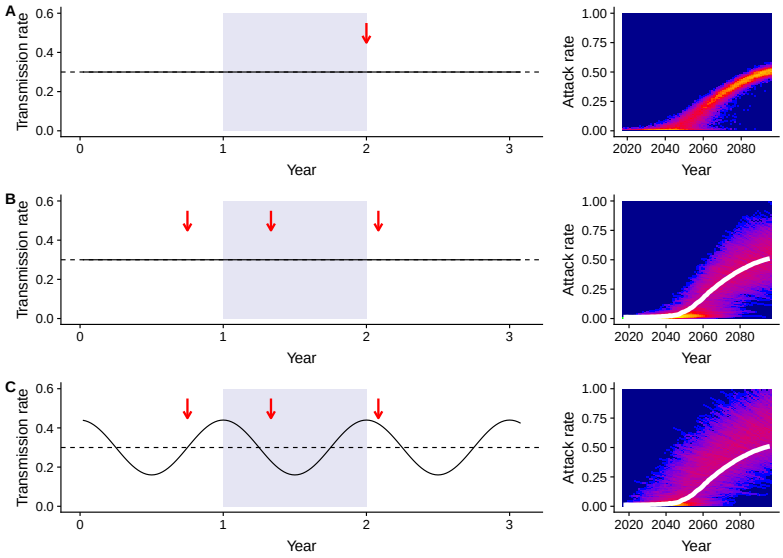


Figure 5: Comparison of the different ABM simulation scenarios taking into different levels of endemicity and seasonality. Red arrows represent ZIKV reintroductions. (A) A single reintroduction per simulation with no seasonal fluctuation of the transmission rate, baseline scenario. (B) Random continuous reintroductions each year without seasonality. (C) Random continuous reintroductions each year with seasonality. The thick white line in panel B and C, represent the median attack rate of the baseline model (A) for comparison.

References

[1] Netto EM, Moreira-Soto A, Pedroso C, Höser C, Funk S, Kucharski AJ, et al. High Zika Virus Seroprevalence in Salvador, Northeastern Brazil Limits the Potential for Further Outbreaks. MBio. 2017;8(6). doi:10.1128/mbio.01390-17.

[2] Kraemer MUG, Reiner RC, Brady OJ, Messina JP, Gilbert M, Pigott DM, et al. Past and future spread of the arbovirus vectors Aedes aegypti and Aedes albopictus. Nat Microbiol. 2019;4(5):854–863. doi:10.1038/s41564-019-0376-y.

[3] Maria A, Acero JL, Aguilera AI, Lozano MG. Central America Urbanization Review: Making Cities Work for Central America. The World Bank; 2017.

[4] Langerak T, Brinkman T, Mumtaz N, Arron G, Hermelijn S, Baldewsingh G, et al. Zika Virus Seroprevalence in Urban and Rural Areas of Suriname, 2017. J Infect Dis. 2019;doi:10.1093/infdis/jiz063.

[5] O'Neill BC, Kriegler E, Ebi KL, Kemp-Benedict E, Riahi K, Rothman DS, et al. The roads ahead: Narratives for shared socioeconomic pathways describing world futures in the 21st century. Global Environmental Change. 2017;42:169 – 180. doi:10.1016/j.gloenvcha.2015.01.004.

[6] International Institute for Applied Systems Analysis . SSP Database (Shared Socioeconomic Pathways) - Version 2.0; 2019. [Online]. Available from: `https://secure.iiasa.ac.at/web-apps/ene/SspDb/dsd?Action=htmlpage&page=citation`.

[7] Althouse BM, Vasilakis N, Sall AA, Diallo M, Weaver SC, Hanley KA. Potential for Zika Virus to Establish a Sylvatic Transmission Cycle in the Americas. PLOS Neglected Tropical Diseases. 2016;10(12):1–11. doi:10.1371/journal.pntd.0005055.

Chapter 7

Discussion

"Epidemiology is something more than the total of its established facts. It includes their orderly arrangement into chains of inference which extends more or less beyond the bounds of direct observation."

– **Wade Hampton Frost** (March 3, 1880 – May 1, 1938)

In this thesis I described different epidemiological aspect of the Zika virus outbreak. In this chapter I will discuss the methods, findings and their implications. This discussion goes beyond the points raised in the discussions in the previous chapters. In the first section, I provide a summary of the findings. In the second, I discuss the interpretation and implications of the findings. In the third section, I discuss the strengths and limitations of the work presented, and in the fourth section, the lessons learnt in the context of outbreak response. In the last section, I present an outlook on future research. I finish this chapter with the overall conclusions of this PhD thesis.

7.1 Summary of the findings

In Chapter 2, I provided insight in how evidence accumulates during an outbreak and more in general during new causal questions. Case reports and case series were the first studies to appear, followed by basic research (*in vivo* and *in vitro* studies). It took more than a year after the onset of the ZIKV outbreak for robust epidemiological studies to be published. Establishing early public health guidance thus requires a broad approach taking into account all evidence available. We have to make do with the low quality evidence. To minimize further delays, evidence should be accessible as soon as it becomes available through rapid and open access dissemination.

In Chapter 3, I extended a systematic review that was conducted earlier [29] and turned it into a living systematic review. I introduced the concept and implementation of living systematic reviews in the context of an emerging disease. We assessed the evidence on the causal relation between ZIKV infection and adverse congenital and auto-immune neurological outcomes, published between May 30, 2016 and January 18, 2017, using a framework based on the causality dimensions of Bradford Hill. During this period, we saw an expansion of the evidence that ZIKV was indeed a cause of congenital abnormalities and GBS. We provided a proof of concept for the use of living systematic reviews to synthesize evidence about an emerging pathogen such as ZIKV. In Chapter 4, I assessed the evidence published between January 18, 2017 and July 1, 2019. We quantified the strength of association of the relation between maternal ZIKV infection and congenital adverse outcomes and between ZIKV infection and GBS. We found that the strength of association between ZIKV infection and adverse outcomes from case-control studies differs according to whether exposure to ZIKV is assessed in the mother (odds ratio (OR) 3.8, 95% CI: 1.7–8.7, I^2=19.8%) or the foetus/infant (OR 37.4, 95% CI: 11.0–127.1, I^2=0%). In cohort studies, the risk of congenital abnormalities was 3.5 times higher after ZIKV infection (95% CI: 0.9–13.5, I^2=0%). The strength of association between ZIKV infection and GBS was higher in studies that enrolled controls from hospital (OR: 55.8, 95% CI: 17.2–181.7, I^2=0%) than in studies that enrolled controls at random from the same community or household (OR: 2.0, 95% CI: 0.8–5.4, I^2=74.6%). The heterogeneity between the studies could be partly explained by the heterogeneity in methods and sampled populations. Studies suffered from bias and uncontrolled residual confounding.

In Chapter 5, I presented a framework to systematically assess the evidence for ZIKV as a sexually transmitted disease. We reviewed all available literature and concluded that the risk of sexual transmission of ZIKV is likely small, but relevant for certain risk groups. We found that in semen viral RNA could be detected for a median period of 34 days (95% CI: 28–41 days) and 35 days (no CI given) based on two cohort studies. Aggregated data about detection of ZIKV RNA from 37 case reports and case series indicate a median duration of 40 days (95% CI: 30–49 days) and a maximum duration of 370 days in semen. In human vaginal fluid, the

median duration was 14 days (95% CI: 7–20 days) and the maximum duration was 37 days. Infectious virus in human semen was detected for a median duration of 12 days (95% CI: 1–21 days) and a maximum of 69 days. We highlighted the poor quality of the evidence and the need for systematic observational studies that evaluate the risk of sexual transmission of ZIKV.

In Chapter 6, I presented predictions on the future risk of ZIKV, based on data from Managua, Nicaragua, using mathematical modelling. We concluded that the risk of a new outbreak in the next decades is low due to herd immunity. However, a next outbreak will disproportionally hit people in the young reproductive age hardest (age 15–29 years). Vaccination could curb this risk: Early introduction of vaccination in 15-year-old girls has the capacity to extend the herd immunity and be of benefit to the whole population. Introduction of a vaccine needs to happen within a decade after the 2016 outbreak to achieve this protection. The duration of immunity following ZIKV infection has impact on the speed at which outbreaks will reoccur.

7.2 Implications and interpretation of the findings

Disease outbreaks have always been part of human history and will remain a challenge in the future. Every new outbreak allows us to learn lessons from it and expand our knowledge. Here I put the results in the wider context of the public health response to ZIKV and disease outbreaks in general. I discuss the implications of the findings in three themes: The accumulation of evidence and causal inference during disease outbreaks, the estimation of the risk of sexual transmission, and the future risk of ZIKV.

7.2.1 The implication and interpretation of the findings on the delay in evidence accumulation and causal inference during disease outbreaks

I showed in Chapter 2-4 that early in disease outbreaks a lot of questions are unanswered, many of which are of a causal nature. During disease outbreaks we want to know what caused the outbreak, what interventions will be of benefit, or if the disease causes a certain adverse outcome. These questions are not only relevant to advance science, they often serve important public health purposes when the answers are translated into action through guidelines. Rapid establishment of evidence-based public health guidance is vital for a proper outbreak response. In Chapter 2, I concluded that in the ZIKV outbreak the quality of the evidence was limited. This pattern is likely similar in other outbreaks of emerging diseases and emerging causal questions in general. Early in an outbreak, we often only have access to surveillance data, case reports and case series. These studies are well suited for discovery and explanation of new phenomena [133]. Fundamental research can provide insight into the biology and pathogenesis of the disease. However, robust epidemiological studies, that provide evidence for guidance, would often not be available. This was confirmed with the findings presented in Chapter 2. At least in the first year after the ZIKV outbreak, no robust epidemiological studies were available yet. This means that we have to make do with the available evidence and look at the full body of evidence. We need to draw causal conclusions from imperfect data.

In Chapter 3, I used a framework that considers the totality of evidence on the causal relation between ZIKV infection and adverse outcomes. The framework and the continuous incorporation of evidence tries to tackle the shortcomings of the evidence illustrated in

Chapter 2. With a living systematic review we manage to integrate new evidence as it becomes available. In Chapter 4, I extend these findings with meta-analyses of the strength of association. The strong heterogeneity observed between the studies might imply that we miss information on important co-factors to fully explain this variation. The heterogeneity could also be a result of a lack of standardized methods and protocols early in an outbreak. By using a living systematic review format, we observed an extension of the evidence and the conclusions that ZIKV caused congenital abnormalities and adverse auto-immune neurological outcomes were reinforced.

Every publication on a suspected causal association contributes a piece of evidence, but none of these pieces of evidence by itself might be enough to answer causal questions. Early in an outbreak evidence helps to discover and explain new phenomena [133]. Here, anecdotal evidence is of great value to initiate other studies and generate hypotheses. Looking at all available evidence during an outbreak, regardless of the study design, helps to understand causal relations [125]. In the absence of plausible alternative explanations one might conclude that the most likely conclusion is that of causality. We saw this in the ZIKV outbreak using the Bradford Hill dimensions [29] and Chapter 3 and Chapter 4. It took three years after the start of the PHEIC for the first meta-analysis of the strength of association between ZIKV infection and adverse outcomes to be presented (Chapter 4). Quantifying the strength of association and the risk of adverse outcomes due to ZIKV infection is important for resource planning and prioritizing the disease: there are many competing public health challenges and resources are limited. A quantification of the risk can be extended to the assessment of the burden and helps to inform policy-makers. From discovery of a causal association to the aggregation of the available evidence, we need to take into account the risk of bias and confounding. The value of a piece of evidence is often dependent on the purpose for which it is used [722].

An implication of the findings is that we have to be aware that during disease outbreaks evidence is imperfect. In a setting of emerging evidence, causal inference is performed using imperfect evidence. Emphasizing and communicating the limited quality of early evidence is vital, and guidance based on this evidence should be updated as soon as more robust evidence becomes available.

7.2.2 The implication and interpretation of the findings on the risk of sexual transmission

The sexual transmission framework presented in Chapter 5 can serve as a blueprint for the systematic investigation of disease transmission. We applied it to break down the complicated concept of transmission into smaller quantifiable parameters. It illustrates the use and limitations of observational data. Not all parameters can be directly measured or inferred from the data. Mathematical modelling and basic research studies can help fill in the gaps. The framework served to identify research gaps during a WHO meeting where the research on sexual transmission of ZIKV was discussed (Chapter 8.2) [47].

Different diagnostic techniques represent the duration of persistence of ZIKV in the genital tract differently; this resulted in the conclusion that the duration of infectiousness is likely underestimated if it is based on the viral culture of genital tract fluids, detection of viral RNA likely overestimates it (Chapter 5). This implies that we need to take into account these patterns for interpreting the findings. We presented all data in a concise format together with the emphasis on the limitations; this helped an expert committee to formulate recommendations. The results presented in Chapter 5 were used as evidence in the WHO

guideline on the sexual transmission of ZIKV (Chapter 8.1). The recommended duration of protected sexual intercourse after possible ZIKV infection was reduced from six months to three months for men, and two months for women.

Case reports and disease surveillance have allowed the discovery of sexual transmission and viral persistence in semen, but larger systematic longitudinal studies provided 'better' evidence for guidance. As with the causal research, taking into account indirect evidence from animal studies and mathematical models, helped to make sense of the observational data. We observed that the anecdotal nature of the data from observations in case reports and case series resulted in a bias. Here, the more extreme results are typically reported; for example: a longer duration of viral persistence in the genital tract. Following the evidence over time, we observed a regression to the mean duration of persistence from the case reports and case series, similar to the values presented in recent cohort studies [49, 619]. Thus, early evidence overestimated the duration of persistence, which was reflected in the initial recommendation by several health agencies to practice six months of abstinence or condom-protected sexual intercourse.

7.2.3 The implication and interpretation of the work on estimating the future risk of Zika virus in Managua, Nicaragua

In Chapter 6, I showed that people of young reproductive age (15–29 year) are more likely to be affected by a next ZIKV outbreak, because of an uneven distribution of protective antibodies across the different ages, which has important public health implications. In this age group most births occur and thus the risk of adverse congenital outcomes is highest. An extension of the herd immunity by vaccination could potentially reduce the risk of future outbreaks and thus prevent adverse congenital outcomes. The later vaccination is introduced, the less effect on herd immunity it has.

An implication of the finding that in the next decade a new large outbreak is unlikely is that it allows time to prepare for it. Vaccination should be introduced within the next ten years to prevent losing the benefit vaccine-induced immunity has on extending the herd immunity. Our model can be refined and applied in other contexts. The conclusion that herd immunity protects people from a next outbreak in the near future, seems to hold true in the Pacific, where large outbreaks after the 2013 outbreaks have not been observed [61]. If acquired immunity against reinfection with ZIKV is not lifelong, the risk of new outbreaks will rise quicker than anticipated. Understanding whether immunity wears off and at which speed is crucial for more accurate risk prediction and public health planning.

7.3 Strengths and limitations of the work presented

7.3.1 Strengths

One of the overall strengths of this thesis is the application of a variety of epidemiological methods to combine imperfect data into coherent narratives, from which we distil relevant public health messages. Similar to how Wade Hampton Frost described the purpose of epidemiology over a century ago, as quoted at the beginning of this Chapter [723]. I combine data with systematic review methodology in conceptual frameworks and mathematical modelling, allowing inference from many lines of evidence [125].

We are the first to formally provide insight into how evidence on a causal question accumulates in an outbreak context (Chapter 2). Meta-research, the study of research

itself, is a young field [724] especially in the context of disease outbreaks. Meta-research has established its value and place in science by its contribution to investigating problems such as the replication crisis [725]. We are also the first to transform a systematic review into a living systematic review in this context. I highlight the benefits and provide a solution to systematic reviews in rapidly emerging fields that become out-dated within months.

I managed to capture and describe the uncertainty in the data throughout the different chapters. In the quantification of the strength of the association between ZIKV and adverse outcomes (Chapter 4), I take into account and describe the heterogeneity and some of its potential sources. In the work on the sexual transmission of ZIKV I comprehensively compared the evidence on the duration of infectiousness and its uncertainty of which its conclusions directly contributed to inform guidelines (Chapter 8.1). In the prediction of the future risk of ZIKV outbreaks (Chapter 6), I considered the uncertainty around parameters and propagate the uncertainty throughout the simulations. The findings have an important public health message and call to action. There, I ensured reproducibility by sharing the model code.

7.3.2 Limitations

Concluding causality from observational data, poses the risk of incorrect conclusions due to uncontrolled unidentified confounding [726]. Conclusions here were reached by group consensus, which might not be the appropriate since groups of people can be biased by pre-existing beliefs [727]. People tend to prefer information that is consistent with a hypothesis rather than information which opposes it [728]. Much research was designed to corroborate the hypothesis that ZIKV was indeed a cause of adverse outcomes increasing the risk of confirmation and publication bias. I did not quantify the publication bias. Where one could argue that research is about challenging hypotheses, or falsifiability and not verifiability [729]. ZIKV as a cause of adverse outcomes is neither sufficient nor necessary [103]. ZIKV is a component cause for adverse outcomes and the effects are not unique to the cause. We merely skimmed the surface of logical reasoning and its application to causality.

Despite the elegance of the living systematic review, if one is conducted in a vacuum and not for public health guidance, the effort remains purely academic, detached from public health needs. Updates of the living review on the sexual transmission are unlikely to cause updates of the guidance, where ideally this should be the case [136]. I defined stopping criteria based on the period of funding, where an endpoint based on the results or certainty of results might be more appropriate. The speed of the living systematic reviews during the first year of conduct was low, because we started with a delay of over a year. It took nearly two years to catch up with the evidence. The dissemination of evidence was also delayed due to lags within the author team and external factors such as institutional clearance procedures.

In the studies presented in this thesis, we could not formally quantify several uncertainties. Diagnosing ZIKV is challenging and diagnostic uncertainty is currently inherent to the disease [730]. In the different studies, we did not incorporate this uncertainty in our analysis, but only discussed its effect on our conclusions. A formal assessment of the effect of the diagnostic uncertainty would increase the reliability of the results and increase the confidence intervals around the estimates to properly reflect the overall uncertainty. Similarly, not all uncertainty could be quantified in the work on the sexual transmission of ZIKV. We conclude that the risk of sexual transmission is 'likely a small risk'. Furthermore, the duration of infectiousness could not be measured directly, and we had to rely on data that suffers from bias. This uncertainty is also propagated in the guidelines on the prevention of sexual transmission

of ZIKV (Chapter 8.1), there is a discrepancy between the strength of evidence and the strength of recommendation. The GRADE score of the evidence is low to very low, where some recommendations are strong. Due to the nature of the evidence as illustrate above, these situations are common in guideline development of emerging outbreaks [731]. A last important source of uncertainty is around the generalizability of the modelling results (Chapter 6). It is unclear how well the conclusions translate to other regions, since there is strong heterogeneity in the distribution of vectors in the real world [732]. Updating our estimates as soon as more data becomes available will improve risk estimates and predictions.

7.4 Perspective and follow-up questions

By conducting the work presented in this thesis, I have identified several gaps in ZIKV research and disease outbreak research in general. Some of these questions have been previously identified in the WHO agenda on ZIKV research, but have remained unanswered [95]. The points below originate from the weaknesses identified in the work presented in this thesis or built upon ideas that arose while conducting the work.

7.4.1 Delay in accumulation of evidence and causal research

Where in this thesis I only explored the accumulation of evidence during the ZIKV outbreak, the conclusions need to be validated using other emerging causal questions. We need to increase the understanding of what causes the variation in the time between the introduction of ZIKV and the publication of reports on adverse outcomes. By identifying these barriers, we might be able to overcome these in future outbreaks or in emerging causal questions, and thereby accelerate the speed at which evidence becomes available.

By distinguishing different stages in emerging questions, we can improve the understanding of the role of evidence available at each point in time. Early on in an outbreak, we set out to discover and hypothesize new associations. Here case reports and case series play a pivotal role [132]. We then continue to look for explanations and try to refute the hypothesis. As time passes and evidence from a wide variety of sources and study designs accumulates, hypotheses become theory and evidence can be implemented into guidance. At this stage, properly conducted cohort studies or case-control studies are a valuable source of evidence to inform guidance and help understand causal relations [726]. Having infrastructure in place, such as dormant cohorts that can be activated and rapidly start collecting data once an outbreak occurs. However, these cohorts need to be funded and maintained for outbreaks of which we do not know where and whether they will occur in the future. Modelling studies can help identify regions at risk and guide planning.

We need to be able to quantify and communicate the uncertainty resulting from conclusions drawn from imperfect data. Quantifying and signalling uncertainty could be performed using semi-quantitative terminology such as used by the GRADE community (very low, low, medium, high certainty). In pharmacovigilance, standardized language on the certainty of the causal relation between a drug and adverse outcomes is used; the causal relation is described as unlikely, possible, probable or definite. However, this quantification is largely subjective and issues with reproducibility remain [733]. Standardized objective methodology would be of great value, but of great difficulty as well. In the context of living systematic reviews, these might serve as a stopping criterion; we would conduct and maintain a living systematic review until our research question has been answered with a pre-specified certainty.

The evidence presented in Chapter 3 and 4 has not answered the role of co-factors or confounders on the causal relation between ZIKV infection and adverse outcomes. The distribution of these factors might partially explain the observed heterogeneity between the different observational studies. Several factors have been hypothesized to play a role in the risk of ZIKV exposure and the subsequent risk of adverse outcomes, such as differences in genetic makeup of individuals in a population [734], cross-reactivity because of previous exposure to arboviruses [73] and variation in vector competence [735]. However, many of these factors have not been measured in a reliable and systematic way.

Living reviews and living guidelines form an opportunity for fields where evidence is emerging. However, many challenges need to be solved. Conducting and maintaining living systematic reviews requires resources. A solution could be that living systematic reviews form part of the outbreak response and are maintained by a community to guarantee continuity and feasibility. Conducting living systematic reviews in the public domain and sharing data, protocols and results increases transparency. An implementation of living systematic reviews within the guideline development pipeline seems a logical next step [136]. However, these efforts might be costly and time-consuming. It can also be difficult to communicate and disseminate a message that changes too often over time.

7.4.2 ZIKV and public health

In my thesis I identified several topics within the public health domain in which we need to increase our understanding or take action:

1) We need to increase our understanding of the duration of immunity. Early evidence indicates that ZIKV-specific antibodies might decline over time [68] and immunity might not be lifelong, which implies that a next outbreak can occur quicker than expected. The 2015–2017 outbreak has affected many people. Up to two-thirds of populations in the Americas may now have antibodies against ZIKV. Although this might protect individuals and populations alike against reinfection in the coming decade, much is unknown about the longer term. Thus, investigating and understanding the duration of immunity in populations that have been in contact with ZIKV is essential. Cohorts from the Pacific region, Asia, or Africa, might shine light on the issue in the near future. The outbreak in the Americas is still too recent to expect conclusive results from re-sampling these populations.

2) We need to move the development of vaccines forward. With the work presented in Chapter 6, we showed that vaccination of 15-year-old girls could form an attractive intervention; introduction of vaccination within the next decade has the potential to extend the herd immunity for the entire population. Shoukat et al. (2018) have shown the economic feasibility of such an intervention [736]. With a later introduction of vaccination, the capacity to extend the effect of the herd immunity is lost.

3) We need to document the complete set of sequelae caused by ZIKV infection. To get a full overview of the burden of the disease, follow-up of children affected by ZIKV is necessary. The true impact of ZIKV infection lies in infants born with congenital abnormalities from mothers infected with ZIKV. Extending the work on the strength of association with birth outcome, with outcomes associated with early childhood, will provide more insight into the true burden and costs of the disease. Fortunately, several cohort studies have continued to follow-up infants born from infected mothers [737, 738].

4) We need to increase surveillance of adverse outcomes. The detection of adverse outcomes has played a large role in identifying the disease. However, non of these adverse outcomes is

unique. We often had limited knowledge of baseline incidence of these adverse outcomes, making it difficult to establish whether an increased prevalence meant indeed a detection of disease, or simply an increased surveillance effort. For early detection, symptomatic surveillance on adverse outcomes might be feasible. However, research is needed to determine the capacity to detect a signal in different settings. This will help us to understand the true impact of ZIKV, also in endemic regions such as Asia and Africa.

5) We need to look at ZIKV from a social perspective. In the studies presented in this thesis I focus on ZIKV infections, its transmission and the different adverse outcomes it causes. I do not take into account risk factors that result from the social and environmental context of disease occurrence. From a public health perspective, we cannot ignore the 'causes' of the 'causes' [739]: Underlying causes such as a low socio-economic position that help explain why some individuals or populations are affected by disease and some are not. Similarly, the capacity to respond and counter outbreaks depends on the wealth and public health capacity of a region. Social determinants play an important role in disease emergence [740]. Thus, we should not ignore these societal causes. Infectious diseases remain too often the diseases of the poor [741]. If the objective of public health is to improve the overall health of a population, reducing inequalities is possibly the most important intervention [739]. I believe that throughout the Zika virus outbreak response, this theme has been largely neglected. More emphasis and identification of these causes of causes will help target underlying problems.

7.4.3 The effect of diagnostic uncertainty

Throughout the work, I have discussed the diagnostic uncertainty and the potential bias introduced by not knowing the exact exposure status of individuals. Capturing the diagnostic uncertainty in a formal way would be more appropriate. Since many studies assess exposure using different techniques and comparative studies assessing diagnostic performance are becoming available [707, 730], we could use this information, for example, in latent-class models [742]. It remains a challenge that diagnostic performance is dependent on other co-factors, such as timing of diagnosis and previous exposure to other flaviviruses. Ideally, one has access to individual based data, where exposure status has been measured using different techniques and relevant co-factors are collected as well. Stored samples can provide additional information, as diagnostic methods continue to improve and samples could thus be re-assessed to establish diagnostic test accuracies.

7.4.4 Better data and better data sharing

As Wade Hampton Frost already noted in 1918 that "statistics of disease have never before been possible on such a large scale. Their collection and tabulation, even if they do not lead to immediate results of value, will undoubtedly prove of great importance to students of later epidemics" [723]. The same holds true a century later: The capacity to collect data keeps increasing and properly collected data, if shared, will always be of great value to the scientific community.

The quality of epidemiological research is for a large part determined by the quality of the available data. The causal research would greatly benefit from data that is collected in a uniform and unbiased way. We need data on adverse outcomes as a result of ZIKV infection, where potential confounders are measured as well. Uniform, per protocol collection of these data, facilitates the joint analysis of individual patient data in an individual patient

data meta-analysis [743]. This would increase the power of the meta-analysis presented in Chapter 4 and allow the stratification and analysis of the effect of confounders. This way we can investigate the effect of – for example – previous exposure to other flaviviruses and socio-economic status. This would help explain the large heterogeneity we have seen between studies.

Likewise, for the prediction of future ZIKV risk (Chapter 5), one would ideally have access to representative data on the presence of antibodies against ZIKV in humans in different regions, allowing a more precise assessment of protective immunity throughout the population. This helps to identify populations that remain vulnerable to outbreaks, and helps model the risk of a new ZIKV outbreak on a larger scale, e.g. country- or even continent-wide. Resampling populations over time will increase our understanding of the duration of protective immunity. The loss of protective immunity, as early evidence might indicate [68], could increase the risk of new outbreaks.

Studies that assess the prevalence of ZIKV antibodies in humans can also serve to answer open questions about sexual transmission. Populations that consist predominantly of travellers, such as soldiers stationed in endemic regions who travel back and forth to non-endemic regions, can serve as a valuable source of information. Investigating them and their sexual partners can help quantify the risk of both symptomatic and asymptomatic sexual transmission. In an endemic setting household contacts have already been shown to be more likely symptomatic after sexual contact with seropositive household members [744]. It remains unclear what an appropriate measure of infectiousness is. Additional data from observational studies and *in vitro* studies are needed to provide further insight.

7.4.5 Future outbreaks

Implementing lessons learnt from previous outbreaks helps to improve the response in a next disease outbreak. The WHO has identified several diseases that pose a risk for future outbreaks or re-emergence. On this so-called blueprint disease list, 'disease X' is added to mark a next outbreak of an unknown or unexpected disease [745]. Disease X might cause adverse outcome Y, and thus the lessons learned from ZIKV causing adverse outcomes can be directly applied. The transmission route of disease X might be new or not well understood and the transmission framework could help to identify key parameters and identify research gaps. We can use the frameworks described in this thesis for the systematic collation of all available evidence. Living systematic reviews form a suitable instrument in the outbreak response toolbox when evidence accumulates rapidly. However, we still need a standardization of methods and critical assessment of its use, since living systematic reviews are more resource intensive than classic systematic reviews.

Every new outbreak increases the collective knowledge about how to respond to a next outbreak; the lessons learnt from Ebola have resulted in a more streamlined outbreak response by the WHO of which the ZIKV outbreak response benefited [746]. Although attempts have been made to rapidly share data through online repositories and websites [94, 183], this can be greatly improved during next outbreaks. Publishing a preprint of study results can reduce publications delays by a few months (Chapter 2). However, every outbreak poses its context-specific challenges, and tailoring the response to the unique setting will be necessary. Responding to disease outbreaks requires much more than the understanding the epidemiology of the disease and requires a transdisciplinary approach [747].

7.5 Conclusion

In this thesis, I established and used different frameworks and methods that helped to make sense of the limited evidence that is available during disease outbreaks. With the ZIKV epidemic, the virus has been introduced on the American continent and is likely there to stay. Due to the climate crisis, we are facing a future where our European temperate region also becomes more suitable for vectors and thus vector-borne disease such as ZIKV [748]. With the ZIKV epidemic on the wane, we now have time to consolidate findings and implement the lessons learnt. We need to be prepared for the re-emergence of ZIKV but also for the emergence of disease X. The tools and methods presented in this thesis help us to be more prepared for a next outbreak.

Chapter 8

Supplementary chapters

Chapters 8.1, 8.2 and 8.3 are publications to which I have contributed as a co-author and that provide additional context to the work presented in this thesis.

Chapter 8.1

WHO Guidelines on sexual transmission of ZIKV

This publication is published on the ***WHO website***: WHO reference number: WHO/RHR/19.4.

Contribution: I collected and synthesised the evidence, I presented the evidence to a WHO expert group, I contributed to writing of the draft of the guidelines, and to the writing of the report.

8.1.1 Introduction

Zika virus is an arthropod-borne flavivirus, which is transmitted primarily by mosquitoes of the *Aedes* genus, but can also be transmitted through sexual intercourse. In 2016, the World Health Organization (WHO) concluded that Zika virus infection during pregnancy is a cause of congenital abnormalities, including microcephaly. The proportion of affected neonates born to mothers infected with Zika virus during pregnancy has not been established with certainty. Published estimates range from 6% of infants born to women with and without symptoms of possible Zika virus infection in the United States of America (USA) to 42% of infants born to women with symptoms of skin rash in pregnancy in Brazil. WHO also concluded that Zika virus can trigger Guillain-Barre syndrome (GBS), an immune-mediated neurological condition. A multi-country assessment estimated that two of 10,000 Zika virus infections result in GBS (95% credible Interval: 0.5–4.5/10,000). Prevention of the sexual transmission of Zika virus can therefore prevent acute infection and neurological complications in a sexual partner, and prevention of transmission to a pregnant woman would prevent congenital Zika virus infection.

As of February 2018, 86 countries and territories have had evidence of Zika virus transmission and, as of January 2018, over 500,000 suspected cases had been reported in Latin America and the Caribbean. In the USA, as of 15 April 2018, 52 of 5,672 reported cases of Zika virus disease were presumed to have been acquired through sexual transmission. In the European Union and European Economic Area, as of 13 March 2017, 20 of 1,737 cases with a known route of transmission were acquired through sexual transmission.

Sexual transmission of Zika virus is much more likely from men to women than from women to men, and same-sex transmission, from man to man, has only been documented once. Where documented, the longest time period between the onset of symptoms in one sexual partner and the other is 44 days, with half of the sexual partners developing symptoms by 12 days. The longest time period for which infectious Zika virus has been detected by viral culture in semen is 69 days. However, Zika virus genetic material in semen has cleared within 50 days in most cases; it is not known whether genetic material detected for longer durations represents infectious virus.

Recommendations for the prevention of sexual transmission of Zika virus need to take into account the risk of ongoing mosquito-borne transmission of Zika virus in geographic areas. In areas with ongoing transmission, people are much more likely to become infected by Zika virus through bites from infected mosquitoes and the contribution of condom use to overall prevention of infection will be low. In areas with no autochthonous mosquito-borne Zika virus transmission, sexual transmission from returning travellers is one of the main routes of transmission. Travellers returning from areas with ongoing Zika virus transmission can therefore substantially reduce the risk of subsequent infections through the correct and consistent use of condoms. Areas with ongoing transmission are defined as regions with active circulation of mosquito-borne Zika virus. These are areas where disease surveillance detects circulation of Zika virus, in accordance with periodic epidemiological updates from WHO. In the absence of adequate disease surveillance, the definition of areas of ongoing transmission depends on the availability of local risk assessments. Adoption of the precautionary principle could result in designation of areas with known previous transmission as areas with ongoing transmission. Areas without ongoing transmission have no active circulation or suspected active circulation of Zika virus.

8.1.1.1 Rationale for the guidelines

WHO published interim guidelines on the prevention of sexual transmission of Zika virus in September 2016 [1], based on a limited amount of evidence under an emergency process during a public health emergency of international concern. The body of evidence has grown considerably since then and WHO experts concluded, at a meeting in March 2017, that the guidelines should be developed under the formal WHO guideline process [2].These guidelines contain updated recommendations on the prevention of sexual transmission of Zika virus, based on the best available evidence as of June 2018.

8.1.1.2 Rationale for the update of interim guidelines

At the time of issuance of the interim guidance, very few data on sexual transmission of Zika virus were available and recommendations were developed under emergency response procedures. In March 2017, WHO convened an expert meeting to review the evidence and identify the research gaps surrounding sexual transmission of Zika virus. At this meeting, participants discussed a conceptual framework. The sexual transmission framework describes key events in sexual transmission of Zika virus between humans, based on variables and time periods that apply to all infectious diseases.

8.1.1.3 What is new in this guideline?

- For the new recommended duration for correct and consistent use of condoms or abstinence to prevent sexual transmission of Zika virus, a distinction is made between men and women, and the recommended duration has been reduced from 6 to 3 months for men, 2 months for women.
- The risk groups women or couples planning to conceive or having sex that could result in conception and pregnant women, are more explicitly targeted in these new recommendations.
- For this guideline, systematic reviews were conducted to assess available evidence on the sexual transmission of Zika virus and all evidence on effectiveness of condom use to prevent sexual transmission of Zika virus.

8.1.1.4 Goal and objectives

The overall goal of these guidelines is to provide guidance and evidence-based recommendations about the prevention of sexual transmission of Zika virus. The absolute risks of different clinical complications of Zika virus are not fully known and the prevention measures may differ. Nevertheless, it is essential for individuals to have information about the risks of sexual intercourse as a mode of transmission in itself. These guidelines are informed by an update of the evidence underpinning the interim guidance and follow the requirements of the formal WHO guideline development process.The specific objectives are:

- to provide recommendations about the prevention of sexual transmission of Zika virus, rather than about the prevention of specific complications or about the prevention of mosquito-borne transmission;
- to update the interim guidelines in accordance with the formal WHO guidelines development process;

- to offer safe and effective options for the prevention of sexual transmission of Zika virus; and
- to provide evidence summaries about the risks of sexual transmission of Zika virus and the effectiveness of condoms for the prevention of sexual transmission of Zika virus.

8.1.1.5 Target audience

These guidelines aim to inform national and sub-national policy-makers, health care providers, other healthcare stakeholders and the general public.

8.1.2 Methods

These guidelines were developed as outlined in the second edition of the WHO handbook for guideline development [3]. Members of the guideline development group, which included experts in sexually transmitted infections, virology, epidemiology, gynaecology, condoms and sexual behaviour, developed key questions to guide the guideline development process. All members declared conflict of interests according to WHO procedures. For each key question, an evidence team from the University of Bern conducted systematic reviews, synthesized the retrieved evidence and assessed its certainty using the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) framework. The guideline development group, based on an evidence-to-decision framework, developed and finalized the recommendations and justifications during a web conference in May 2018 and through subsequent communication by email. Recommendations were formulated as "strong" or "conditional" using the evidence-to-decision framework. The strength of individual recommendations is indicated after the recommendation in parentheses.The quality of the body of evidence was assessed using the GRADE framework. After external review, these guidelines were published.

8.1.3 Recommendations

1. Recommendations for individuals living in areas with ongoing transmission of Zika virus

   (a) Recommendations for all sexually active women and men

      i. All women and men with Zika virus infection and their sexual partners, particularly pregnant women [4], should receive information about the risks of sexual transmission of Zika virus (strong recommendation, very low certainty of evidence).

      ii. All women and men should be offered a full range of contraceptives and be counselled to be able to make an informed choice about whether and when to prevent pregnancy in order to avoid possible adverse outcomes of Zika virus infection during pregnancy (strong recommendation, best practice recommendation).

      iii. Men should be informed about the possible risk of sexual transmission of Zika virus during the 3 months after known or presumptive infection.[1] Men should be informed about the correct and consistent use of condoms or abstinence during that time period to prevent Zika virus infection

through sexual transmission (conditional recommendation, low certainty of evidence).

iv. Women should be informed about the possible risk of sexual transmission of Zika virus during the 2 months after known or presumptive infection.[1] Women should be informed about the correct and consistent use of condoms or abstinence during that time period to prevent Zika virus infection through sexual transmission (conditional recommendation, very low certainty of evidence).

(b) Recommendations for women or couples planning to conceive or having sex that could result in conception

i. Women who have had sex that could result in conception and do not wish to become pregnant due to concerns about Zika virus infection should have ready access to emergency contraceptive services and counselling (best practice).

ii. Women should receive information about the possible risk of vertical transmission of Zika virus to the foetus. Women should avoid sex that could result in conception for 2 months after known or presumptive infection,[1] to ensure that a possible Zika virus infection has cleared before becoming pregnant (strong recommendation, very low certainty of evidence).

iii. Male sexual partners should receive information about the possible risk of sexual transmission of Zika virus during the 3 months after known or presumptive infection.1 Men should use condoms correctly and consistently or abstain from having sex for that time period to prevent Zika virus infection through sexual transmission (strong recommendation, low certainty of evidence).

iv. Taking into account current and projected local transmission rates[2] of Zika virus, women or couples planning to conceive should be informed about the option to delay conception until the risk of Zika virus infection in the local area has substantially decreased, in accordance with local risk assessment (conditional recommendation, very low certainty of evidence).

(c) Recommendations for pregnant women [4] and their sexual partners

i. Pregnant women and their sexual partners should use condoms correctly and consistently or abstain from sex for the whole duration of the pregnancy to prevent Zika virus infection through sexual transmission and possible adverse outcomes of Zika virus infection during pregnancy (strong recommendation, very low certainty of evidence).

2. Recommendations for individuals living in areas without ongoing transmission of Zika virus travelling to or from areas with ongoing Zika virus transmission

(a) Recommendations for all sexually active women and men returning from areas with ongoing Zika virus transmission

i. All women and men travelling to or returning from areas with ongoing Zika virus transmission,and their sexual partners, particularly pregnant women [4], should receive information about the risks of sexual transmission of Zika virus (strong recommendation, very low certainty of evidence).

ii. All women and men travelling to or returning from areas with ongoing transmission of Zika virus should be offered a full range of contraceptives and be counselled to be able to make an informed choice about whether and when to prevent pregnancy in order to avoid possible adverse outcomes of Zika virus infection during pregnancy (strong recommendation, very low certainty of evidence).

iii. Men returning from areas with ongoing Zika virus transmission and their sexual partners should use condoms correctly and consistently or abstain from sex for at least 3 months after the last possible exposure1to prevent Zika virus infection through sexual transmission (strong recommendation, low certainty of evidence).

iv. Women returning from areas with ongoing Zika virus transmission and their sexual partners should use condoms correctly and consistently or abstain from sex for at least 2 months after the last possible exposure[3] to prevent Zika virus infection through sexual transmission (strong recommendation, very low certainty of evidence).

(b) Recommendations for women or couples planning to conceive or having sex that could result in conception and returning from areas with ongoing Zika virus transmission

i. Women returning from areas with ongoing Zika virus transmission should avoid sex that could result in conception for at least 2 months after the last possible exposure[3] (strong recommendation, very low certainty of evidence).

ii. Male sexual partners returning from areas with ongoing Zika virus transmission should use condoms correctly and consistently or abstain from sex for at least 3 months after the last possible exposure1to prevent Zika virus infection through sexual transmission and reduce the risk of conception (strong recommendation, low certainty of evidence).

(c) Recommendations for pregnant women [4] and their sexual partners travelling to or returning from areas with ongoing Zika virus transmission

i. Pregnant women and their sexual partners should use condoms correctly and consistently or abstain from sex for the whole duration of the pregnancy if the sexual partner is returning from areas with ongoing Zika virus transmission. This recommendation aims to prevent Zika virus infection through sexual transmission and possible adverse pregnancy and foetal outcomes (strong recommendation, very low certainty of evidence).

ii. Pregnant women should consider delaying non-essential travel to areas with ongoing Zika virus transmission (conditional recommendation, very low certainty of evidence).

3. Recommendations about safer sex WHO always recommends the use of safer sexual practices. Safer sex is a behavioural concept that promotes the reduction of sexual risk-taking behaviour. It emphasizes measures to reduce the risk of contracting or spreading sexually transmitted infections (STIs), including postponing sexual debut, non-penetrative sex, correct and consistent use of male or female condoms, and reducing the number of sexual partners. Men and women should receive counselling, and be informed, about safer sex. Health authorities should ensure affordable and

equitable access to condoms and other contraception methods, especially in the context of Zika virus transmission and other STIs. The correct and consistent use of condoms reduces the risk of an unintended pregnancy as well as STIs, including the human immunodeficiency virus (HIV).

8.1.4 References

1. Prevention of sexual transmission of Zika virus: interim guidance update. Geneva: World Health Organization; 2016 (WHO/ZIKV/MOC/16.1 Rev.3).
2. Kim CR, Counotte M, Bernstein K, Deal C, Mayaud P, Low N et al. Investigating the sexual transmission of Zika virus. Lancet Glob Health. 2018;6(1):e24-e5.
3. WHO handbook for guideline development, second edition. Geneva: World Health Organization; 2014
4. Pregnancy management in the context of Zika virus infection. Geneva; World Health Organization; 2016 (WHO/ZIKV/MOC/16.2 Rev.1).
5. Laboratory testing for Zika virus infection: interim guidance. Geneva: World Health Organization; 2016 (WHO/ZIKV/LAB/16.1).

[1] After known or presumptive infection: after onset of symptoms compatible with Zika virus infection or, if asymptomatic, a positive test result for Zika virus. Most Zika virus infections are asymptomatic. Sexual transmission from a partner with asymptomatic Zika virus infection has been reported. Whether a person is infected or not may be hard to establish, given the low diagnostic accuracy of some available tests and the absence of resources for testing in some areas. Further guidance on the diagnosis of Zika virus infection can be found in reference [5].

[2] Local or projected transmission rates: in areas with high levels of current ongoing Zika virus transmission, delaying conception until the transmission rate decreases can reduce the risk of Zika virus infection during pregnancy.

[3] After the last possible exposure: after the last day of stay in an area with ongoing Zika virus transmission or the last day of sexual contact with a possibly Zika virus-infected person.

Chapter 8.2

Investigating the sexual transmission of ZIKV

Caron R Kim[1]
Michel Counotte[2]
Kyle Bernstein[3]
Carolyn Deal[4]
Philippe Mayaud[5]
Nicola Low[2]
Nathalie Broutet[1]

[1] Department of Reproductive Health and Research, World Health Organization, Geneva, Switzerland
[2] Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland
[3] Division of Sexually Transmitted Disease Prevention, Centers for Disease Control and Prevention, Atlanta, GA, USA
[4] National Institute of Allergy and Infectious Diseases, Rockville, MD, USA
[5] London School of Hygiene & Tropical Medicine, London, UK

This article is published in ***The Lancet Global Health***: Investigating the sexual transmission of Zika virus. (2018) DOI: 10.1016/S2214-109X(17)30419-9.

Contribution: I contributed to the conception and writing of the manuscript.

8.2.1 Investigating sexual transmission of ZIKV

The sexual transmissibility of Zika virus, a pathogen that is transmitted primarily by *Aedes* mosquitoes, has important implications [749], particularly for women because infection during pregnancy causes adverse pregnancy and fetal outcomes, including microcephaly [29]. WHO has included transmission through sexual intercourse and bodily fluids as a priority in its Zika Virus Research Agenda, which was a crucial component of the public health response to the 2015–16 Zika virus outbreak in South America. However, in the absence of methodologically rigorous population-based studies, the epidemiology of sexually transmitted Zika virus remains poorly understood. To help to understand and quantify aspects of sexual transmission, the WHO Zika Sexual Transmission Research Group developed a sexual transmission framework (appendix). The proposed framework describes seven variables and their inter-relationships: incubation period, serial interval, duration of infectiousness, probability of transmission per sex act, reproductive number, transmission rate through sexual contact, and susceptibility to Zika virus infection through sexual contact [750]. Through a combination of empirical research and modelling, this framework aims to determine the transmission dynamics of sexually transmitted Zika virus and thereby establish its epidemic potential.

To discuss the applicability of the framework and to address the dearth of data and research related to sexually transmissible Zika virus, a meeting of experts was convened in Geneva, Switzerland, on March 20–21, 2017. Experts in the fields of sexually transmitted infections, mathematical modelling, reproductive health, public health, and arboviral biology from public health and academic institutions reviewed the existing evidence about sexual transmission of Zika virus, identified critical research gaps, and discussed methods for investigation of sexual transmission. This Comment summarises the main findings of the meeting.

Evidence from epidemiological, biological, and animal studies was reviewed. First, a systematic review of 18 observational studies and case reports summarised evidence of sexual transmission of Zika virus in 27 sexual partnerships [568]. No studies of sexual transmission in endemic areas have been identified to date; the cases of sexual transmission were identified in sexual partners of travellers returning from areas affected by Zika virus. Second, a prospective cohort study in Puerto Rico [49] showed more frequent and longer persistence of Zika virus RNA in semen than in vaginal fluid when detected by quantitative reverse transcription PCR (qRT-PCR). Experimental studies in a mouse model have shown that the virus persists in the testis and can infect vaginal mucosa, yet only male-to-female, not female-to-male, sexual transmission has been documented in this model [592]. Third, animal studies have provided additional insights into the role of immunity and the correlation between the detection of Zika virus RNA through RT-PCR and infectiousness as determined by culture [592]. A review of the pathophysiology of the virus noted that the limited understanding of the identity of cellular receptors that mediate Zika virus entry might have implications for research on sexual transmissibility and diagnostics.

The Zika virus sexual transmission framework served as a springboard for discussion to highlight existing gaps in the evidence for sexual transmission and to identify research questions. Key questions include: how can episodes of sexual transmission be differentiated from vector transmission? Is RT-PCR positivity a predictor of infectiousness? Do coexisting sexually transmitted infections and HIV affect duration of viral persistence or the susceptibility to acquisition? Is there a difference between sexual and mosquito-borne acquisition of

infection regarding effects on fetal development? Furthermore, as viral persistence studies include mostly male participants, more data are needed to understand viral localisation and persistence in the female reproductive tract [751]. Investigation of these research questions is complicated by the asymptomatic nature of many Zika virus infections and the need for more accurate diagnostic tests.

Methodological approaches to address the research gaps were also discussed. In Zika-virus-endemic areas, studies of the risk of sexual transmission should require enrolment of couples who live, work, or travel in distinct geographical areas with or without risk of mosquitoborne Zika virus transmission (eg, in areas with the vector or at elevation and without the vector). Observational epidemiological studies should be conducted among discordant couples with Zika virus infection, household contacts of people with diagnosed Zika virus infection returning to areas where there is no mosquito-borne transmission of Zika virus, and groups at high risk of sexually transmitted infections and HIV. A working group has been established to develop a standardised protocol to address the methodological challenges of this issue that could be easily adapted and implemented should new epidemics of Zika virus arise. Particular attention will be given to methods for the valid and consistent collection of sensitive information about sexual practices between partners. Finally, experimental animal and basic science studies were also identified as essential to determine whether the presence of distinct genital mucosal receptors, viral RNA signatures, or immune responses correlates with the mode of transmission.

The expert group underlined the complementary roles of basic science, animal, epidemiological, and mathematical modelling studies. They also highlighted the importance of mobilising adequate funds to move this research agenda forward. A multidisciplinary research approach and adaptation of the sexual transmission framework will not only inform the current questions on Zika virus, but can serve as a template to study and to anticipate the sexual transmission of other emerging pathogens.

8.2.2 Supplementary Material

Supplementary material is available online in the published version of the manuscript http://doi.org/10.1016/S2214-109X(17)30419-9.

Chapter 8.3

Re-emerging and newly recognized sexually transmitted infections: Can prior experiences shed light on future identification and control?

Kyle Bernstein[1]
Virginia B. Bowen[1]
Caron R. Kim[2]
Michel J. Counotte[3]
Robert D. Kirkcaldy[1]
Edna Kara[2]
Gail Bolan[1]
Nicola Low[3]
Nathalie Broutet[2]

[1] Division of STD Prevention, Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America
[2] Department of Reproductive Health and Research, World Health Organization, Geneva, Switzerland
[3] Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland

This article is published in ***PLOS Medicine***: Re-emerging and newly recognized sexually transmitted infections: Can prior experiences shed light on future identification and control? (2017) DOI: 10.1371/journal.pmed.1002474

Contribution: I contributed to the paragraph on Zika virus infection, and the writing of the manuscript.

8.3.1 Summary points

- Determining sexual contact as a mode of pathogen transmission and quantifying the risk of sexual transmission pose epidemiologic challenges.
- Prior experiences with nontraditional sexually transmitted infections present valuable epidemiologic lessons, including comparisons of disease rates by sex, molecular analyses among sexually linked clusters, and methods to control for other potential modes of transmission.
- Applying lessons learned from prior infections might be critical for rapid and effective detection, prevention, and control of other reemerging and newly recognized sexually transmitted infections.

8.3.2 Introduction

The spectrum of pathogens that have a sexually transmitted component is broad. Globally, there are more than 30 recognized sexually transmitted infections (STIs), including those transmitted primarily by sexual contact and those that are sexually transmissible but whose primary mode of transmission is by food, vector, or droplet [752]. This latter group of nontraditional STIs poses unique methodologic and epidemiologic challenges for public health practitioners and researchers, who need to anticipate, identify, and contain the next new STI outbreak. In this paper, we explore these challenges using examples of nontraditional STIs, including 2 (shigellosis and *Neisseria meningitidis*) that have recently reemerged as sexually transmissible and 2 (Zika and Ebola) that are newly recognized as being sexually transmissible (Table 8.3.1).

8.3.3 *Shigella*: Raised male-to-female ratios in routine surveillance data

Shigellosis is a diarrheal illness caused by several species of the bacterium *Shigella*. *Shigella* is transmitted by direct or indirect contact with human feces, often via contaminated food, water, or fomites [753]. Prior to the 1970s, *Shigella* incidence was highest among children <5 years of age, their caretakers, and travelers to less developed countries. Recognition of *Shigella* as a potential STI began in the 1970s with outbreaks among men who have sex with men (MSM) in the US [754–757]. Sexual transmission of *Shigella* likely occurs during oral-anal sex (e.g., anilingus or rimming) or digital-anal sex (e.g., fisting) [758, 759]. During the 1970s and 1980s, *Shigella flexneri* rates increased in the US among adult males, even as overall rates and rates among children declined [760]. Routine case reports for *Shigella* do not include information about sexual practices, but the widening disparity between adult male and female case rates strongly suggested male-male sexual transmission. Increases in *Shigella* among men in the US and England between 2004 and 2015—despite declining or steady rates among women and children—support the reemergence of *Shigella* as an STI among MSM [761, 762]. *Shigella* strains among MSM have demonstrated increasing multidrug resistance over the past 5 years [763–770], and recent genomic analyses suggest international spread of an antimicrobial-resistant *S. flexneri* serotype among MSM [765].

Table 8.3.1: Modes of emergence and key characteristics of selected sexually transmitted infections.

	HIV	*N. meningitidis*	*Mycoplasma genitalium*	*Shigella* spp., *Entamoeba histolytica*, and *Giardia lamblia*	Zika virus	Ebola virus
Mode of emergence	New pathogen in humans, newly recognized disease	Newly discovered pathogen, known genital disease	Newly discovered pathogen, known genital disease	Recognized pathogen, extragenital site	Recognized pathogen, newly recognized mode of transmission	Recognized pathogen, newly recognized mode of transmission
Mode of discovery	New disease, AIDS, United States, 1981 [771]. HIV transmission began much earlier in subSaharan Africa but was unrecognized [772].	Isolation from men with urethritis, US, 1942 [773]	Isolation from men with nongonococcal urethritis, United Kingdom, 1981 [774]	Outbreaks in MSM, proctitis, US, 1960s and 1970s [754, 755, 775, 776]	Sexual partner in US of returning traveler from Senegal, 2011[44]	Tail end of epidemic, Liberia, 2015 [777]
Year pathogen first reported	1983 [777]	1942 [773]	1981 [774]	Early 20th century	1947 [778]	1977 [753]
Reservoir	Human	Human	Human	Environmental	Human	Zoonotic
Primary portal of entry/exit	Mucosal surfaces (genitourinary and anorectum); percutaneous	Mucosal surfaces (mouth and genitourinary)	Mucosal surfaces (genitourinary and anorectum)	Mucosal surfaces (mouth and anorectum)	Percutaneous	Mucosal surfaces
Primary mode of transmission	Direct contact (sexual intercourse), injection, and vertical transmission	Direct contact (sexual intercourse) and droplet transmission	Direct contact (sexual intercourse)	Ingestion	Vector-borne (mosquito)	Direct contact (touching)
Disease	HIV infection and AIDS	Nonspecific urethritis and invasive meningococcal disease	Nonspecific urethritis	Proctitis	Zika virus infection	Ebola virus disease
Type of disease	Systemic	Genital and systemic	Genital	Extragenital	Systemic	Systemic
Genital symptoms	No	Yes, when the infection is localized to the genital tract; no genital symptoms in the systemic (invasive) form of the disease	Yes, typical	Extragenital	Not usual; hematospermia reported	Not usual
Persistence in genital secretions	Lifelong	Not known	Not known; up to a year	Not present in genital secretions	Usually less than a year	Usually less than a year
Curable?	No	Yes	Yes	Yes	No	No

8.3.4 *N. meningitidis*: The role of dyad and cluster analyses

N. meningitidis, the bacterium that causes invasive meningococcal disease (IMD), spreads primarily by droplet transmission and infection of respiratory mucosa. Approximately 5%–10% of healthy adults are nasopharyngeal carriers. *N. meningitidis* has been isolated from men with urethritis [773]. A 1972 study described the transmission of *N. meningitidis* from a male chimpanzee's nasopharynx to his own urethra via oral-genital autoinoculation [779]. The authors concluded that *N. meningitidis* in the human urogenital tract might be the result of oral-genital sexual contact. Dyad and cluster analyses showing related strains of meningococci among partners epidemiologically linked by female-to-male oral sex strengthen the argument for sexual transmission leading to meningococcal urethritis [780, 781]. Recent molecular analyses suggest that *N. meningitidis* has genetically adapted to the urogenital tract [782]. Recent IMD outbreaks among MSM in Europe, Canada, and the US have also raised questions about the role that sexual networks play in *N. meningitidis* transmission [783–788]. Droplet transmission within MSM sexual networks could explain consistently higher *N. meningitidis* nasopharyngeal carriage rates relative to heterosexual men [789, 790]. It remains challenging, however, to determine whether the primary mode of transmission in IMD outbreaks among MSM is oral-genital contact, open-mouth kissing, or droplet transmission via "close contact," including the sharing of living and sleeping spaces.

8.3.5 Ebola virus: Viral persistence in semen and genetic epidemiology

The largest outbreak of Ebola virus disease started in December 2013 in West Africa and led to >28,000 confirmed cases and 11,310 deaths [791]. Almost all infections resulted from exposure to acutely symptomatic infected persons or recently deceased Ebola patients. Concern about possible sexual transmission of Ebola grew as the outbreak continued [792]. Anecdotal reports of new Ebola infections occurring among persons not in close proximity to a symptomatic or recently deceased person were followed by a report of a Liberian woman with Ebola, whose only possible source of infection was her husband, a convalescing Ebola survivor [793]. The husband had a positive PCR test for Ebola RNA in his semen 199 days after symptom onset, and the homology between genetic sequences of the Ebola RNA from the man and woman suggested that the only possible source of her infection was through sexual transmission [793, 794]. Ebola virus persistence in the semen of male survivors was documented in previous sporadic outbreaks [795, 796]. However, in the most recent West African outbreak, more robust systematic assessments found male survivors with Ebola virus RNA detected by PCR up to 565 days after symptom onset [796–798]. There is little evidence supporting viral persistence in other body fluids [792]. Female-to-male sexual transmission of Ebola is likely inefficient, but data are limited. Although the risk of transmission from semen exposure is considered small, the sheer number of male Ebola survivors raised concern about potential flare-ups and new clusters as the West African outbreak waned [799, 800]. Little is known about the public health impact of Ebola persistence among high-risk groups such as sex workers and MSM.

8.3.6 Zika virus: Infections in sexual partners of travelers returning from endemic areas

A large outbreak of Zika virus in Latin America and the Caribbean in 2015–2016 drew international attention because of its reported association with microcephaly. By 2017, 84 countries and territories had evidence of Zika transmission [292]. While the predominant mode of Zika transmission is through the bite of an infected Aedes spp. mosquito, sexual transmission was documented when a scientist returned to the US from Senegal in 2008 and transmitted Zika to his female sex partner who had not travelled [44]. Case reports from 13 countries have since described probable sexual transmission of Zika—via oral, anal, and vaginal sex—to partners of travelers returning from endemic areas. Suspected male-to-female sexual transmission was reported in 27 couples, while only 1 case of female-to-male and 1 case of male-to-male sexual transmission have been documented [568]. Most sexual transmission events occurred in symptomatic couples, but the timing of suspected transmission relative to symptom onset ranges greatly.

Persistent detection of Zika in genital fluids by reverse transcriptase (RT)-PCR and culture provides additional evidence for the biological plausibility of sexual transmission. In Puerto Rico, Zika was detected in the semen of 56% of convalescing men, with a median of 34 days between symptom onset and undetectable virus levels [49]. The maximum reported durations of RNA detection in genital fluids are as follows: 188 days in semen by RT-PCR [661], 69 days in semen by culture [621], 3 days in vaginal fluid in RT-PCR, and 11 days in cervical mucus by RT-PCR [665]. Investigation of Zika sexual transmission is complicated by several factors, including difficulty distinguishing vector and sexual transmission in endemic settings and difficulty obtaining viral cultures to confirm that viral persistence represents infectiousness. It is unclear whether controlling sexual transmission of Zika will contribute substantially to overall control of Zika epidemics; mathematical models estimate the population attributable risk of sexual transmission to be from 3% to 23% [168, 169]. Sustained sexual transmission of Zika is unlikely in a general population, but clusters may occur within high-risk sexual networks [749].

8.3.7 Lessons learned to inform future efforts

Lessons learned from the STIs discussed above can help the public health community identify newly emerging STIs and estimate the potential for an epidemic by sexual transmission. Sexual transmissibility of Zika and Ebola viruses was identified when infections were detected in persons who could only have been infected through sexual intercourse. In the case of meningococcal urethritis, detection of the pathogen in an unexpected anatomic niche and molecular linkage between index patients and their sexual partners established sexual transmissibility. Sexual transmission of *Shigella* was identified by attention to epidemiological changes in the affected populations. *M. genitalium*, another predominantly sexually transmitted pathogen, was discovered in 1981 by scientists searching for causes of nonspecific urethritis (Table 8.3.1) [774]. This discussion harkens back to the global HIV/AIDS epidemic. HIV crossed a species barrier and spread undetected as a new pathogen and STI among humans for years, before its identification as AIDS in MSM with unexplained immune suppression in San Francisco in 1981 [771, 772].

Now is the optimal time to establish methods to assess whether an infectious agent is sexually transmissible and prepare for a new STI with pandemic potential. New or

reemerging pathogens that are sexually transmissible will continue to arise. Since HIV was discovered, new molecular tools, such as phylogenetics and the omics, have become available to complement etiological epidemiological investigations of causal associations. For example, genotypic data were critical in linking sexual partners in the context of Ebola [794] and *Shigella* [765]. Standardized definitions and approaches to the investigation of sexual transmission of infectious agents and criteria for considering a pathogen to be sexually transmissible would greatly aid this effort. For example, is transmission by close, intimate contact (i.e., skin-to-skin) considered sexual transmission? Bradford Hill's classic viewpoints about causation could be adapted; for example, molecular concordance of strains between sexual partners might be a requirement for demonstrating specificity of association. The establishment of an objective criteria for determining the necessary components to consider a pathogen sexually transmitted could complement the existing framework developed by Hill. If sexual transmission is established, quantitative information about parameters such as incubation period, serial interval, transmission probability per coital act, and reproductive number will be needed for mathematical modelling studies of transmission dynamics, the proportion of cases attributable to sexual transmission, the potential for epidemic spread, and the effects of control measures. These quantities are particularly difficult to estimate when the agent has an alternative, predominant mode of transmission in endemic areas, e.g., mosquito-borne Zika. Developing criteria and methodologic approaches to sexual transmission could prove invaluable if they can be applied to key pathogens with epidemic potential, including those that the World Health Organization has published in its blueprint of action to prevent epidemics [61]. A proactive initiative to understand the potential for sexual transmission of such pathogens will help us to stay ahead of the curve.

8.3.8 Acknowledgements

Disclaimer: The views expressed in this article are those of the authors and do not necessarily represent the official positions of the World Health Organization (WHO), the University of Berne, or the Centers for Disease Control and Prevention (CDC).

List of abbreviations

CDC	Centers for Disease Control and Prevention
CHIKV	Chikungunya virus
CM	Congenital malformations
CNS	Central nerve system
DAG	Directional acyclic graphs
DENV	Dengue virus
ECDC	European Centre for Disease Prevention and Control
GBS	Guillain-Barré syndrome
GRADE	Grading of recommendations assessment, development and evaluation
Ig	Immunoglobulin
IMD	invasive meningococcal disease
ITP	Idiopathic thrombocytopenic purpura
LSR	Living systematic review
MC	Microcephaly
MERS	Middle East Respiratory Syndrome
MSM	Men who have sex with men
NHP	Non-human primate
OR	Odds ratio
PAHO	Pan American Health Organization
PHEIC	Public health emergency of international concern
RCT	Randomized controlled trial
RR	Risk ratio
RT-PCR	Reverse transcription polymerase chain reaction
SARS	Severe acute respiratory syndrome
TORCH	Toxoplasmosis, Others (including syphilis, listeriosis, varicella, parvovirus B19), Rubella, Cytomegalovirus (CMV), and Herpes simplex virus (HSV)
WHO	World Health Organization
WNV	West Nile virus
ZIKV	Zika virus

Bibliography

[1] Pan American Health Organization. PAHO - Cumulative Incidence; 2019. [Online]. Available from: https://www.paho.org/hq/index.php?option=com_content&view=article&id=12390:zika-cumulative-cases&Itemid=42090&lang=en.

[2] Moore SM, Oidtman RJ, Soda KJ, Siraj AS, Robert C, Barker CM, et al. Leveraging multiple data types to estimate the true size of the Zika epidemic in the Americas. medRxiv. 2019;doi:10.1101/19002865.

[3] World Health Organization. ZIKA EPIDEMIOLOGY UPDATE July 2019; 2019. [Online]. Available from: https://www.who.int/emergencies/diseases/zika/zika-epidemiology-update-july-2019.pdf?ua=1.

[4] Centro De Operações De Emergências Em Saúde Pública Sobre Microcefalias. Monitoramento dos Casos de Microcefalia no Brasil; 2015. [Online]. Available from: http://portalarquivos2.saude.gov.br/images/pdf/2016/fevereiro/17/coes-microcefalia-inf-epi-13-se06-2016.pdf.

[5] Morens DM, Folkers GK, Fauci AS. Emerging infections: a perpetual challenge. Lancet Infect Dis. 2008;8(11):710–719. doi:10.1016/S1473-3099(08)70256-1.

[6] Kazanjian P. Ebola in Antiquity? Clin Infect Dis. 2015;61(6):963–968. doi:10.1093/cid/civ418.

[7] Littman RJ. The plague of Athens: Epidemiology and paleopathology. Mt Sinai J Med. 2009;76(5):456–467. doi:10.1002/msj.20137.

[8] Papagrigorakis MJ, Yapijakis C, Synodinos PN, Baziotopoulou-Valavani E. DNA examination of ancient dental pulp incriminates typhoid fever as a probable cause of the Plague of Athens. Int J Infect Dis. 2006;10(3):206–214. doi:10.1016/j.ijid.2005.09.001.

[9] Morens DM, Littman RJ. Epidemiology of the Plague of Athens. Trans Am Philol Assoc. 1992;122:271. doi:10.2307/284374.

[10] Soupios MA. Impact of the plague in Ancient Greece. Infect Dis Clin North Am. 2004;18(1):45–51. doi:10.1016/S0891-5520(03)00101-6.

[11] Bianchine PJ, Russo TA. The Role of Epidemic Infectious Diseases in the Discovery of America. Allergy Asthma Proc. 1992;13(5):225–232. doi:10.2500/108854192778817040.

[12] Sanders J, Fuhrer G, Johnson M, Riddle M. The epidemiological transition: The current status of infectious diseases in the developed world versus the developing world. Sci Prog. 2008;91(1):1–38.

[13] Heymann DL. The international response to the outbreak of SARS in 2003. Philos Trans R Soc B Biol Sci. 2004;359(1447):1127–1129. doi:10.1098/rstb.2004.1484.

[14] Chen Y, Trovão NS, Wang G, Zhao W, He P, Zhou H, et al. Emergence and Evolution of Novel Reassortant Influenza A Viruses in Canines in Southern China. MBio. 2018;9(3). doi:10.1128/mbio.00909-18.

[15] Oboho IK, Tomczyk SM, Al-Asmari AM, Banjar AA, Al-Mugti H, Aloraini MS, et al. 2014 MERS-CoV Outbreak in Jeddah — A Link to Health Care Facilities. N Engl J Med. 2015;372(9):846–854. doi:10.1056/nejmoa1408636.

[16] Leroy EM, Epelboin A, Mondonge V, Pourrut X, Gonzalez JP, Muyembe-Tamfum JJ, et al. Human Ebola Outbreak Resulting from Direct Exposure to Fruit Bats in Luebo, Democratic Republic of Congo, 2007. Vector-Borne Zoonotic Dis. 2009;9(6):723–728. doi:10.1089/vbz.2008.0167.

[17] Smith RD. Responding to global infectious disease outbreaks: Lessons from SARS on the role of risk perception, communication and management. Soc Sci Med. 2006;63(12):3113–3123. doi:10.1016/j.socscimed.2006.08.004.

[18] World Health Organization. Managing Epidemics: Key facts about major deadly diseases; 2018. [Online]. Available from: https://www.who.int/emergencies/diseases/managing-epidemics-interactive.pdf.

[19] Sands P, Mundaca-Shah C, Dzau VJ. The neglected dimension of global security-a framework for countering infectious-disease crises. N Engl J Med. 2016;374(13):1281–1287. doi:10.1056/NEJMsr1600236.

[20] Dick GW, Kitchen SF HA. Zika virus. I. Isolations and serological specificity. Trans R Soc Trop Med Hyg. 1952;46(5):509–520.

[21] Dick GWA. Zika virus (II). Pathogenicity and physical properties. Trans R Soc Trop Med Hyg. 1952;46(5):521–534. doi:10.1016/0035-9203(52)90043-6.

[22] Hayes EB. Zika virus outside Africa. Emerg Infect Dis. 2009;15(9):1347–1350. doi:10.3201/eid1509.090442.

[23] Haddow AD, Schuh AJ, Yasuda CY, Kasper MR, Heang V, Huy R, et al. Genetic characterization of zika virus strains: Geographic expansion of the asian lineage. PLoS Negl Trop Dis. 2012;6(2):e1477. doi:10.1371/journal.pntd.0001477.

[24] Duffy MR, Chen TH, Hancock WT, Powers AM, Kool JL, Lanciotti RS, et al. Zika Virus Outbreak on Yap Island, Federated States of Micronesia. N Engl J Med. 2009;360(24):2536–2543. doi:10.1056/NEJMoa0805715.

[25] Aubry M, Teissier A, Huart M, Merceron S, Vanhomwegen J, Mapotoeke M, et al. Seroprevalence of dengue and chikungunya virus antibodies, French Polynesia, 2014–2015. Emerg Infect Dis. 2018;24(3):558–561. doi:10.3201/eid2403.171149.

[26] European Centre for Disease Prevention and Control. Rapid risk assessment: Zika virus infection outbreak, French Polynesia; 2014. [Online]. Available from: https://www.ecdc.europa.eu/en/publications-data/rapid-risk-assessment-zika-virus-infection-outbreak-french-polynesia.

[27] Dupont-Rouzeyrol M, Diancourt L, Calvez E, Vandenbogaert M, O'Connor O, Teissier A, et al. Zika virus evolution on the edges of the Pacific ocean. Emerg Microbes Infect. 2017;6(12):e111–e111. doi:10.1038/emi.2017.102.

[28] Hills SL, Fischer M, Petersen LR. Epidemiology of Zika Virus Infection. J Infect Dis. 2017;216(suppl_10):S868–S874. doi:10.1093/infdis/jix434.

[29] Krauer F, Riesen M, Reveiz L, Oladapo OT, Martínez-Vega R, Porgo TV, et al. Zika Virus Infection as a Cause of Congenital Brain Abnormalities and Guillain–Barré Syndrome: Systematic Review. PLoS Med. 2017;14(1):e1002203. doi:10.1371/journal.pmed.1002203.

[30] World Health Organization. WHO | Zika causality statement; 2016. [Online]. Available from: https://www.who.int/emergencies/zika-virus/situation-

report/31-march-2016/en/.

[31] Boyer S, Calvez E, Chouin-Carneiro T, Diallo D, Failloux AB. An overview of mosquito vectors of Zika virus. Microbes Infect. 2018;20(11-12):646–660. doi:10.1016/j.micinf.2018.01.006.

[32] World Health Organization. Zika virus vectors and risk of spread in the WHO European Region; 2016. [Online]. Available from: http://www.euro.who.int/__data/assets/pdf_file/0007/304459/WEB-news_competence-of-Aedes-aegypti-and-albopictus-vector-species.pdf.

[33] Liebman KA, Stoddard ST, Reiner RC, Perkins TA, Astete H, Sihuincha M, et al. Determinants of Heterogeneous Blood Feeding Patterns by Aedes aegypti in Iquitos, Peru. PLoS Negl Trop Dis. 2014;8(2):e2702. doi:10.1371/journal.pntd.0002702.

[34] Brown JE, Evans BR, Zheng W, Obas V, Barrera-Martinez L, Egizi A, et al. Human impacts have shaped historical and recent evolution in Aedes aegypti, the dengue and yellow fever mosquito. Evolution (N Y). 2014;68(2):514–525. doi:10.1111/evo.12281.

[35] Hill SC, Vasconcelos J, Neto Z, Jandondo D, Zé-Zé L, Aguiar RS, et al. Emergence of the Asian lineage of Zika virus in Angola: an outbreak investigation. Lancet Infect Dis. 2019;doi:10.1016/S1473-3099(19)30293-2.

[36] Althouse BM, Vasilakis N, Sall AA, Diallo M, Weaver SC, Hanley KA. Potential for Zika Virus to Establish a Sylvatic Transmission Cycle in the Americas. PLoS Negl Trop Dis. 2016;10(12):e0005055–e0005055. doi:10.1371/journal.pntd.0005055.

[37] Terzian ACB, Zini N, Sacchetto L, Rocha RF, Parra MCP, Del Sarto JL, et al. Evidence of natural Zika virus infection in neotropical non-human primates in Brazil. Sci Rep. 2018;8(1):16034. doi:10.1038/s41598-018-34423-6.

[38] Miner JJ, Diamond MS. Zika Virus Pathogenesis and Tissue Tropism. Cell Host Microbe. 2017;21(2):134–142. doi:10.1016/j.chom.2017.01.004.

[39] Calvet GA, Dos Santos FB, Sequeira PC. Zika virus infection: Epidemiology, clinical manifestations and diagnosis. Curr Opin Infect Dis. 2016;29(5):459–466. doi:10.1097/QCO.0000000000000301.

[40] Martines RB, Bhatnagar J, de Oliveira Ramos AM, Davi HPF, Iglezias SDA, Kanamura CT, et al. Pathology of congenital Zika syndrome in Brazil: a case series. Lancet. 2016;388(10047):898–904. doi:10.1016/S0140-6736(16)30883-2.

[41] Colt S, Garcia-Casal MN, Peña-Rosas JP, Finkelstein JL, Rayco-Solon P, Weise Prinzo ZC, et al. Transmission of Zika virus through breast milk and other breastfeeding-related bodily-fluids: A systematic review. PLoS Negl Trop Dis. 2017;11(4):e0005528. doi:10.1371/journal.pntd.0005528.

[42] Sampieri CL, Montero H. Breastfeeding in the time of Zika: a systematic literature review. Peer J. 2019; p. 7:e6452. doi:10.7717/peerj.6452.

[43] Grischott F, Puhan M, Hatz C, Schlagenhauf P. Non-vector-borne transmission of Zika virus: A systematic review. Travel Med Infect Dis. 2016;14(4):313–330. doi:10.1016/j.tmaid.2016.07.002.

[44] Foy BD, Kobylinski KC, Foy JLC, Blitvich BJ, da Rosa AT, Haddow AD, et al. Probable Non-Vector-borne Transmission of Zika Virus, Colorado, USA. Emerg Infect Dis. 2011;17(5):880–882. doi:10.3201/eid1705.101939.

[45] Centers for Disease Control and Prevention. Cumulative Zika Virus Disease Case Counts in the United States, 2015-2018; 2018. [Online]. Available from: https://www.cdc.gov/zika/reporting/case-counts.html.

[46] Spiteri G, Sudre B, Septfons A, Beauté J. Surveillance of zika virus infection in the EU/EEA, june 2015 to january 2017. Eurosurveillance. 2017;22(41). doi:10.2807/1560-7917.ES.2017.22.41.17-00254.

[47] Kim CR, Counotte M, Bernstein K, Deal C, Mayaud P, Low N, et al. Investigating the sexual transmission of Zika virus. Lancet Glob Heal. 2018;6(1):e24–e25. doi:10.1016/S2214-109X(17)30419-9.

[48] Morrison TE, Diamond MS. Animal Models of Zika Virus Infection, Pathogenesis, and Immunity. J Virol. 2017;91(8). doi:10.1128/jvi.00009-17.

[49] Paz-Bailey G, Rosenberg ES, Doyle K, Munoz-Jordan J, Santiago GA, Klein L, et al. Persistence of Zika Virus in Body Fluids — Final Report. N Engl J Med. 2017;379(13):1234–1243. doi:10.1056/nejmoa1613108.

[50] De Noronha L, Zanluca C, Burger M, Suzukawa AA, Azevedo M, Rebutini PZ, et al. Zika virus infection at different pregnancy stages: Anatomopathological findings, target cells and viral persistence in placental tissues. Front Microbiol. 2018;9(SEP):2266. doi:10.3389/fmicb.2018.02266.

[51] Done JT, Terlecki S, Richardson C, Harkness JW, Sands JJ, Patterson DS, et al. Bovine virus diarrhoea-mucosal disease virus: pathogenicity for the fetal calf following maternal infection. Vet Rec. 1980;106(23):473–479.

[52] Zhang Y, Jing J, Li X, Wang J, Feng X, Cao R, et al. Integration analysis of miRNA and mRNA expression profiles in swine testis cells infected with Japanese encephalitis virus. Infect Genet Evol. 2015;32:342–347. doi:10.1016/j.meegid.2015.03.037.

[53] Yuan L, Huang XY, Liu ZY, Zhang F, Zhu XL, Yu JY, et al. A single mutation in the prM protein of Zika virus contributes to fetal microcephaly. Science. 2017;358(6365):933–936. doi:10.1126/science.aam7120.

[54] Mitchell PK, Mier-Y-Teran-Romero L, Biggerstaff BJ, Delorey MJ, Aubry M, Cao-Lormeau VM, et al. Reassessing Serosurvey-Based Estimates of the Symptomatic Proportion of Zika Virus Infections. Am J Epidemiol. 2019;188(1):206–213. doi:10.1093/aje/kwy189.

[55] Lessler J, Ott CT, Carcelen AC, Konikoff JM, Williamson J, Bi Q, et al. Times to key events in Zika virus infection and implications for blood donation: A systematic review. Bull World Health Organ. 2016;94(11):841–849. doi:10.2471/BLT.16.174540.

[56] Paixão ES, Teixeira MG, Rodrigues LC. Zika, chikungunya and dengue: the causes and threats of new and re-emerging arboviral diseases. BMJ Glob Heal. 2018;3(Suppl 1). doi:10.1136/bmjgh-2017-000530.

[57] Lessler J, Chaisson LH, Kucirka LM, Bi Q, Grantz K, Salje H, et al. Assessing the global threat from Zika virus. Science. 2016;353(6300):aaf8160. doi:10.1126/science.aaf8160.

[58] Oehler E, Watrin L, Larre P, Lastère S, Valour F, Baudouin L, et al. Zika virus infection complicated by Guillain-Barré. Euro Surveill. 2014;1(December 2013):7–9.

[59] Coyne CB, Lazear HM. Zika virus-reigniting the TORCH. Nat Rev Microbiol. 2016;14(11):707–715. doi:10.1038/nrmicro.2016.125.

[60] Moore CA, Staples JE, Dobyns WB, Pessoa A, Ventura CV, Da Fonseca EB, et al. Characterizing the pattern of anomalies in congenital zika syndrome for pediatric clinicians. JAMA Pediatr. 2017;171(3):288–295. doi:10.1001/jamapediatrics.2016.3982.

[61] Musso D, Ko AI, Baud D. Zika Virus Infection — After the Pandemic. N Engl J Med. 2019;381(15):1444–1457. doi:10.1056/nejmra1808246.

[62] Vucic S, Kiernan MC, Cornblath DR. Guillain-Barré syndrome: An update. J Clin Neurosci. 2009;16(6):733–741. doi:10.1016/j.jocn.2008.08.033.

[63] Mier-y Teran-Romero L, Delorey MJ, Sejvar JJ, Johansson MA. Guillain-Barré syndrome risk among individuals infected with Zika virus: A multi-country assessment.

BMC Med. 2018;16(1):67. doi:10.1186/s12916-018-1052-4.

[64] Pierson TC, Graham BS. Zika Virus: Immunity and Vaccine Development. Cell. 2016;167(3):625–631. doi:10.1016/j.cell.2016.09.020.

[65] Pasquier C, Joguet G, Mengelle C, Chapuy-Regaud S, Pavili L, Prisant N, et al. Kinetics of anti-ZIKV antibodies after Zika infection using two commercial enzyme-linked immunoassays. Diagn Microbiol Infect Dis. 2018;doi:10.1016/j.diagmicrobio.2017.09.001.

[66] Theel ES, Jane Hata D. Diagnostic testing for Zika virus: A postoutbreak update. J Clin Microbiol. 2018;56(4). doi:10.1128/JCM.01972-17.

[67] Forshey BM, Stoddard ST, Morrison AC. Dengue Viruses and Lifelong Immunity: Reevaluating the Conventional Wisdom. J Infect Dis. 2016;214(7):979–981. doi:10.1093/infdis/jiw102.

[68] Henderson AD, Aubry M, Kama M, Vanhomwegen J, Teissier A, Mariteragi-Helle T, et al. Zika virus seroprevalence declines and neutralization antibodies wane in adults following outbreaks in French Polynesia and Fiji. bioRxiv. 2019; p. 578211. doi:10.1101/578211.

[69] Halstead SB. Biologic evidence required for zika disease enhancement by dengue antibodies. Emerg Infect Dis. 2017;doi:10.3201/eid2304.161879.

[70] George J, Valiant WG, Mattapallil MJ, Walker M, Huang YJS, Vanlandingham DL, et al. Prior Exposure to Zika Virus Significantly Enhances Peak Dengue-2 Viremia in Rhesus Macaques. Sci Rep. 2017;doi:10.1038/s41598-017-10901-1.

[71] Gordon A, Gresh L, Ojeda S, Katzelnick LC, Sanchez N, Mercado JC, et al. Prior dengue virus infection and risk of Zika: A pediatric cohort in Nicaragua. PLoS Med. 2019;doi:10.1371/journal.pmed.1002726.

[72] Langerak T, Mumtaz N, Tolk VI, Van Gorp ECM, Martina BE, Rockx B, et al.. The possible role of cross-reactive dengue virus antibodies in Zika virus pathogenesis; 2019.

[73] Priyamvada L, Hudson W, Ahmed R, Wrammert J. Humoral cross-reactivity between Zika and dengue viruses: implications for protection and pathology. Emerg Microbes Infect. 2017;6(5):e33. doi:10.1038/emi.2017.42.

[74] Rabe IB, Staples JE, Villanueva J, Hummel KB, Johnson JA, Rose L, et al. Interim guidance for interpretation of Zika virus antibody test results. Morb Mortal Wkly Rep. 2016;65(21):543–546. doi:10.15585/mmwr.mm6521e1.

[75] Centers for Disease Control and Prevention. Updated Guidance for US Laboratories Testing for Zika Virus Infection; 2017. [Online]. Available from: https://www.cdc.gov/zika/pdfs/laboratory-guidance-zika.pdf.

[76] Fischer C, Pedroso C, Mendrone A, de Filippis AMB, Vallinoto ACR, Morais Ribeiro B, et al. External quality assessment for Zika virus molecular diagnostic testing, Brazil. Emerg Infect Dis. 2018;24(5):888–892. doi:10.3201/eid2405.171747.

[77] Atkinson B, Hearn P, Afrough B, Lumley S, Carter D, Aarons EJ, et al. Detection of Zika Virus in Semen. Emerg Infect Dis. 2016;22(5):940–940. doi:10.3201/eid2205.160107.

[78] Javed F, Manzoor KN, Ali M, Haq IU, Khan AA, Zaib A, et al. Zika virus: what we need to know? J Basic Microbiol. 2018;58(1):3–16. doi:10.1002/jobm.201700398.

[79] Flores HA, O'Neill SL. Controlling vector-borne diseases by releasing modified mosquitoes. Nat Rev Microbiol. 2018;16(8):508–518. doi:10.1038/s41579-018-0025-0.

[80] Nazni WA, Hoffmann AA, NoorAfizah A, Cheong YL, Mancini MV, Golding N, et al. Establishment of Wolbachia Strain wAlbB in Malaysian Populations of Aedes aegypti for Dengue Control. Curr Biol. 2019;29(24):4241–4248.e5. doi:10.1016/j.cub.2019.11.007.

[81] Anders KL, Indriani C, Ahmad RA, Tantowijoyo W, Arguni E, Andari B, et al. The AWED trial (Applying Wolbachia to Eliminate Dengue) to assess the efficacy of Wolbachia-infected mosquito deployments to reduce dengue incidence in Yogyakarta, Indonesia: Study protocol for a cluster randomised controlled trial. Trials. 2018;19(1). doi:10.1186/s13063-018-2670-z.

[82] Carvalho DO, McKemey AR, Garziera L, Lacroix R, Donnelly CA, Alphey L, et al. Suppression of a field population of Aedes aegypti in Brazil by sustained release of transgenic male mosquitoes. PLoS Negl Trop Dis. 2015;9(7). doi:10.1371/journal.pntd.0003864.

[83] Evans BR, Kotsakiozi P, Costa-da Silva AL, Ioshino RS, Garziera L, Pedrosa MC, et al. Transgenic Aedes aegypti Mosquitoes Transfer Genes into a Natural Population. Sci Rep. 2019;9(1):13047. doi:10.1038/s41598-019-49660-6.

[84] Achee NL, Gould F, Perkins TA, Reiner RC, Morrison AC, Ritchie SA, et al. A Critical Assessment of Vector Control for Dengue Prevention. PLoS Negl Trop Dis. 2015;9(5):e0003655. doi:10.1371/journal.pntd.0003655.

[85] Moyes CL, Vontas J, Martins AJ, Ng LC, Koou SY, Dusfour I, et al. Contemporary status of insecticide resistance in the major Aedes vectors of arboviruses infecting humans. PLoS Negl Trop Dis. 2017;11(7). doi:10.1371/journal.pntd.0005625.

[86] Gaudinski MR, Houser KV, Morabito KM, Hu Z, Yamshchikov G, Rothwell RS, et al. Safety, tolerability, and immunogenicity of two Zika virus DNA vaccine candidates in healthy adults: randomised, open-label, phase 1 clinical trials. Lancet. 2018;391(10120):552–562. doi:10.1016/S0140-6736(17)33105-7.

[87] Modjarrad K, Lin L, George SL, Stephenson KE, Eckels KH, De La Barrera RA, et al. Preliminary aggregate safety and immunogenicity results from three trials of a purified inactivated Zika virus vaccine candidate: phase 1, randomised, double-blind, placebo-controlled clinical trials. Lancet. 2018;391(10120):563–571. doi:10.1016/S0140-6736(17)33106-9.

[88] National Institute of Allergy and Infectious Diseases. ClinicalTrials.gov - VRC 705: A Zika Virus DNA Vaccine in Healthy Adults and Adolescents (DNA); 2019. [Online]. Available from: https://clinicaltrials.gov/ct2/show/NCT03110770.

[89] Poland GA, Kennedy RB, Ovsyannikova IG, Palacios R, Ho PL, Kalil J. Development of vaccines against Zika virus. Lancet Infect Dis. 2018;18(7):e211–e219. doi:10.1016/S1473-3099(18)30063-X.

[90] Diamond MS, Ledgerwood JE, Pierson TC. Zika Virus Vaccine Development: Progress in the Face of New Challenges. Annu Rev Med. 2018;70(1):121–135. doi:10.1146/annurev-med-040717-051127.

[91] Halstead SB. Licensed dengue vaccine: Public health conundrum and scientific challenge. Am J Trop Med Hyg. 2016;95(4):741–745. doi:10.4269/ajtmh.16-0222.

[92] Hadinegoro SR, Arredondo-García JL, Capeding MR, Deseda C, Chotpitayasunondh T, Dietze R, et al. Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. N Engl J Med. 2015;373(13):1195–1206. doi:10.1056/NEJMoa1506223.

[93] Brazillian Ministry of Health. Portaria n° 1.813, de 11 de Novembro de 2015. Declara Emergência em Saúde Pública de importância Nacional (ESPIN) por alteração do padrão de ocorrência de microcefalias no Brasil, Brazilian Ministry of Health, Brazil (2016); 2015. [Online]. Available from: http://bvsms.saude.gov.br/bvs/saudelegis/gm/2015/prt1813_11_11_2015.html.

[94] World Health Organization. WHO statement on the first meeting of the International Health Regulations (2005) (IHR 2005) Emergency Committee on Zika virus and observed increase in neurological disorders and neonatal

malformations. WHO. 2016;.

[95] World Health Organization. WHO Zika virus research agenda; 2016. [Online]. Available from: https://www.who.int/reproductivehealth/zika/zika-virus-research-agenda/en/.

[96] World Health Organization. Laboratory testing for Zika virus infection – Interim guidance; 2016. [Online]. Available from: https://www.who.int/csr/resources/publications/zika/laboratory-testing/en/.

[97] World Health Organization. Pregnancy management in the context of Zika virus infection – Interim guidance; 2016. [Online]. Available from: https://www.who.int/csr/resources/publications/zika/pregnancy-management/en/.

[98] Polen KD, Gilboa SM, Hills S, Oduyebo T, Kohl KS, Brooks JT, et al. Update: Interim guidance for preconception counseling and prevention of sexual transmission of zika virus for men with possible zika virus exposure — United States, August 2018. Morb Mortal Wkly Rep. 2018;67(31):868–871. doi:10.15585/mmwr.mm6731e2.

[99] World Health Organization. Vector control operations framework for Zika virus – Operations framework; 2016. [Online]. Available from: https://www.who.int/csr/resources/publications/zika/vector-control/en/.

[100] Centers for Disease Control and Prevention. Mosquito Control; 2017. [Online]. Available from: https://www.cdc.gov/zika/vector/index.html.

[101] World Health Organization. Publications, technical guidance on Zika virus; 2019. [Online]. Available from: https://www.who.int/csr/resources/publications/zika/en/.

[102] Centers for Disease Control and Prevention. Sexual Transmission and Prevention; 2019. [Online]. Available from: https://www.cdc.gov/zika/prevention/sexual-transmission-prevention.html.

[103] Lyons J, Ward B. Causal Inference. Kenneth Rothman; 2017. Available from: https://www.taylorfrancis.com/books/9781134881819/chapters/10.4324/9781315542287-6.

[104] Aristotle. Aristotle on Causality: The Four Causes. Stanford Encyclopedia of Philosophy. 2015; p. 1–3.

[105] Mill JS. A system of logic, ratiocinative and inductive : being a connected view of the principles of evidence and the methods of scientific investigation / by John Stuart Mill. No. v. 1 in A System of Logic, Ratiocinative and Inductive: Being a Connected View of the Principles of Evidence and the Methods of Scientific Investigation. John W. Parker; 2011. Available from: https://books.google.ch/books?id=y4MEAAAAQAAJ.

[106] Maclure M. Popperian refutation in epidemiology. Am J Epidemiol. 1985;121(3):343–350. doi:10.1093/oxfordjournals.aje.a114005.

[107] Karl R Popper. Science as Falsification. In: Conjectures and Refutations; 1963.

[108] Henle J, Marchand F. Klassiker der Medizin 3: Von den Miasmen und Kontagien und von den miasmatisch-kontagiösen Krankheiten (1840). Klassiker der Medizin ; Bd. 3. Leipzig: J. A. Barth; 1910.

[109] Keim A, Lumet L. Louis Pasteur. New York: A. Stokes; 1914. Available from: http://catalog.hathitrust.org/api/volumes/oclc/715525.html.

[110] Brody H, Rip MR, Vinten-Johansen P, Paneth N, Rachman S. Map-making and myth-making in Broad Street: The London cholera epidemic, 1854. Lancet. 2000;356(9223):64–68. doi:10.1016/S0140-6736(00)02442-9.

[111] Carter KC. The Koch-Pasteur dispute on establishing the cause of anthrax. Bull Hist Med. 1988;62(1):42–57.

[112] Koch R. Die Ätiologie der Milzbrand-Krankheit, begründet auf die Entwicklungsgeschichte des Bacillus Anthracis (1876). Robert Koch-Institut; 2018.

[113] Rothman KJ, Greenland S. Causation and causal inference in epidemiology. Am J Public Health. 2005;95(SUPPL. 1):S144–50. doi:10.2105/AJPH.2004.059204.

[114] Krieger N. Epidemiology and the web of causation: Has anyone seen the spider? Soc Sci Med. 1994;39(7):887–903. doi:10.1016/0277-9536(94)90202-X.

[115] Lenhard J. Models and statistical inference: The controversy between Fisher and Neyman-Pearson. Br J Philos Sci. 2006;57(1):69–91. doi:10.1093/bjps/axi152.

[116] Beebee H, Hitchcock C, Menzies P. The Oxford Handbook of Causation (Oxford Handbooks). Oxford University Press; 2012. Available from: http://www.amazon.com/The-Oxford-Handbook-Causation-Handbooks/dp/0199642583.

[117] Liebetrau A. Correlation and Causation. Meas Assoc. 2012;20(7):89–89. doi:10.4135/9781412984942.n7.

[118] Hall NS. R. A. Fisher and his advocacy of randomization. J Hist Biol. 2007;40(2):295–325. doi:10.1007/s10739-006-9119-z.

[119] Vandenbroucke JP. Is 'the causes of cancer' a miasma theory for the end of the twentieth century? Int J Epidemiol. 1988;17(4):708–709. doi:10.1093/ije/17.4.708.

[120] García-Cortés M, Stephens C, Lucena MI, Fernández-Castañer A, Andrade RJ. Causality assessment methods in drug induced liver injury: Strengths and weaknesses. J Hepatol. 2011;55(3):683–691. doi:10.1016/j.jhep.2011.02.007.

[121] Blackburn H, Labarthe D. Stories from the evolution of guidelines for causal inference in epidemiologic associations: 1953-1965. Am J Epidemiol. 2012;176(12):1071–1077. doi:10.1093/aje/kws374.

[122] United States Surgeon General's Advisory Committee on Smoking and Health. Smoking and Health, Report of the Advisory Committee to the Surgeon General of the Public Health Service. U.S. Department of Health, Education, and Welfare, Public Health Service; 1964. Available from: https://profiles.nlm.nih.gov/NN/B/B/M/Q/{_}/nnbbmq.pdf.

[123] Hill A. The Environment and Disease: Association or causation? Proc R Soc Med. 1965;58(58):295–300.

[124] Krieger N, Smith GD. The tale wagged by the DAG: Broadening the scope of causal inference and explanation for epidemiology. Int J Epidemiol. 2016;45(6):1787–1808. doi:10.1093/ije/dyw114.

[125] Munafò MR, Davey Smith G. Repeating experiments is not enough. Nature. 2018;553(7689):399–401. doi:10.1038/d41586-018-01023-3.

[126] Rosenbaum PR. Observation and experiment : an introduction to causal inference. Cambridge, Massachusetts : Harvard University Press, 2017.; 2017.

[127] Grimes DA, Schulz KF. Bias and causal associations in observational research. Lancet. 2002;359(9302):248–252. doi:10.1016/S0140-6736(02)07451-2.

[128] Rothman KJ, Greenland S, Lash TL. Modern Epidemiology. Wolters Kluwer Health/Lippincott Williams & Wilkins; 2008. Available from: https://books.google.ch/books?id=Z3vjT9ALxHUC.

[129] Greenland S, Morgenstern H. Confounding in Health Research. Annu Rev Public Health. 2001;22(1):189–212. doi:10.1146/annurev.publhealth.22.1.189.

[130] Newhouse JP, McClellan M. ECONOMETRICS IN OUTCOMES RESEARCH: The Use of Instrumental Variables. Annu Rev Public Health. 1998;19(1):17–34. doi:10.1146/annurev.publhealth.19.1.17.

[131] Agostino RBD. Tutorial in biostatistics: propensity score methods for bias reduction in the comparison of a treatment to a non-randomized control group. Stat Med. 1998;2281:2265–2281.

[132] Vandenbroucke JP. In defense of case reports and case series. Ann Intern Med. 2001;134(4):330–334. doi:10.7326/0003-4819-134-4-200102200-00017.

[133] Vandenbroucke JP. Observational research, randomised trials, and two views of medical science. PLoS Med. 2008;5(3):0339–0343. doi:10.1371/journal.pmed.0050067.

[134] Moher D, Liberati A, Tetzlaff J, Altman DG, Altman D, Antes G, et al. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement (Chinese edition). J Chinese Integr Med. 2009;7(9):889–896. doi:10.3736/jcim20090918.

[135] Egger M, Smith GD, Altman DG. Systematic Reviews in Health Care. Meta-analysis in Context. Second Edition. John Wiley & Sons; 2008.

[136] Elliott JH, Turner T, Clavisi O, Thomas J, Higgins JPT, Mavergames C, et al. Living Systematic Reviews: An Emerging Opportunity to Narrow the Evidence-Practice Gap. PLoS Med. 2014;11(2):e1001603. doi:10.1371/journal.pmed.1001603.

[137] Thomas J, Noel-Storr A, Marshall I, Wallace B, McDonald S, Mavergames C, et al. Living systematic reviews: 2. Combining human and machine effort. J Clin Epidemiol. 2017;91:31–37. doi:10.1016/j.jclinepi.2017.08.011.

[138] Khamis AM, Kahale LA, Pardo-Hernandez H, Schünemann HJ, Akl EA. Methods of conduct and reporting of living systematic reviews: A protocol for a living methodological survey [version 1; peer review: 2 approved]. F1000Research. 2019;doi:10.12688/f1000research.18005.1.

[139] Wallace BC, Noel-Storr A, Marshall IJ, Cohen AM, Smalheiser NR, Thomas J. Identifying reports of randomized controlled trials (RCTs) via a hybrid machine learning and crowdsourcing approach. J Am Med Informatics Assoc. 2017;24(6):1165–1168. doi:10.1093/jamia/ocx053.

[140] Van Noorden R. Trouble at the text mine. Nature. 2012;483(7388):134–135. doi:10.1038/483134a.

[141] Marshall IJ, Kuiper J, Wallace BC. RobotReviewer: Evaluation of a system for automatically assessing bias in clinical trials. J Am Med Informatics Assoc. 2016;23(1):193–201. doi:10.1093/jamia/ocv044.

[142] Higgins JPT, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ. 2011;343(7829):d5928. doi:10.1136/bmj.d5928.

[143] Hultcrantz M, Rind D, Akl EA, Treweek S, Mustafa RA, Iorio A, et al. The GRADE Working Group clarifies the construct of certainty of evidence. J Clin Epidemiol. 2017;87:4–13. doi:10.1016/j.jclinepi.2017.05.006.

[144] Glass GV. Primary, Secondary, and Meta-Analysis of Research. Educ Res. 2007;5(10):3–8. doi:10.3102/0013189x005010003.

[145] Deeks JJ, Higgins JP, Altman DG. Chapter 10: Analysing data and undertaking meta-analyses. In: Cochrane Handb. Syst. Rev. Interv.. version 6 ed.; 2019. p. Chapter 10.

[146] Higgins JPT, Thompson SG, Spiegelhalter DJ. A re-evaluation of random-effects meta-analysis. J R Stat Soc Ser A Stat Soc. 2009;172(1):137–159. doi:10.1111/j.1467-985X.2008.00552.x.

[147] Higgins JPT, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med. 2002;21(11):1539–1558. doi:10.1002/sim.1186.

[148] Thompson SG, Higgins JPT. How should meta-regression analyses be undertaken and interpreted? Stat Med. 2002;21(11):1559–1573. doi:10.1002/sim.1187.

[149] Alonso-Coello P, Schünemann HJ, Moberg J, Brignardello-Petersen R, Akl EA, Davoli M, et al. GRADE Evidence to Decision (EtD) frameworks: a systematic and transparent approach to making well informed healthcare choices. 1: Introduction. Gac Sanit. 2018;32(2):166.e1–166.e10. doi:10.1016/j.gaceta.2017.02.010.

[150] Norris SL, Sawin VI, Ferri M, Sastre LR, Porgo TV. An evaluation of emergency guidelines issued by the World Health Organization in response to four infectious disease outbreaks. PLoS One. 2018;13(5). doi:10.1371/journal.pone.0198125.

[151] Brauer F. Compartmental Models in Epidemiology. In: Mathematical Epidemiology. SpringerLink; 2008. p. 19–79.

[152] Allen LJS, Burgin AM. Comparison of deterministic and stochastic SIS and SIR models in discrete time. Math Biosci. 2000;163(1):1–33. doi:10.1016/S0025-5564(99)00047-4.

[153] Porgo TV, Norris SL, Salanti G, Johnson LF, Simpson JA, Low N, et al. The use of mathematical modeling studies for evidence synthesis and guideline development: A glossary. Res Synth Methods. 2019;10(1):125–133. doi:10.1002/jrsm.1333.

[154] Dietz K. The estimation of the basic reproduction number for infectious diseases. Stat Methods Med Res. 1993;2(1):23–41. doi:10.1177/096228029300200103.

[155] Heesterbeek JAP. A brief history of R0 and a recipe for its calculation. Acta Biotheor. 2002;50(3):189–204. doi:10.1023/A:1016599411804.

[156] Dietz K. Transmission and control of arbovirus diseases. Epidemiology. 1975;104:104–121.

[157] Grassly NC, Fraser C. Mathematical models of infectious disease transmission. Nat Rev Microbiol. 2008;6(6):477–487. doi:10.1038/nrmicro1845.

[158] Wildner M. In memory of William of Occam. Lancet. 1999;354(9196):2172. doi:10.1016/S0140-6736(05)77085-9.

[159] Seino T. Understanding the Role of Assumptions in Mathematical Modeling : Analysis of Lessons with Emphasis on ' the awareness of assumptions ' The Construction of the Lesson Based on ' the awareness of assumptions '. Build Connect Theory, Res Pract. 2005; p. 664–671.

[160] Smith DL, Battle KE, Hay SI, Barker CM, Scott TW, McKenzie FE. Ross, Macdonald, and a theory for the dynamics and control of mosquito-transmitted pathogens. PLoS Pathog. 2012;8(4). doi:10.1371/journal.ppat.1002588.

[161] Funk S, Kucharski AJ, Camacho A, Eggo RM, Yakob L, Murray LM, et al. Comparative Analysis of Dengue and Zika Outbreaks Reveals Differences by Setting and Virus. PLoS Negl Trop Dis. 2016;10(12):e0005173. doi:10.1371/journal.pntd.0005173.

[162] Pandey A, Mubayi A, Medlock J. Comparing vector-host and SIR models for dengue transmission. Math Biosci. 2013;246(2):252–259. doi:10.1016/j.mbs.2013.10.007.

[163] Champagne C, Salthouse DG, Paul R, Cao-Lormeau VM, Roche B, Cazelles B. Structure in the variability of the basic reproductive number (R0) for Zika epidemics in the Pacific islands. Elife. 2016;5(NOVEMBER2016). doi:10.7554/eLife.19874.

[164] Kucharski AJ, Funk S, Eggo RM, Mallet HP, Edmunds WJ, Nilles EJ. Transmission Dynamics of Zika Virus in Island Populations: A Modelling Analysis of the 2013–14 French Polynesia Outbreak. PLoS Negl Trop Dis.

2016;10(5):e0004726. doi:10.1371/journal.pntd.0004726.

[165] Nishiura H, Kinoshita R, Mizumoto K, Yasuda Y, Nah K. Transmission potential of Zika virus infection in the South Pacific. Int J Infect Dis. 2016;45:95–97. doi:10.1016/j.ijid.2016.02.017.

[166] Riou J, Poletto C, Boëlle PY. A comparative analysis of Chikungunya and Zika transmission. Epidemics. 2017;19:43–52. doi:10.1016/j.epidem.2017.01.001.

[167] Zhang Q, Sun K, Chinazzi M, Pastore Y Piontti A, Dean NE, Rojas DP, et al. Spread of Zika virus in the Americas. Proc Natl Acad Sci U S A. 2017;114(22):E4334–E4343. doi:10.1073/pnas.1620161114.

[168] Gao D, Lou Y, He D, Porco TC, Kuang Y, Chowell G, et al. Prevention and Control of Zika as a Mosquito-Borne and Sexually Transmitted Disease: A Mathematical Modeling Analysis. Sci Rep. 2016;6:28070. doi:10.1038/srep28070.

[169] Towers S, Brauer F, Castillo-Chavez C, Falconar AKI, Mubayi A, Romero-Vivas CME. Estimate of the reproduction number of the 2015 Zika virus outbreak in Barranquilla, Colombia, and estimation of the relative role of sexual transmission. Epidemics. 2016;17:50–55. doi:10.1016/j.epidem.2016.10.003.

[170] Moghadas SM, Shoukat A, Espindola AL, Pereira RS, Abdirizak F, Laskowski M, et al. Asymptomatic Transmission and the Dynamics of Zika Infection. Sci Rep. 2017;7(1):5829. doi:10.1038/s41598-017-05013-9.

[171] Netto EM, Moreira-Soto A, Pedroso C, Höser C, Funk S, Kucharski AJ, et al. High Zika Virus Seroprevalence in Salvador, Northeastern Brazil Limits the Potential for Further Outbreaks. MBio. 2017;8(6). doi:10.1128/mbio.01390-17.

[172] Lourenço J, Maia de Lima M, Faria NR, Walker A, Kraemer MU, Villabona-Arenas CJ, et al. Epidemiological and ecological determinants of Zika virus transmission in an urban setting. eLife. 2017;6(February 2015):1–25. doi:10.7554/eLife.29820.

[173] Ferguson NM, Cucunubá ZM, Dorigatti I, Nedjati-Gilani GL, Donnelly CA, Basáñez MG, et al. Countering the Zika epidemic in Latin America. Science. 2016;353(6297):353–354. doi:10.1126/science.aag0219.

[174] Ioos S, Mallet HP, Leparc Goffart I, Gauthier V, Cardoso T, Herida M. Current Zika virus epidemiology and recent epidemics. Med Mal Infect. 2014;44(7):302–307. doi:10.1016/j.medmal.2014.04.008.

[175] Pan American Health Organization. Epidemiological Alert - Neurological syndrome, congenital malformations, and Zika virus infection. Implications for public health in the Americas; 2015. [Online]. Available from: https://www.paho.org/hq/dmdocuments/2015/2015-dec-1-cha-epi-alert-zika-neuro-syndrome.pdf.

[176] Cao-Lormeau VM, Blake A, Mons S, Lastère S, Roche C, Vanhomwegen J, et al. Guillain-Barré Syndrome outbreak associated with Zika virus infection in French Polynesia: A case-control study. Lancet. 2016;387(10027):1531–1539. doi:10.1016/S0140-6736(16)00562-6.

[177] Counotte MJ, Egli-Gany D, Riesen M, Abraha M, Porgo TV, Wang J, et al. Zika virus infection as a cause of congenital brain abnormalities and Guillain-Barré syndrome: From systematic review to living systematic review. F1000Research. 2018;7(196):196. doi:10.12688/f1000research.13704.1.

[178] Counotte MJ, Meili KW, Taghavi K, Calvet G, Sejvar J, Low N. Zika virus infection as a cause of congenital brain abnormalities and Guillain-Barré syndrome: A living systematic review [version 1; peer review: awaiting peer review]. F1000Research. 2019;8(1433). doi:10.12688/f1000research.19918.1.

[179] Pan American Health Organization. Timeline of Emergence of Zika virus in the Americas; 2016. [Online]. Available from: https://www.paho.org/hq/index.php?option=com_content&view=article&id=11959:timeline-of-emergence-of-zika-virus-in-the-americas&Itemid=41711&lang=en.

[180] Musso D, Bossin H, Mallet HP, Besnard M, Broult J, Baudouin L, et al. Zika virus in French Polynesia 2013–14: anatomy of a completed outbreak. Lancet Infect Dis. 2018;doi:10.1016/S1473-3099(17)30446-2.

[181] Johansson MA, Reich NG, Meyers LA, Lipsitch M. Preprints: An underutilized mechanism to accelerate outbreak science. PLoS Med. 2018;15(4). doi:10.1371/journal.pmed.1002549.

[182] Zambrana JV, Bustos Carrillo F, Burger-Calderon R, Collado D, Sanchez N, Ojeda S, et al. Seroprevalence, risk factor, and spatial analyses of Zika virus infection after the 2016 epidemic in Managua, Nicaragua. Proc Natl Acad Sci. 2018;115(37):9294–9299. doi:10.1073/pnas.1804672115.

[183] Dye C, Bartolomeos K, Moorthy V, Kieny MP. Data sharing in public health emergencies: A call to researchers. Bull World Health Organ. 2016;94(3):158. doi:10.2471/BLT.16.170860.

[184] Abdill RJ, Blekhman R. Tracking the popularity and outcomes of all bioRxiv preprints. Elife. 2019;8. doi:10.7554/eLife.45133.

[185] McMichael C. Climate change-related migration and infectious disease. Virulence. 2015;6(6):548–553. doi:10.1080/21505594.2015.1021539.

[186] Cauchemez S, Besnard M, Bompard P, Dub T, Guillemette-Artur P, Eyrolle-Guignot D, et al. Association Between Zika Virus and Microcephaly in French Polynesia, 2013–2015. Obstet Gynecol Surv. 2016;71(9):512–514. doi:10.1097/01.ogx.0000491260.72445.aa.

[187] Broutet N, Krauer F, Riesen M, Khalakdina A, Almiron M, Aldighieri S, et al. Zika Virus as a Cause of Neurologic Disorders. N Engl J Med. 2016;374(16):1506–9. doi:10.1056/NEJMp1602708.

[188] Rasmussen SA, Jamieson DJ, Honein MA, Petersen LR. Zika Virus and Birth Defects — Reviewing the Evidence for Causality. N Engl J Med. 2016;374(20):1981–1987. doi:10.1056/NEJMsr1604338.

[189] Chammard TB, Schepers K, Breurec S, Messiaen T, Destrem AL, Mahevas M, et al. Severe thrombocytopenia after zika virus infection, guadeloupe, 2016. Emerg Infect Dis. 2017;23(4):696–698. doi:10.3201/eid2304.161967.

[190] Chraïbi S, Najioullah F, Bourdin C, Pegliasco J, Deligny C, Résière D, et al. Two cases of thrombocytopenic purpura at onset of Zika virus infection. J Clin Virol. 2016;83(34):61–62. doi:10.1016/j.jcv.2016.08.299.

[191] de Laval F, Matheus S, Maquart M, Yvrard E, Barthes N, Combes C, et al. Prospective Zika virus disease cohort: systematic screening. Lancet. 2016;388(10047):868. doi:10.1016/s0140-6736(16)31429-5.

[192] Zea-Vera AF, Parra B. Zika virus (ZIKV) infection related with immune thrombocytopenic purpura (ITP) exacerbation and antinuclear antibody positivity. Lupus. 2017;26(8):890–892. doi:10.1177/0961203316671816.

[193] Adams L, Bello-Pagan M, Lozier M, Ryff KR, Espinet C, Torres J, et al. Update: Ongoing Zika Virus Transmission — Puerto Rico, November 1, 2015–July 7, 2016. MMWR Morb Mortal Wkly Rep. 2016;65(30):774–779. doi:10.15585/mmwr.mm6530e1.

[194] Bhadelia N. Prospective cohort study of pregnant Brazilian women elucidates link between Zika virus infection and fetal abnormalities. Evid Based Med. 2016;21(5):193–193. doi:10.1136/ebmed-2016-110476.

[195] Bhatnagar J, Rabeneck DB, Martines RB, Reagan-Steiner S, Ermias Y, Estetter LBC, et al. Zika virus RNA replication and

persistence in brain and placental tissue. Emerg Infect Dis. 2017;23(3):405–414. doi:10.3201/eid2303.161499.

[196] Rubin R. A young woman contemplating travel abroad: What she needs to know about Zika virus infection. Consultant. 2016;56(11):1024–1025. doi:10.1056/NEJMoa1602412.

[197] Campos AGdM, Lira RPC, Faria e Arantes TE. Optical coherence tomography of macular atrophy associated with microcephaly and presumed intrauterine Zika virus infection. Arq Bras Oftalmol. 2016;79(6):400–401. doi:10.5935/0004-2749.20160112.

[198] Carvalho FHC, Cordeiro KM, Peixoto AB, Tonni G, Moron AF, Feitosa FEL, et al. Associated ultrasonographic findings in fetuses with microcephaly because of suspected Zika virus (ZIKV) infection during pregnancy. Prenat Diagn. 2016;36(9):882–887. doi:10.1002/pd.4882.

[199] de Araújo TVB, Rodrigues LC, de Alencar Ximenes RA, de Barros Miranda-Filho D, Montarroyos UR, de Melo APL, 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(12):1356–1363. doi:10.1016/S1473-3099(16)30318-8.

[200] De Paiva Cavalcante T, De Campos JJG, De Freire MRM, Ferreira FG, Barboza GH, De Ferreira TLR, et al. 25th Congress of the European Society for Pediatric Neurosurgery (ESPN) Paris-France, 8-11 May 2016. Child's Nerv Syst. 2016;32(5):905–1012. doi:10.1007/s00381-016-3044-z.

[201] de Paula Freitas B, Ko AI, Khouri R, Mayoral M, Henriques DF, Maia M, et al. Glaucoma and Congenital Zika Syndrome. Ophthalmology. 2017;124(3):407–408. doi:10.1016/j.ophtha.2016.10.004.

[202] França GVA, Schuler-Faccini L, Oliveira WK, Henriques CMP, Carmo EH, Pedi VD, et al. Congenital Zika virus syndrome in Brazil: a case series of the first 1501 livebirths with complete investigation. Lancet. 2016;388(10047):891–897. doi:10.1016/S0140-6736(16)30902-3.

[203] Herrera K, Bernasko J, Garry D, Vahanian S, Kaplan C. Vertical transmission of Zika virus (ZIKV) in early pregnancy: two cases, two different courses. Case Reports Perinat Med. 2016;5(2):131–133. doi:10.1515/crpm-2016-0027.

[204] Honein MA, Dawson AL, Petersen EE, Jones AM, Lee EH, Yazdy MM, et al. Birth defects among fetuses and infants of US women with evidence of possible zika virus infection during pregnancy. JAMA - J Am Med Assoc. 2017;317(1):59–68. doi:10.1001/jama.2016.19006.

[205] Custódio João E, Da Silveira Gouvea MIF, De Lourdes Benamor Teixeira M, Mendes-Silva W, Silva Esteves J, Motta Santos E, et al. Zika virus infection associated with congenital birth defects in a HIV-infected pregnant woman. Pediatr Infect Dis J. 2016;36(5):500–501. doi:10.1097/INF.0000000000001482.

[206] Kumar M, Ching L, Astern J, Lim E, Stokes AJ, Melish M, et al. Prevalence of Antibodies to Zika Virus in Mothers from Hawaii Who Delivered Babies with and without Microcephaly between 2009-2012. PLoS Negl Trop Dis. 2016;10(12):e0005262. doi:10.1371/journal.pntd.0005262.

[207] Leal MdC, Muniz LF, Caldas Neto SdS, van der Linden V, Ramos RCF. Sensorineural hearing loss in a case of congenital Zika virus. Braz J Otorhinolaryngol. 2016;0:0. doi:10.1016/j.bjorl.2016.06.001.

[208] Leal MC, Muniz LF, Ferreira TSA, Santos CM, Almeida LC, Van Der Linden V, et al. Hearing Loss in Infants with Microcephaly and Evidence of Congenital Zika Virus Infection — Brazil, November 2015–May 2016. MMWR Morb Mortal Wkly Rep. 2016;65(34):917–919. doi:10.15585/mmwr.mm6534e3.

[209] Meaney-Delman D, Oduyebo T, Polen KND, White JL, Bingham AM, Slavinski SA, et al. Prolonged Detection of Zika Virus RNA in Pregnant Women. Obstet Gynecol. 2016;128(4):724–730. doi:10.1097/AOG.0000000000001625.

[210] De Oliveira Melo AS, Aguiar RS, Amorim MMR, Arruda MB, De Oliveira Melo F, Ribeiro STC, et al. Congenital Zika virus infection: Beyond neonatal microcephaly. JAMA Neurol. 2016;73(12):1407–1416. doi:10.1001/jamaneurol.2016.3720.

[211] da Silva AAM, Ganz JSS, Sousa PdS, Doriqui MJR, Ribeiro MRC, Branco MdRFC, et al. Early growth and neurologic outcomes of infants with probable congenital Zika virus syndrome. Emerg Infect Dis. 2016;22(11):1953–1956. doi:10.3201/eid2211.160956.

[212] Pomar L, Malinger G, Benoist G, Carles G, Ville Y, Rousset D, et al. Association between Zika virus and fetopathy: a prospective cohort study in French Guiana. Ultrasound Obstet Gynecol. 2017;49(6):729–736. doi:10.1002/uog.17404.

[213] Schaub B, Monthieux A, Najioullah F, Adenet C, Muller F, Césaire R. Persistent maternal Zika viremia: a marker of fetal infection. Ultrasound Obstet Gynecol. 2017;49(5):658–660. doi:10.1002/uog.17210.

[214] Soares de Souza A, Moraes Dias C, Braga FDCB, Terzian ACB, Estofolete CF, Oliani AH, et al. Fetal Infection by Zika Virus in the Third Trimester: Report of 2 Cases. Clin Infect Dis. 2016;63(12):1622–1625. doi:10.1093/cid/ciw613.

[215] Suy A, Sulleiro E, Rodó C, Vázquez É, Bocanegra C, Molina I, et al. Prolonged Zika Virus Viremia during Pregnancy. N Engl J Med. 2016;375(26):2611–2613. doi:10.1056/NEJMc1607580.

[216] de Araújo TVB, Rodrigues LC, de Alencar Ximenes RA, de Barros Miranda-Filho D, Montarroyos UR, de Melo APL, 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(12):1356–1363. doi:10.1016/S1473-3099(16)30318-8.

[217] van der Eijk AA, van Genderen PJ, Verdijk RM, Reusken CB, Mögling R, van Kampen JJA, et al. Miscarriage Associated with Zika Virus Infection. N Engl J Med. 2016;375(10):1002–1004. doi:10.1056/nejmc1605898.

[218] Van Der Linden V, Filho ELR, Lins OG, Van Der Linden A, Aragão MDFVV, Brainer-Lima AM, et al. Congenital Zika syndrome with arthrogryposis: Retrospective case series study. BMJ. 2016;354(8):i3899. doi:10.1136/bmj.i3899.

[219] van der Linden V, Pessoa A, Dobyns W, Barkovich AJ, Júnior HvdL, Filho ELR, et al. Description of 13 Infants Born During October 2015–January 2016 With Congenital Zika Virus Infection Without Microcephaly at Birth — Brazil. MMWR Morb Mortal Wkly Rep. 2016;65(47):1343–1348. doi:10.15585/mmwr.mm6547e2.

[220] Vargas A, Saad E, Dimech GS, Santos RH, Sivini MAVC, Albuquerque LC, et al. Características dos primeiros casos de microcefalia possivelmente relacionados ao vírus Zika notificados na Região Metropolitana de Recife, Pernambuco. Epidemiol e Serviços Saúde. 2016;25(4):691–700. doi:10.5123/S1679-49742016000400003.

[221] Ventura CV, Fernandez MP, Gonzalez IA, Rivera-Hernandez DM, Lopez-Alberola R, Peinado M, et al. First Travel-Associated Congenital Zika Syndrome in the US: Ocular and Neurological Findings in the Absence of Microcephaly. Ophthalmic Surgery, Lasers Imaging Retin. 2016;47(10):952–955. doi:10.3928/23258160-20161004-09.

[222] Villamil-Gómez WE, Rodríguez-Morales AJ, Uribe-García AM, González-Arismendy E, Castellanos JE, Calvo EP, et al. Zika, dengue, and chikungunya co-infection in a pregnant woman from Colombia. Int J Infect Dis. 2016;51:135–138. doi:10.1016/j.ijid.2016.07.017.

[223] Werner H, Fazecas T, Guedes B, Lopes Dos Santos J, Daltro P, Tonni G, et al. Intrauterine Zika virus infection and microcephaly: Correlation of perinatal imaging and three-dimensional virtual physical models. Ultrasound Obstet Gynecol. 2016;47(5):657–660. doi:10.1002/uog.15901.

[224] Cuevas EL, Tong VT, Rozo N, Valencia D, Pacheco O, Gilboa SM, et al. Preliminary Report of Microcephaly Potentially Associated with Zika Virus Infection During Pregnancy — Colombia, January–November 2016. MMWR Morb Mortal Wkly Rep. 2016;65(49):1409–1413. doi:10.15585/mmwr.mm6549e1.

[225] Sanin-Blair J, Cumplido A, Gutierrez-Marin J, Campo-Campo M, Garcia-Posada R, Londono A, et al. Fetal ultrasound findings in zika infection. Preliminary report in a Colombian selected population. J Matern Neonatal Med. 2016;29(2):163–164.

[226] de Magalhães-Barbosa MC, Prata-Barbosa A, Robaina JR, Raymundo CE, Lima-Setta F, da Cunha AJLA. Trends of the microcephaly and Zika virus outbreak in Brazil, January–July 2016. Travel Med Infect Dis. 2016;14(5):458–463. doi:10.1016/j.tmaid.2016.09.006.

[227] Aldo P, You Y, Szigeti K, Horvath TL, Lindenbach B, Mor G. HSV-2 enhances ZIKV infection of the placenta and induces apoptosis in first-trimester trophoblast cells. Am J Reprod Immunol. 2016;76(5):348–357. doi:10.1111/aji.12578.

[228] Barreras P, Pamies D, Kumar A, Munoz LS, Parra B, Abreu C, et al. 141st Annual Meeting of the American Neurological Association. Ann Neurol. 2016;80(s20):S1–S265. doi:10.1002/ana.24759.

[229] Bayless NL, Greenberg RS, Swigut T, Wysocka J, Blish CA. Zika Virus Infection Induces Cranial Neural Crest Cells to Produce Cytokines at Levels Detrimental for Neurogenesis. Cell Host Microbe. 2016;20(4):423–428. doi:10.1016/j.chom.2016.09.006.

[230] Chan JFW, Yip CCY, Tsang JOL, Tee KM, Cai JP, Chik KKH, et al. Differential cell line susceptibility to the emerging Zika virus: implications for disease pathogenesis, non-vector-borne human transmission and animal reservoirs. Emerg Microbes Infect. 2016;5:e93. doi:10.1038/emi.2016.99.

[231] El Costa H, Gouilly J, Mansuy JM, Chen Q, Levy C, Cartron G, et al. ZIKA virus reveals broad tissue and cell tropism during the first trimester of pregnancy. Sci Rep. 2016;6:35296. doi:10.1038/srep35296.

[232] Garcez PP, Nascimento JM, Vasconcelos JM, Costa RM, Delvecchio R, Trindade P, et al. Combined proteome and transcriptome analyses reveal that Zika virus circulating in Brazil alters cell cycle and neurogenic programmes in human neurospheres. PeerJ Prepr. 2016;4(e2033v1):0. doi:10.7287/peerj.preprints.2033v1.

[233] Hanners NW, Eitson JL, Usui N, Richardson RB, Wexler EM, Konopka G, et al. Western Zika Virus in Human Fetal Neural Progenitors Persists Long Term with Partial Cytopathic and Limited Immunogenic Effects. Cell Rep. 2016;15(11):2315–2322. doi:10.1016/j.celrep.2016.05.075.

[234] Hughes BW, Addanki KC, Sriskanda AN, McLean E, Bagasra O. Infectivity of Immature Neurons to Zika Virus: A Link to Congenital Zika Syndrome. EBioMedicine. 2016;10(10038):65–70. doi:10.1016/j.ebiom.2016.06.026.

[235] Jurado KA, Simoni MK, Tang Z, Uraki R, Hwang J, Householder S, et al. Zika virus productively infects primary human placenta-specific macrophages. JCI Insight. 2016;1(13):0. doi:10.1172/jci.insight.88461.

[236] Kodani AT, Reiter JF, Knopp K, DeRisi J. Zika infection disrupts centriole biogenesis. Mol Biol Cell. 2016;27(25):4182–4183. doi:10.1091/mbc.E16-10-0736.

[237] Kumar A, Singh HN, Pareek V, Raza K, Dantham S, Kumar P, et al. A Possible Mechanism of Zika Virus Associated Microcephaly: Imperative Role of Retinoic Acid Response Element (RARE) Consensus Sequence Repeats in the Viral Genome. Front Hum Neurosci. 2016;10:403. doi:10.3389/fnhum.2016.00403.

[238] Li Y, Muffat J, Omer A, Bosch I, Lancaster MA, Sur M, et al. Induction of Expansion and Folding in Human Cerebral Organoids. Cell Stem Cell. 2017;20(3):385–396.e3. doi:10.1016/j.stem.2016.11.017.

[239] Liang Q, Luo Z, Zeng J, Chen W, Foo SS, Lee SA, et al. Zika Virus NS4A and NS4B Proteins Deregulate Akt-mTOR Signaling in Human Fetal Neural Stem Cells to Inhibit Neurogenesis and Induce Autophagy. Cell Stem Cell. 2016;19(5):663–671. doi:10.1016/j.stem.2016.07.019.

[240] Liu S, Delalio LJ, Isakson BE, Wang TT. AXL-Mediated Productive Infection of Human Endothelial Cells by Zika Virus. Circ Res. 2016;119(11):1183–1189. doi:10.1161/CIRCRESAHA.116.309866.

[241] Lucchese G, Kanduc D. Minimal immune determinants connect Zika virus, human Cytomegalovirus, and Toxoplasma gondii to microcephaly-related human proteins. Am J Reprod Immunol. 2017;77(2). doi:10.1111/aji.12608.

[242] Nogueira FC, Velasquez E, Melo AS, Domont GB. Zika virus may not be alone: proteomics associates a bovine-like viral diarrhea virus to microcephaly. bioRxiv. 2016;16(September):062596. doi:10.1101/062596.

[243] Onorati M, Li Z, Liu F, Sousa AMM, Nakagawa N, Li M, et al. Zika Virus Disrupts Phospho-TBK1 Localization and Mitosis in Human Neuroepithelial Stem Cells and Radial Glia. Cell Rep. 2016;16(10):2576–2592. doi:10.1016/j.celrep.2016.08.038.

[244] Retallack H, Di Lullo E, Arias C, Knopp KA, Laurie MT, Sandoval-Espinosa C, et al. Zika virus cell tropism in the developing human brain and inhibition by azithromycin. Proc Natl Acad Sci. 2016;113(50):14408–14413. doi:10.1073/pnas.1618029113.

[245] Retallack H, Di Lullo E, Arias C, Knopp KA, Sandoval-Espinosa C, Laurie MT, et al. Zika Virus in the Human Placenta and Developing Brain: Cell Tropism and Drug Inhibition. DoiOrg. 2016;0:058883. doi:10.1101/058883.

[246] Simonin Y, Loustalot F, Desmetz C, Foulongne V, Constant O, Fournier-Wirth C, et al. Zika Virus Strains Potentially Display Different Infectious Profiles in Human Neural Cells. EBioMedicine. 2016;12:161–169. doi:10.1016/j.ebiom.2016.09.020.

[247] Sironi M, Forni D, Clerici M, Cagliani R. Nonstructural Proteins Are Preferential Positive Selection Targets in Zika Virus and Related Flaviviruses. PLoS Negl Trop Dis. 2016;10(9):e0004978. doi:10.1371/journal.pntd.0004978.

[248] Souza BSF, Sampaio GLA, Pereira CS, Campos GS, Sardi SI, Freitas LAR, et al. Zika virus infection induces mitosis abnormalities and apoptotic cell death of human neural progenitor cells. Sci Rep. 2016;6:39775. doi:10.1038/srep39775.

[249] Tabata T, Petitt M, Puerta-Guardo H, Michlmayr D, Wang C, Fang-Hoover J, et al. Zika Virus Targets Different Primary Human Placental Cells, Suggesting Two Routes for Vertical Transmission. Cell Host Microbe. 2016;20(2):155–166. doi:10.1016/j.chom.2016.07.002.

[250] Tsetsarkin KA, Kenney H, Chen R, Liu G, Manukyan H, Whitehead SS, et al. A Full-Length Infectious cDNA Clone

of Zika Virus from the 2015 Epidemic in Brazil as a Genetic Platform for Studies of Virus-Host Interactions and Vaccine Development. MBio. 2016;7(4):e01114–16. doi:10.1128/mbio.01114-16.

[251] Veilleux C, Eugenin E. Abstracts from the 14th International Symposium on NeuroVirology October 25–28, 2016, Toronto, Ontario, Canada. J Neurovirol. 2016;22(S1):1–89. doi:10.1007/s13365-016-0478-8.

[252] Wang Z, Ma'ayan A. An open RNA-Seq data analysis pipeline tutorial with an example of reprocessing data from a recent Zika virus study. F1000Research. 2016;5(1574):1574. doi:10.12688/f1000research.9110.1.

[253] Weisblum Y, Oiknine-Djian E, Vorontsov OM, Haimov-Kochman R, Zakay-Rones Z, Meir K, et al. Zika Virus Infects Early- and Midgestation Human Maternal Decidual Tissues, Inducing Distinct Innate Tissue Responses in the Maternal-Fetal Interface. J Virol. 2017;91(4):pii: e01905–16. doi:10.1128/jvi.01905-16.

[254] Xu M, Lee EM, Wen Z, Cheng Y, Huang WK, Qian X, et al. Identification of small-molecule inhibitors of Zika virus infection and induced neural cell death via a drug repurposing screen. Nat Med. 2016;22(10):1101–1107. doi:10.1038/nm.4184.

[255] Yaffe Y, Dellibovi-Ragheb TA, Chen Y, Elkabetz Y, Altan-Bonnet N. Board Number: B456 The role of centrosomes in the pathogenesis of Zika virus (ZIKV)-related microcephaly. Mol Biol Cell. 2016;27(25):4919. doi:10.1091/mbc.E16-10-0736.

[256] Zhang F, Hammack C, Ogden SC, Cheng Y, Lee EM, Wen Z, et al. Molecular signatures associated with ZIKV exposure in human cortical neural progenitors. Nucleic Acids Res. 2016;44(18):8610–8620. doi:10.1093/nar/gkw765.

[257] Oliveira DBL, Almeida FJ, Durigon EL, Mendes ÉA, Braconi CT, Marchetti I, et al. Prolonged Shedding of Zika Virus Associated with Congenital Infection. N Engl J Med. 2016;375(12):1202–1204. doi:10.1056/nejmc1607583.

[258] Adams Waldorf KM, Stencel-Baerenwald JE, Kapur RP, Studholme C, Boldenow E, Vornhagen J, et al. Fetal brain lesions after subcutaneous inoculation of Zika virus in a pregnant nonhuman primate. Nat Med. 2016;22(11):1256–1259. doi:10.1038/nm.4193.

[259] Brault JB, Khou C, Basset J, Coquand L, Fraisier V, Frenkiel MP, et al. Comparative Analysis Between Flaviviruses Reveals Specific Neural Stem Cell Tropism for Zika Virus in the Mouse Developing Neocortex. EBioMedicine. 2016;10(2):71–76. doi:10.1016/j.ebiom.2016.07.018.

[260] Cugola FR, Fernandes IR, Russo FB, Freitas BC, Dias JLM, Guimarães KP, et al. The Brazilian Zika virus strain causes birth defects in experimental models. Nature. 2016;534(7606):267–271. doi:10.1038/nature18296.

[261] Doobin DJ, Rosenfeld A, Carabalona A, Racaniello V, Vallee RB. Genetic and infectious causes of microcephaly caused by NDE1 mutations and Zika virus. Mol Biol Cell. 2016;27(25):4129–4130. doi:10.1091/mbc.E16-10-0736.

[262] Goodfellow FT, Tesla B, Simchick G, Zhao Q, Hodge T, Brindley MA, et al. Zika Virus Induced Mortality and Microcephaly in Chicken Embryos. Stem Cells Dev. 2016;25(22):1691–1697. doi:10.1089/scd.2016.0231.

[263] Huang WC, Abraham R, Shim BS, Choe H, Page DT. Zika virus infection during the period of maximal brain growth causes microcephaly and corticospinal neuron apoptosis in wild type mice. Sci Rep. 2016;6:34793. doi:10.1038/srep34793.

[264] Manangeeswaran M, Ireland DDC, Verthelyi D. Zika (PRVABC59) Infection Is Associated with T cell Infiltration and Neurodegeneration in CNS of Immunocompetent Neonatal C57Bl/6 Mice. PLoS Pathog. 2016;12(11):e1006004. doi:10.1371/journal.ppat.1006004.

[265] Shao Q, Herrlinger S, Yang SL, Lai F, Moore JM, Brindley MA, et al. Zika virus infection disrupts neurovascular development and results in postnatal microcephaly with brain damage. Development. 2016;143(22):4127–4136. doi:10.1242/dev.143768.

[266] Yockey LJ, Varela L, Rakib T, Khoury-Hanold W, Fink SL, Stutz B, et al. Vaginal Exposure to Zika Virus during Pregnancy Leads to Fetal Brain Infection. Cell. 2016;166(5):1247–1256.e4. doi:10.1016/j.cell.2016.08.004.

[267] Panchaud A, Stojanov M, Ammerdorffer A, Vouga M, Baud D. Emerging role of Zika virus in adverse fetal and neonatal outcomes. Clin Microbiol Rev. 2016;29(3):659–694. doi:10.1128/CMR.00014-16.

[268] Torres JR, Falleiros-Arlant LH, Dueñas L, Pleitez-Navarrete J, Salgado DM, Castillo JBD. Congenital and perinatal complications of chikungunya fever: a Latin American experience. Int J Infect Dis. 2016;51:85–88. doi:10.1016/j.ijid.2016.09.009.

[269] Sarno M, Aquino M, Pimentel K, Cabral R, Costa G, Bastos F, et al. Progressive lesions of central nervous system in microcephalic fetuses with suspected congenital Zika virus syndrome. Ultrasound Obstet Gynecol. 2017;50(6):717–722. doi:10.1002/uog.17303.

[270] Soares de Oliveira-Szejnfeld P, Levine D, Melo ASdO, Amorim MMR, Batista AGM, Chimelli L, et al. Congenital Brain Abnormalities and Zika Virus: What the Radiologist Can Expect to See Prenatally and Postnatally. Radiology. 2016;281(1):203–218. doi:10.1148/radiol.2016161584.

[271] Strafela P, Vizjak A, Mraz J, Mlakar J, Pizem J, Tul N, et al. Zika virus-associated micrencephaly: A thorough description of neuropathologic findings in the fetal central nervous system. Arch Pathol Lab Med. 2017;141(1):73–81. doi:10.5858/arpa.2016-0341-SA.

[272] Vesnaver TV, Tul N, Mehrabi S, Parissone F, Štrafela P, Mlakar J, et al. Zika virus associated microcephaly/micrencephaly—fetal brain imaging in comparison with neuropathology. BJOG An Int J Obstet Gynaecol. 2017;124(3):521–525. doi:10.1111/1471-0528.14423.

[273] Acosta-Reyes J, Navarro E, Herrera MJ, Goenaga E, Ospina ML, Parra E, et al. Severe neurologic disorders in 2 fetuses with Zika virus infection, Colombia. Emerg Infect Dis. 2017;23(6):982–984. doi:10.3201/eid2306.161702.

[274] Anaya JM, Rodríguez Y, Monsalve DM, Vega D, Ojeda E, González-Bravo D, et al. A comprehensive analysis and immunobiology of autoimmune neurological syndromes during the Zika virus outbreak in Cúcuta, Colombia. J Autoimmun. 2017;77:123–138. doi:10.1016/j.jaut.2016.12.007.

[275] Arias A, Torres-Tobar L, Hernández G, Paipilla D, Palacios E, Torres Y, et al. Guillain-Barré syndrome in patients with a recent history of Zika in Cúcuta, Colombia: A descriptive case series of 19 patients from December 2015 to March 2016. J Crit Care. 2017;37:19–23. doi:10.1016/j.jcrc.2016.08.016.

[276] Dirlikov E, Major CG, Mayshack M, Medina N, Matos D, Ryff KR, et al. Guillain-Barré Syndrome During Ongoing Zika Virus Transmission — Puerto Rico, January 1–July 31, 2016. MMWR Morb Mortal Wkly Rep. 2016;65(34):910–914. doi:10.15585/mmwr.mm6534e1.

[277] Do Rosário MS, De Jesus PAP, Vasilakis N, Farias DS, Novaes MAC, Rodrigues SG, et al. Case report: Guillain-barré syndrome after zika virus infection in Brazil. Am J Trop Med Hyg. 2016;95(5):1157–1160. doi:10.4269/ajtmh.16-0306.

[278] Fabrizius RG, Anderson K, Hendel-Paterson B, Kaiser RM, Maalim S, Walker PF. Case report: Guillain-barré syndrome associated with zika virus infection in a traveler returning from Guyana. Am J Trop Med Hyg.

2016;95(5):1161–1165. doi:10.4269/ajtmh.16-0397.

[279] Da Silva IRF, Frontera JA, Do Nascimento OJM. News from the battlefront: Zika virus-associated Guillain-Barré syndrome in Brazil. Neurology. 2016;87(15):e180–e181. doi:10.1212/WNL.0000000000003024.

[280] Fontes CAP, dos Santos AASMD, Marchiori E. Magnetic resonance imaging findings in Guillain-Barré syndrome caused by Zika virus infection. Neuroradiology. 2016;58(8):837–838. doi:10.1007/s00234-016-1687-9.

[281] Langerak T, Yang H, Baptista M, Doornekamp L, Kerkman T, Codrington J, et al. Zika virus infection and Guillain-Barré syndrome in three patients from suriname. Front Neurol. 2016;7(DEC):233. doi:10.3389/fneur.2016.00233.

[282] Medina MT, England JD, Lorenzana I, Medina-Montoya M, Alvarado D, De Bastos M, et al. Zika virus associated with sensory polyneuropathy. J Neurol Sci. 2016;369(30):271–272. doi:10.1016/j.jns.2016.08.044.

[283] Parra B, Lizarazo J, Jiménez-Arango JA, Zea-Vera AF, González-Manrique G, Vargas J, et al. Guillain–Barré Syndrome Associated with Zika Virus Infection in Colombia. N Engl J Med. 2016;375(16):1513–1523. doi:10.1056/NEJMoa1605564.

[284] Siu R, Bukhari W, Todd A, Gunn W, Huang QS, Timmings P. Acute Zika infection with concurrent onset of Guillain-Barré Syndrome. Neurology. 2016;87(15):1623–1624. doi:10.1212/WNL.0000000000003038.

[285] Skrove JL, Salhab J, Colella DM, Michel G, Sobrado J, Almeida M. Clinical Vignettes/Case Reports – Small Intestine/Unclassified. Am J Gastroenterol. 2016;111:S971–S1076. doi:10.1038/ajg.2016.374.

[286] Zambrano H, Waggoner JJ, Almeida C, Rivera L, Benjamin JQ, Pinsky BA. Case report: Zika virus and chikungunya virus coinfections: A series of three cases from a single center in Ecuador. Am J Trop Med Hyg. 2016;95(4):894–896. doi:10.4269/ajtmh.16-0323.

[287] Zambrano H, Waggoner JJ, Almeida C, Rivera L, Benjamin JQ, Pinsky BA. Case report: Zika virus and chikungunya virus coinfections: A series of three cases from a single center in Ecuador. Am J Trop Med Hyg. 2016;95(4):894–896. doi:10.4269/ajtmh.16-0323.

[288] Septfons A, Leparc-Goffart I, Couturier E, Franke F, Deniau J, Balestier A, et al. Travel-associated and autochthonous Zika virus infection in mainland France, 1 January to 15 July 2016. Eurosurveillance. 2016;21(32):0. doi:10.2807/1560-7917.ES.2016.21.32.30315.

[289] Walker WL, Lindsey NP, Lehman JA, Krow-Lucal ER, Rabe IB, Hills SL, et al. Zika Virus Disease Cases — 50 States and the District of Columbia, January 1–July 31, 2016. MMWR Morb Mortal Wkly Rep. 2016;65(36):983–986. doi:10.15585/mmwr.mm6536e5.

[290] dos Santos T, Rodriguez A, Almiron M, Sanhueza A, Ramon P, de Oliveira WK, et al. Zika Virus and the Guillain–Barré Syndrome — Case Series from Seven Countries. N Engl J Med. 2016;375(16):1598–1601. doi:10.1056/NEJMc1609015.

[291] Machado-Alba JE, Machado-Duque ME, Gaviria-Mendoza A, Orozco-Giraldo V. Diagnosis of neurological disorders and the Zika virus epidemic in Colombia 2014 –2016. Int J Infect Dis. 2016;51:133–134. doi:10.1016/j.ijid.2016.09.010.

[292] World Health Organization. WHO | Zika situation report; 2017. [Online]. Available from: http://www.who.int/emergencies/zika-virus/situation-report/10-march-2017/en/.

[293] Lee CT, Vora NM, Bajwa W, Boyd L, Harper S, Kass D, et al. Zika Virus Surveillance and Preparedness — New York City, 2015–2016. MMWR Morb Mortal Wkly Rep. 2016;65(24):629–635. doi:10.15585/mmwr.mm6524e3.

[294] Van Kerkhove MD, Reveiz L, Souza JP, Jaenisch T, Carson G, Broutet N. Harmonisation of Zika virus research protocols to address key public health concerns. Lancet Glob Heal. 2016;4(12):e911–e912. doi:10.1016/s2214-109x(16)30255-8.

[295] Akl EA, Kahale LA, Hakoum MB, Matar CF, Sperati F, Barba M, et al. Parenteral anticoagulation in ambulatory patients with cancer. Cochrane Database Syst Rev. 2017;2017(9):Cd006652. doi:10.1002/14651858.CD006652.pub5.

[296] Spurling GKP, Del Mar CB, Dooley L, Foxlee R, Farley R. Delayed antibiotic prescriptions for respiratory infections. Cochrane Database Syst Rev. 2017;2017(9):Cd004417. doi:10.1002/14651858.CD004417.pub5.

[297] Leis AA, Stokic DS. Zika virus and Guillain-Barre syndrome: Is there sufficient evidence for causality? Front Neurol. 2016;7(SEP):170. doi:10.3389/fneur.2016.00170.

[298] Rozé B, Najioullah F, Fergé JL, Apetse K, Brouste Y, Cesaire R, et al. Zika virus detection in urine from patients with Guillain-Barré syndrome on Martinique, January 2016. Eurosurveillance. 2016;21(9):30154. doi:10.2807/1560-7917.es.2016.21.9.30154.

[299] Thomas DL, Sharp TM, Torres J, Armstrong PA, Munoz-Jordan J, Ryff KR, et al. Local Transmission of Zika Virus — Puerto Rico, November 23, 2015–January 28, 2016. MMWR Morb Mortal Wkly Rep. 2016;65(6):154–158. doi:10.15585/mmwr.mm6506e2.

[300] Akl EA, Meerpohl JJ, Elliott J, Kahale LA, Schünemann HJ, Agoritsas T, et al. Living systematic reviews: 4. Living guideline recommendations. J Clin Epidemiol. 2017;91:47–53. doi:10.1016/j.jclinepi.2017.08.009.

[301] Butler D. Drop in cases of Zika threatens large-scale trials. Nature. 2017;545(7655):396–397. doi:10.1038/545396a.

[302] Hamer DH, Chen LH. Zika in Angola and India. J Travel Med. 2019;doi:10.1093/jtm/taz012.

[303] Umapathi T, Kam YW, Ohnmar O, Ng BCJ, Ng Y, Premikha M, et al. The 2016 Singapore Zika virus outbreak did not cause a surge in Guillain-Barré syndrome. J Peripher Nerv Syst. 2018;23(3):197–201. doi:10.1111/jns.12284.

[304] Ruchusatsawat K, Wongjaroen P, Posanacharoen A, Rodriguez-Barraquer I, Sangkitporn S, Cummings DAT, et al. Long-term circulation of Zika virus in Thailand: an observational study. Lancet Infect Dis. 2019;19(4):439–446. doi:10.1016/S1473-3099(18)30718-7.

[305] Heymann DL, Hodgson A, Sall AA, Freedman DO, Staples JE, Althabe F, et al. Zika virus and microcephaly: why is this situation a PHEIC? Lancet. 2016;387(10020):719–721. doi:10.1016/S0140-6736(16)00320-2.

[306] Counotte MJ, Egli-Gany D, Riesen M, Abraha M, Porgo TV, Wang J, et al. Zika virus infection as a cause of congenital brain abnormalities and Guillain-Barré syndrome: From systematic review to living systematic review. F1000Research. 2018;7(196):196. doi:10.12688/f1000research.13704.1.

[307] Devakumar D, Bamford A, Ferreira MU, Broad J, Rosch RE, Groce N, et al. Infectious causes of microcephaly: epidemiology, pathogenesis, diagnosis, and management. Lancet Infect Dis. 2018;18(1):e1–e13. doi:10.1016/S1473-3099(17)30398-5.

[308] Frenkel LD, Gomez F, Sabahi F. The pathogenesis of microcephaly resulting from congenital infections: why is my baby's head so small? Eur J Clin Microbiol Infect Dis. 2018;37(2):209–226. doi:10.1007/s10096-017-3111-8.

[309] Charrel R, Mögling R, Pas S, Papa A, Baronti C, Koopmans M, et al. Variable Sensitivity in Molecular Detection of Zika Virus in European Expert Laboratories: External Quality Assessment, November 2016. J Clin Microbiol.

2017;55(11):3219–3226. doi:10.1128/jcm.00987-17.

[310] Fischer C, Pedroso C, Mendrone A, de Filippis AMB, Vallinoto ACR, Morais Ribeiro B, et al. External quality assessment for Zika virus molecular diagnostic testing, Brazil. Emerg Infect Dis. 2018;24(5):888–892. doi:10.3201/eid2405.171747.

[311] Koopmans M, de Lamballerie X, Jaenisch T, Rosenberger KD, Morales I, Marques ETA, et al. Familiar barriers still unresolved—a perspective on the Zika virus outbreak research response. Lancet Infect Dis. 2019;19(2):e59–e62. doi:10.1016/S1473-3099(18)30497-3.

[312] Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)-A metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform. 2009;42(2):377–381. doi:10.1016/j.jbi.2008.08.010.

[313] Viechtbauer W. Conducting Meta-Analyses in R with the metafor Package . J Stat Softw. 2015;36(3):1–48. doi:10.18637/jss.v036.i03.

[314] R Core Team. R: A Language and Environment for Statistical Computing; 2018. Available from: https://www.R-project.org/.

[315] Hernán MA, Hernández-Díaz S, Robins JM. A structural approach to selection bias. Epidemiology. 2004;15(5):615–625. doi:10.1097/01.ede.0000135174.63482.43.

[316] Acosta-Reyes J, Navarro E, Herrera MJ, Goenaga E, Ospina ML, Parra E, et al. Severe neurologic disorders in 2 fetuses with Zika virus infection, Colombia. Emerg Infect Dis. 2017;23(6):982–984. doi:10.3201/eid2306.161702.

[317] Adhikari EH, McKiever M, Nelson DB, Johnson KA, Stocks ME, Rogers VL, et al. 860: Neonatal surveillance among asymptomatic Zika-exposed infants through 6 months of life. Am J Obstet Gynecol. 2018;218(1):S512–S513. doi:10.1016/j.ajog.2017.11.397.

[318] Adhikari EH, Nelson DB, Johnson KA, Jacobs S, Rogers VL, Roberts SW, et al. Infant outcomes among women with Zika virus infection during pregnancy: results of a large prenatal Zika screening program. Am J Obstet Gynecol. 2017;216(3):292.e1–292.e8. doi:10.1016/j.ajog.2017.01.018.

[319] Alayed MS, Qureshi MA, Ahmed S, Alqahtani AS, Alqahtani AM, Alshaybari K, et al. Seroprevalence of Zika virus among asymptomatic pregnant mothers and their newborns in the Najran region of southwest Saudi Arabia. Ann Saudi Med. 2018;38(6):408–412. doi:10.5144/0256-4947.2018.408.

[320] Aleman TS, Ventura CV, Cavalcanti MM, Serrano LW, Traband A, Nti AA, et al. Quantitative assessment of microstructural changes of the retina in infants with congenital Zika syndrome. JAMA Ophthalmol. 2017;135(10):1069–1076. doi:10.1001/jamaophthalmol.2017.3292.

[321] de Almeida IMLM, Ramos CV, Rodrigues DC, de Sousa AC, do Nascimento MdLCAPC, da Silva MVB, et al. Clinical and epidemiological aspects of microcephaly in the state of Piauí, northeastern Brazil, 2015–2016. J Pediatr (Rio J). 2018;95(4):466–474. doi:10.1016/j.jped.2018.04.013.

[322] Alves LV, Mello MJG, Bezerra PG, Alves JGB. Congenital Zika Syndrome and Infantile Spasms: Case Series Study. J Child Neurol. 2018;33(10):664–666. doi:10.1177/0883073818780105.

[323] Angelidou A, Michael Z, Hotz A, Friedman K, Emani S, Larovere K, et al. Is There More to Zika? Complex Cardiac Disease in a Case of Congenital Zika Syndrome. Neonatology. 2018;113(2):177–182. doi:10.1159/000484656.

[324] Aragao MFVV, Brainer-Lima AM, Holanda AC, Van Der Linden V, Aragao LV, Silva MLM, et al. Spectrum of spinal cord, spinal root, and brain mri abnormalities in congenital Zika syndrome with and without Arthrogryposis. Am J Neuroradiol. 2017;38(5):1045–1053. doi:10.3174/ajnr.A5125.

[325] Aragao MFVV, Holanda AC, Brainer-Lima AM, Petribu NCL, Castillo M, Van Der Linden V, et al. Nonmicrocephalic infants with congenital zika syndrome suspected only after neuroimaging evaluation compared with those with microcephaly at birth and postnatally: How large is the Zika virus "iceberg"? Am J Neuroradiol. 2017;38(7):1427–1434. doi:10.3174/ajnr.A5216.

[326] Azevedo RSS, Araujo MT, Oliveira CS, Filho AJM, Nunes BTD, Henriques DF, et al. Zika Virus Epidemic in Brazil. II. Post-Mortem Analyses of Neonates with Microcephaly, Stillbirths, and Miscarriage. J Clin Med. 2018;7(12):496. doi:10.3390/jcm7120496.

[327] Baran LCP, da Costa MF, Vidal KS, Damico FM, Barboni MTS, Da SLD, et al. Alterations in visual acuity and visual development in infants 1-24 months old either exposed to or infected by Zika virus during gestation, with and without microcephaly. J AAPOS. 2019;doi:10.1016/j.jaapos.2019.03.005.

[328] Bautista LE. Maternal Zika virus infection and newborn microcephaly—an analysis of the epidemiological evidence. Ann Epidemiol. 2018;28(2):111–118. doi:10.1016/j.annepidem.2017.11.010.

[329] Beaufrère A, Bessières B, Bonnière M, Driessen M, Alfano C, Couderc T, et al. A clinical and histopathological study of malformations observed in fetuses infected by the Zika virus. Brain Pathol. 2019;29(1):114–125. doi:10.1111/bpa.12644.

[330] Benjamin I, Fernández G, Figueira JV, Parpacén L, Urbina MT, Medina R. Zika virus detected in amniotic fluid and umbilical cord blood in an in vitro fertilization-conceived pregnancy in Venezuela. Fertil Steril. 2017;107(6):1319–1322. doi:10.1016/j.fertnstert.2017.02.112.

[331] Brady OJ, Osgood-Zimmerman A, Kassebaum NJ, Ray SE, de Araujo VEM, da Nobrega AA, et al. The association between Zika virus infection and microcephaly in Brazil 2015-2017: An observational analysis of over 4 million births. PLoS Med. 2019;16(3):e1002755. doi:10.1371/journal.pmed.1002755.

[332] Brito KGDS, dos Santos EB, Lucas LDSM, Orsini M, Fiorelli R, Teixeira S, et al. Prevalence of neurological complications associated with zika virus in a Brazilian metropolis. Neurol Int. 2018;10(2):61–64. doi:10.4081/ni.2018.7638.

[333] Brock M, Magalhaes AQ, Di Tommaso Leao J, De Oliveira Soeiro CM, Do Carmo Oliveira Monteiro Penalber A, DeMenezes CB, et al. Zika virus infection in pregnant women in Manaus. Int J Gynecol Obstet. 2018;143(Supplement 3):725–726. doi:10.1002/ijgo.12583.

[334] Bulas D, Mulkey S, Vezina G, Fourzali Y, Arroyave-Wessel M, Cristante C, et al. Spr 2018. Pediatr Radiol. 2018;48(S1):1–298. doi:10.1007/s00247-018-4130-z.

[335] Cabral CM, da Nóbrega MEB, Leite PLe, de Souza MSF, Teixeira DCP, Cavalcante TF, et al. Descrição clínico-epidemiológica dos nascidos vivos com microcefalia no estado de Sergipe, 2015. Epidemiol e Serviços Saúde. 2017;26(2):245–254. doi:10.5123/S1679-49742017000200002.

[336] Caixeta R, Magalhaes AC, De Brito WI, Barboza A. Vertical transmission of zika virus and perinatal microcephaly. Int J Gynecol Obstet. 2018;143(Supplement 3):725. doi:10.1002/ijgo.12583.

[337] Calle-Giraldo JP, Rojas CA, Hurtado IC, Barco C, Libreros

D, Sanchez PJ, et al. Outcomes of Congenital Zika Virus Infection During an Outbreak in Valle del Cauca, Colombia. Pediatr Infect Dis J. 2019;38(7):735–740. doi:10.1097/INF.0000000000002307.

[338] Campos MC, Dombrowski JG, Phelan J, Marinho CRF, Hibberd M, Clark TG, 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. 2018;13(8):e0201452. doi:10.1371/journal.pone.0201452.

[339] Cano M, Esquivel R. Zika virus infection in Hospital del Niño 'Dr José Renán Esquivel' (Panamá): Case Review since the introduction in Latin America. Pediátr Panamá. 2018;47(3):15–19.

[340] Cardoso TF, dos Santos RS, Corrêa RM, Campos JV, Silva RdB, Tobias CC, et al. Congenital Zika infection: neurology can occur without microcephaly. Arch Dis Child. 2019;104(2):199–200. doi:10.1136/archdischild-2018-314782.

[341] Carvalho FR, Medeiros T, Vianna RAdO, Douglass-Jaimes G, Nunes PCG, Quintans MDS, et al. Simultaneous circulation of arboviruses and other congenital infections in pregnant women in Rio de Janeiro, Brazil. Acta Trop. 2019;192:49–54. doi:10.1016/j.actatropica.2019.01.020.

[342] de Castro JDV, Pereira LP, Dias DA, Aguiar LB, Maia JCN, da Costa JIF, et al. Presumed Zika virus-related congenital brain malformations: the spectrum of CT and MRI findings in fetuses and newborns. Arq Neuropsiquiatr. 2017;75(10):703–710. doi:10.1590/0004-282x20170134.

[343] Di Cavalcanti D, Alves LV, Furtado GJ, Santos CC, Feitosa FG, Ribeiro MC, et al. Echocardiographic findings in infants with presumed congenital Zika syndrome: Retrospective case series study. PLoS One. 2017;12(4):e0175065. doi:10.1371/journal.pone.0175065.

[344] Chimelli L, Melo ASO, Avvad-Portari E, Wiley CA, Camacho AHS, Lopes VS, et al. The spectrum of neuropathological changes associated with congenital Zika virus infection. Acta Neuropathol. 2017;133(6):983–999. doi:10.1007/s00401-017-1699-5.

[345] Chimelli L, Pone SM, Avvad-Portari E, Vasconcelos ZFM, Zin AA, Cunha DP, et al. Persistence of Zika virus after birth: Clinical, virological, neuroimaging, and neuropathological documentation in a 5-month infant with congenital Zika syndrome. J Neuropathol Exp Neurol. 2018;77(3):193–198. doi:10.1093/jnen/nlx116.

[346] Chu V, Petersen LR, Moore CA, Meaney-Delman D, Nelson G, Christian Sonne D, et al. Possible Congenital Zika Syndrome in Older Children Due to Earlier Circulation of Zika Virus. Am J Med Genet Part A. 2018;176(9):1882–1889. doi:10.1002/ajmg.a.40378.

[347] Collier ARY, Barouch DH. Maternal immune response to zika virus. Reprod Sci. 2018;25 (1)(1):163A–164A.

[348] Contreras-Capetillo SN, Valadéz-González N, Manrique-Saide P, Carcaño-Castillo RE, Pacheco-Tugores F, Barrera-Pérez HAM, et al. Birth Defects Associated With Congenital Zika Virus Infection in Mexico. Clin Pediatr (Phila). 2018;57(8):927–936. doi:10.1177/0009922817738341.

[349] da Maia JTS, Vidal SJA, Mendes TV, Lins MG, Zacarkim MR, Rolim DD, et al. Perinatal Case Fatality Rate in Cases of Congenital Zika Syndrome: a Cross-Sectional Study (P5.160). Neurology. 2018;90(15 Supplement):P5.160.

[350] Dávila-Camargo A, Durán-Nah JJ, Dupinet-Sánchez A. Ophthalmologic findings in newborns of women with Zika virus infection during pregnancy: A case series. Rev Mex Oftalmol. 2018;92(4):159–190. doi:10.24875/RMOE.M18000001.

[351] Davila-Castrodad NM, Reyes-Bou Z, Correa-Rivas M, Perez-Berenguer JL, Di-Pasquale L, Garcia-Garcia I. First Autopsy of a Newborn with Congenital Zika Syndrome in Puerto Rico. P R Heal Sci J. 2018;37(Special Issue):S81–s84.

[352] De Alcantara T, da Silva Maia JT, Aronoff D, Rolim DD, Zacarkim MR, LaBeaud AD, et al. Radiological Findings and Neurological Disorders in Microcephaly Cases Related to Zika Virus: A Cohort Study (P2.314). Neurology. 2018;90(15 Supplement):P2.314.

[353] De Alcantara T, LaBeaud AD, Aronoff D, Zacarkim MR, da Silva Maia JT, Fernandes KE, et al. CT Scan Findings in Microcephaly Cases During 2015–2016 Zika Outbreak: A Cohort Study (P2.310). Neurology. 2018;90(15 Supplement):P2.310.

[354] de Araújo TVB, Ximenes RAdA, Miranda-Filho DdB, Souza WV, Montarroyos UR, de Melo APL, et al. Association between microcephaly, Zika virus infection, and other risk factors in Brazil: final report of a case-control study. Lancet Infect Dis. 2018;18(3):328–336. doi:10.1016/S1473-3099(17)30727-2.

[355] De Fatima Viana Vasco Aragao M, Van Der Linden V, Petribu NC, De Mello RJV, Holanda AC, Araujo LC, et al. Congenital Zika Syndrome: Comparison of brain CT scan with postmortem histological sections from the same subjects. Neuroradiology. 2018;60(Supplement 1):367–368.

[356] de Fatima Viana Vasco Aragao M, van der Linden V, Petribu NC, Valenca MM, Parizel PM, de Mello RJV. Congenital Zika Syndrome: The Main Cause of Death and Correspondence Between Brain CT and Postmortem Histological Section Findings From the Same Individuals. Top Magn Reson Imaging. 2019;28(1):29–33. doi:10.1097/RMR.0000000000000194.

[357] de Freitas Ribeiro BN, Muniz BC, Gasparetto EL, Marchiori E. Congenital involvement of the central nervous system by the Zika virus in a child without microcephaly – spectrum of congenital syndrome by the Zika virus. J Neuroradiol. 2018;45(2):152–153. doi:10.1016/j.neurad.2017.11.007.

[358] De Magalhães-Barbosa MC, Prata-Barbosa A, Robaina JR, Raymundo CE, Lima-Setta F, Da Cunha AJLA. Prevalence of microcephaly in eight south-eastern and midwestern Brazilian neonatal intensive care units: 2011-2015. Arch Dis Child. 2017;102(8):728–734. doi:10.1136/archdischild-2016-311541.

[359] De Moraes CG, Pettito M, Yepez JB, Sakuntabhai A, Simon-Loriere E, Zaidi MB, et al. Corrigendum: Optic neuropathy and congenital glaucoma associated with probable Zika virus infection in Venezuelan patients. JMM case reports. 2018;5(8):e005161. doi:10.1099/jmmcr.0.005161.

[360] de Oliveira WK, Carmo EH, Henriques CM, Coelho G, Vazquez E, Cortez-Escalante J, et al. Zika Virus Infection and Associated Neurologic Disorders in Brazil. N Engl J Med. 2017;376(16):1591–1593. doi:10.1056/NEJMc1608612.

[361] de Oliveira WK, de França GVA, Carmo EH, Duncan BB, de Souza Kuchenbecker R, Schmidt MI. Infection-related microcephaly after the 2015 and 2016 Zika virus outbreaks in Brazil: a surveillance-based analysis. Lancet. 2017;390(10097):861–870. doi:10.1016/S0140-6736(17)31368-5.

[362] de Paula Freitas B, Zin A, Ko A, Maia M, Ventura CV, Belfort R. Anterior-Segment Ocular Findings and Microphthalmia in Congenital Zika Syndrome. Ophthalmology. 2017;124(12):1876–1878. doi:10.1016/j.ophtha.2017.06.009.

[363] del Campo M, Feitosa IML, Ribeiro EM, Horovitz DDG, Pessoa ALS, França GVA, et al. The phenotypic spectrum of congenital Zika syndrome. Am J Med Genet Part A. 2017;173(4):841–857. doi:10.1002/ajmg.a.38170.

[364] del Carpio-Orantes L, Rosas-Lozano AL, García-Méndez S. Zika virus infection in pregnant women in a General Hospital of Veracruz, Mexico. J Matern Neonatal Med. 2019; p. 1–5. doi:10.1080/14767058.2019.1582627.

[365] Delaney A, Mai C, Smoots A, Cragan J, Ellington S, Langlois P, et al. Population-Based Surveillance of Birth Defects Potentially Related to Zika Virus Infection — 15 States and U.S. Territories, 2016. MMWR Morb Mortal Wkly Rep. 2018;67(3):91–96. doi:10.15585/mmwr.mm6703a2.

[366] Eppes C, Rac M, Dempster C, Ballas J, Davidson C, Aagaard K. Zika Virus in a Non-Endemic Urban Population. Obstet Gynecol. 2017;129:S135. doi:10.1097/01.aog.0000514683.06169.08.

[367] Felix A, Hallet E, Favre A, Kom-Tchameni R, Defo A, Fléchelles O, et al. Cerebral injuries associated with Zika virus in utero exposure in children without birth defects in French Guiana: Case report. Med (United States). 2017;96(51):e9178. doi:10.1097/MD.0000000000009178.

[368] Fernandez MP, Parra Saad E, Ospina Martinez M, Corchuelo S, Mercado Reyes M, Herrera MJ, et al. Ocular histopathologic features of congenital Zika syndrome. JAMA Ophthalmol. 2017;135(11):1163–1169. doi:10.1001/jamaophthalmol.2017.3595.

[369] Fernandez Martinez B, Martinez Sanchez EV, Diaz Garcia O, Gomez Barroso D, Sierra Moros MJ, Cano Portero R, et al. Zika virus disease in Spain. Surveillance results and epidemiology on reported cases, 2015-2017. Med Clin. 2019;doi:10.1016/j.medcli.2018.12.014.

[370] Ferreira HNC, Schiariti V, Regalado ICR, Sousa KG, Pereira SA, Fechine CPNdS, et al. Functioning and disability profile of children with microcephaly associated with congenital Zika virus infection. Int J Environ Res Public Health. 2018;15(6). doi:10.3390/ijerph15061107.

[371] Figueredo LF, Franco-Zuluaga JA, Carrere-Rivera C, Rodriguez-Silva DS, Akle V. Anatomical findings of fetuses vertically-infected with Zika virus. FASEB Journal Conf Exp Biol. 2018;32(Supplement 1).

[372] Fonteles CSR, Marques Ribeiro E, Sales Aragão Santos M, Ferreira Pequeno Leite R, Sales Assunção G, Monteiro AJ, et al. Lingual frenulum phenotypes in Brazilian infants with congenital zika syndrome. Cleft Palate-Craniofacial J. 2018;55(10):1391–1398. doi:10.1177/1055665618766999.

[373] de França GVA, Pedi VD, Garcia MHdO, do Carmo GMI, Leal MB, Garcia LP. Síndrome congênita associada à infecção pelo vírus Zika em nascidos vivos no Brasil: descrição da distribuição dos casos notificados e confirmados em 2015-2016. Epidemiol e Serviços Saúde. 2018;27(2):e2017473. doi:10.5123/S1679-49742018000200014.

[374] Gely-Rojas L, García-Fragoso L, Negrón J, Deynes D, García-García I, Zorrilla CD. Congenital Zika Syndrome in Puerto Rico, Beyond Microcephaly, A Multiorgan Approach. P R Health Sci J. 2018;37(Special Issue):S73–S76.

[375] Gely-Rojas L, Pérez R, García-Fragoso L, García-García I. Association of Zika Virus Exposure in Utero with Ocular Phenotypes in a Group of Newborns in Puerto Rico Exposure with ocular phenotypes in newborns in Puerto Rico. P R Health Sci J. 2018;37(Special Issue):S77–S80.

[376] Giovanetti M, Goes de Jesus J, Lima de Maia M, Junior JX, Castro Amarante MF, Viana P, et al. Genetic evidence of Zika virus in mother's breast milk and body fluids of a newborn with severe congenital defects. Clin Microbiol Infect. 2018;24(10):1111–1112. doi:10.1016/j.cmi.2018.06.008.

[377] Gregianini TS, Ranieri T, Favreto C, Nunes ZMA, Tumioto Giannini GL, Sanberg ND, et al. Emerging arboviruses in Rio Grande do Sul, Brazil: Chikungunya and Zika outbreaks, 2014-2016. Rev Med Virol. 2017;27(6). doi:10.1002/rmv.1943.

[378] Guevara JG, Agarwal-Sinha S. Ocular abnormalities in congenital Zika syndrome: A case report, and review of the literature. J Med Case Rep. 2018;12(1):161. doi:10.1186/s13256-018-1679-y.

[379] Gunturiz ML, Cortés L, Cuevas EL, Chaparro P, Ospina ML. Toxoplasmosis cerebral congénita e infección por el virus del Zika y del chikunguña: reporte de un caso. Biomédica. 2018;38(2):144–152. doi:10.7705/biomedica.v38i0.3652.

[380] Halai UA, Nielsen-Saines K, Moreira ML, De Sequeira PC, Pereira JP, De Araujo Zin A, et al. Maternal Zika virus disease severity, virus load, prior dengue antibodies, and their relationship to birth outcomes. Clin Infect Dis. 2017;65(6):877–883. doi:10.1093/cid/cix472.

[381] Hall NB, Broussard K, Evert N, Canfield M. Notes from the Field : Zika Virus-Associated Neonatal Birth Defects Surveillance — Texas, January 2016–July 2017 . MMWR Morb Mortal Wkly Rep. 2017;66(31):835–836. doi:10.15585/mmwr.mm6631a5.

[382] Hay JA, Nouvellet P, Donnelly CA, Riley S. Potential inconsistencies in Zika surveillance data and our understanding of risk during pregnancy. PLoS Negl Trop Dis. 2018;12(12):e0006991. doi:10.1371/journal.pntd.0006991.

[383] Herber S, Silva AA, Sanseverino MTV, Friedrich L, Ranieri TMS, Favreto C, et al. Prevalence and causes of congenital microcephaly in the absence of a Zika virus outbreak in southern Brazil. J Pediatr (Rio J). 2018;95(5):600–606. doi:10.1016/j.jped.2018.05.013.

[384] Hernández-Ávila JE, Palacio-Mejía LS, López-Gatell H, Alpuche-Aranda CM, Molina-Vélez D, González-González L, et al. Zika virus infection estimates, Mexico. Bull World Health Organ. 2018;96(5):306–313. doi:10.2471/BLT.17.201004.

[385] Ho CY, Castillo N, Encinales L, Porras A, Mendoza AR, Lynch R, et al. Second-trimester Ultrasound and Neuropathologic Findings in Congenital Zika Virus Infection. Pediatr Infect Dis J. 2018;37(12):1290–1293. doi:10.1097/INF.0000000000002080.

[386] Hoen B, Schaub B, Funk AL, Ardillon V, Boullard M, Cabié A, et al. Pregnancy Outcomes After ZIKV Infection in French Territories in the Americas. Obstet Anesth Dig. 2018;38(3):131–132. doi:10.1097/01.aoa.0000542352.41212.4b.

[387] Howard A, Visintine J, Fergie J, Deleon M. Two infants with presumed congenital zika syndrome, Brownsville, Texas, USA, 2016-2017. Emerg Infect Dis. 2018;24(4):625–630. doi:10.3201/eid2404.171545.

[388] Hurtado-Villa P, Puerto AK, Victoria S, Gracia G, Guasmayán L, Arce P, et al. Raised Frequency of Microcephaly Related to Zika Virus Infection in Two Birth Defects Surveillance Systems in Bogotá and Cali, Colombia. Pediatr Infect Dis J. 2017;36(10):1017–1019. doi:10.1097/INF.0000000000001670.

[389] Jaenisch T, Rosenberger KD, Brito C, Brady O, Brasil P, Marques ETA. Risque de microcéphalie après une infection à virus Zika au Brésil, de 2015 à 2016. Bull World Health Organ. 2017;95(3):191–198. doi:10.2471/BLT.16.178608.

[390] James-Powell T, Brown Y, Christie CDC, Melbourne-Chambers R, Moore JT, Morgan O, et al. Trends of microcephaly and severe arthrogryposis in three urban hospitals following the Zika, chikungunya and dengue fever epidemics of 2016 in Jamaica. West Indian Med J. 2017;66(1):10–19. doi:10.7727/wimj.2017.124.

[391] João EC, Ferreira OdC, Gouvêa MI, Teixeira MdLB, Tanuri A, Higa LM, et al. Pregnant women co-infected with HIV and zika: Outcomes and birth defects in infants according to maternal symptomatology. PLoS One. 2018;13(7):e0200168. doi:10.1371/journal.pone.0200168.

[392] Journel I, Andrécy LL, Metellus D, Pierre JS, Faublas RM, Juin S, et al. Transmission of Zika Virus — Haiti, October 12, 2015–September 10, 2016. MMWR Morb Mortal Wkly Rep. 2017;66(6):172–176. doi:10.15585/mmwr.mm6606a4.

[393] Juca E, Pessoa A, Ribeiro E, Menezes R, Kerbage S, Lopes T, et al. Hydrocephalus associated to congenital Zika syndrome: does shunting improve clinical features? Childs Nerv Syst. 2018;34(1):101–106. doi:10.1007/s00381-017-3636-2.

[394] Kam YW, Leite JA, Lum FM, Tan JJL, Lee B, Judice CC, et al. Specific biomarkers associated with neurological complications and congenital central nervous system abnormalities from Zika virus-infected patients in Brazil. J Infect Dis. 2017;216(2):172–181. doi:10.1093/infdis/jix261.

[395] Gurcharanjeet K, Manganas L. EEG findings in a Case of Congenital Zika Virus Syndrome (P4.076). Neurology. 2017;88(16 Supplement):P4.076.

[396] Khatib A, Showler AJ, Kain D, Melvin R, Lecce C, Boggild AK. A diagnostic gap illuminated by a sexually-transmitted case of congenital Zika virus infection. Travel Med Infect Dis. 2019;27:117–118. doi:10.1016/j.tmaid.2018.10.017.

[397] Kikuti M, Cardoso CW, Prates APB, Paploski IAD, Kitron U, Reis MG, et al. Congenital brain abnormalities during a zika virus epidemic in Salvador, Brazil, april 2015 to july 2016. Eurosurveillance. 2018;23(45). doi:10.2807/1560-7917.ES.2018.23.45.1700757.

[398] Krow-Lucal ER, de Andrade MR, Cananéa JNA, Moore CA, Leite PL, Biggerstaff BJ, et al. Association and birth prevalence of microcephaly attributable to Zika virus infection among infants in Paraíba, Brazil, in 2015–16: a case-control study. Lancet Child Adolesc Heal. 2018;2(3):205–213. doi:10.1016/S2352-4642(18)30020-8.

[399] Leal MC, van der Linden V, Bezerra TP, de Valois L, Borges ACG, Antunes MMC, et al. Characteristics of dysphagia in infants with microcephaly caused by congenital zika virus infection, Brazil, 2015. Emerg Infect Dis. 2017;23(8):1253–1259. doi:10.3201/eid2308.170354.

[400] Lee EH, Cooper H, Iwamoto M, Lash M, Conners EE, Bloch D, et al. First 12 Months of Life for Infants in New York City, New York, With Possible Congenital Zika Virus Exposure. J Pediatr Infect Dis Soc. 2019;doi:10.1093/jpids/piz027.

[401] de Carvalho AL, Brites C, Taguchi TB, Pinho SF, Campos G, Lucena R. Congenital zika virus infection with normal neurodevelopmental outcome, Brazil. Emerg Infect Dis. 2018;24(11):2128–2130. doi:10.3201/eid2411.180883.

[402] Lima GP, Rozenbaum D, Pimentel C, Frota ACC, Vivacqua D, Machado ES, et al. Factors associated with the development of Congenital Zika Syndrome: a case-control study. BMC Infect Dis. 2019;19(1):277. doi:10.1186/s12879-019-3908-4.

[403] van der Linden V, van der Linden Junior H, Leal MdC, Rolim Filho EL, van der Linden A, Aragão MdFVV, et al. Discordant clinical outcomes of congenital Zika virus infection in twin pregnancies. Arq Neuropsiquiatr. 2017;75(6):381–386. doi:10.1590/0004-282x20170066.

[404] Lockrow J, Tully H, Saneto RP. Epileptic spasms as the presenting seizure type in a patient with a new "O" of TORCH, congenital Zika virus infection. Epilepsy Behav Case Reports. 2019;11:1–3. doi:10.1016/j.ebcr.2018.09.002.

[405] Frutos MGL, Ochoa JH, Barbás MG, Lucchini HD, Moya M, Malinger G. New Insights into the Natural History of Congenital Zika Virus Syndrome. Fetal Diagn Ther. 2018;44(1):72–76. doi:10.1159/000479866.

[406] de Magalhães-Barbosa MC, Prata-Barbosa A, Robaina JR, Raymundo CE, Lima-Setta F, da Cunha AJLA. New trends of the microcephaly and Zika virus outbreak in Brazil, July 2016–December 2016. Travel Med Infect Dis. 2017;16:52–57. doi:10.1016/j.tmaid.2017.03.009.

[407] Majumder MS, Hess R, Ross R, Piontkivska H. Seasonality of birth defects in West Africa: could congenital Zika syndrome be to blame? F1000Research. 2018;7:159. doi:10.12688/f1000research.13858.1.

[408] Abramov DM, Saad T, Gomes-Junior SC, De Souza E Silva D, Araújo I, Moreira MEL, et al. Auditory brainstem function in microcephaly related to Zika virus infection. Neurology. 2018;90(7):e606–e614. doi:10.1212/WNL.0000000000004974.

[409] Martins RS, Froes MH, Saad LDC, Ignacio Junior SM, Prado WDA, Figueiredo EM, et al. Descriptive report of cases of congenital syndrome associated with Zika virus infection in the state of Sao Paulo, Brazil, from 2015 to 2017. Epidemiol Serv Saude. 2018;27(3):e2017382. doi:10.5123/S1679-49742018000300012.

[410] Mattar S, Ojeda C, Arboleda J, Arrieta G, Bosch I, Botia I, et al. Case report: Microcephaly associated with Zika virus infection, Colombia. BMC Infect Dis. 2017;17(1):423. doi:10.1186/s12879-017-2522-6.

[411] Maykin M, Avaad-Portari E, Esquivel M, Pereira JP, Fisher SJ, Moreira ME, et al. 874: Placental Histopathologic Findings in Zika-infected Pregnancies. Am J Obstet Gynecol. 2018;218(1):S520–S521. doi:10.1016/j.ajog.2017.11.411.

[412] Mejdoubi M, Monthieux A, Cassan T, Lombard C, Flechelles O, Adenet C. Brain MRI in Infants after Maternal Zika Virus Infection during Pregnancy. N Engl J Med. 2017;377(14):1399–1400. doi:10.1056/NEJMc1612813.

[413] Mendes Neto NN, da Silva Maia JT, Zacarkim MR, Queiroz I, Labeaud AD, Aronoff DM. Perinatal Case Fatality Rate Related to Congenital Zika Syndrome in Brazil: A Cross-Sectional Study. Pediatr Neurol. 2018;81:47–48. doi:10.1016/j.pediatrneurol.2017.11.012.

[414] Méndez N, Oviedo-Pastrana M, Mattar S, Caicedo-Castro I, Arrieta G. Zika virus disease, microcephaly and Guillain-Barré syndrome in Colombia: Epidemiological situation during 21 months of the Zika virus outbreak, 2015-2017. Arch Public Heal. 2017;75(1):65. doi:10.1186/s13690-017-0233-5.

[415] Meneses JDA, Ishigami AC, De Mello LM, De Albuquerque LL, De Brito CAA, Cordeiro MT, et al. Lessons Learned at the Epicenter of Brazil's Congenital Zika Epidemic: Evidence from 87 Confirmed Cases. Clin Infect Dis. 2017;64(10):1302–1308. doi:10.1093/cid/cix166.

[416] Merriam AA, Nhan-Chang CL, Huerta-Bogdan BI, Wapner R, Gyamfi-Bannerman C. A Single-Center Experience with a Pregnant Immigrant Population and Zika Virus Serologic Screening in New York City. Am J Perinatol. 2019;doi:10.1055/s-0039-1688819.

[417] Merriam AA, Nhan-Chang CL, Huerta-Bogdan BI, Wapner RJ, Gyamfi-Bannerman C. 393: A single-center experience with a pregnant immigrant population and zika virus in new york city. Am J Obstet Gynecol. 2016;216(1):S236. doi:10.1016/j.ajog.2016.11.651.

[418] Lage MLC, de Carvalho AL, Ventura PA, Taguchi TB, Fernandes AS, Pinho SF, et al. Clinical, neuroimaging, and neurophysiological findings in children with microcephaly related to congenital zika virus infection. Int J Environ Res Public Health. 2019;16(3). doi:10.3390/ijerph16030309.

[419] Mletzko J, Schildgen O. Absence of Zika virus in abortion and placental tissue in a German cohort. Rev Med Microbiol. 2018;29(1):17–19. doi:10.1097/MRM.0000000000000121.

[420] Moi ML, Nguyen TTT, Nguyen CT, Vu TBH, Tun MMN, Pham TD, et al. Zika virus infection and microcephaly in Vietnam. Lancet Infect Dis. 2017;17(8):805–806.

doi:10.1016/S1473-3099(17)30412-7.

[421] Moreira-Soto A, Cabral R, Pedroso C, Eschbach-Bludau M, Rockstroh A, Vargas LA, et al. Exhaustive TORCH Pathogen Diagnostics Corroborate Zika Virus Etiology of Congenital Malformations in Northeastern Brazil. mSphere. 2018;3(4):e00278–18. doi:10.1128/msphere.00278-18.

[422] Moreira-Soto A, Sarno M, Pedroso C, Netto EM, Rockstroh A, Luz E, et al. Evidence for Congenital Zika Virus Infection from Neutralizing Antibody Titers in Maternal Sera, Northeastern Brazil. J Infect Dis. 2017;216(12):1501–1504. doi:10.1093/infdis/jix539.

[423] Mulkey SB, Bulas DI, Vezina G, Fourzali Y, Morales A, Arroyave-Wessel M, et al. Sequential Neuroimaging of the Fetus and Newborn with in Utero Zika Virus Exposure. JAMA Pediatr. 2019;173(1):52–59. doi:10.1001/jamapediatrics.2018.4138.

[424] Mulkey SB, Vezina G, Bulas DI, Khademian Z, Blask A, Kousa Y, et al. Neuroimaging Findings in Normocephalic Newborns With Intrauterine Zika Virus Exposure. Pediatr Neurol. 2018;78:75–78. doi:10.1016/j.pediatrneurol.2017.10.012.

[425] Nogueira Mendes Neto N, Thais Da Silva Maia J, Duarte Rolim D, Jayne Alves Vidal S, Vidal Mendes T, Rodrigues Zacarkim M, et al. Mortality in Newborns With Microcephaly Due to Maternal Zika Virus Infection in Rio Grande do Norte State—Northeast Brazil: A Cross-Sectional Analysis. Open Forum Infect Dis. 2017;3(suppl_1). doi:10.1093/ofid/ofw195.2.

[426] Nogueira ML, Nery Júnior NRR, Estofolete CF, Bernardes Terzian AC, Guimarães GF, Zini N, et al. Adverse birth outcomes associated with Zika virus exposure during pregnancy in São José do Rio Preto, Brazil. Clin Microbiol Infect. 2018;24(6):646–652. doi:10.1016/j.cmi.2017.11.004.

[427] Oliveira-Filho J, Felzemburgh R, Costa F, Nery N, Mattos A, Henriques DF, et al. Seizures as a complication of congenital Zika syndrome in early infancy. Am J Trop Med Hyg. 2018;98(6):1860–1862. doi:10.4269/ajtmh.17-1020.

[428] Orofino DHG, Passos SRL, de Oliveira RVC, Farias CVB, Leite MdFMP, Pone SM, et al. Cardiac findings in infants with in utero exposure to Zika virus- a cross sectional study. PLoS Negl Trop Dis. 2018;12(3):e0006362. doi:10.1371/journal.pntd.0006362.

[429] Parra-Saavedra M, Reefhuis J, Piraquive JP, Gilboa SM, Badell ML, Moore CA, et al. Serial Head and Brain Imaging of 17 Fetuses with Confirmed Zika Virus Infection in Colombia, South America. Obstet Gynecol. 2017;130(1):207–212. doi:10.1097/AOG.0000000000002105.

[430] Pedroso C, Fischer C, Feldmann M, Sarno M, Luz E, Moreira-Soto A, et al. Cross-Protection of Dengue Virus Infection against Congenital Zika Syndrome, Northeastern Brazil. Emerg Infect Dis. 2019;25(8). doi:10.3201/eid2508.190113.

[431] Peixoto Filho AAA, de Freitas SB, Ciosaki MM, Oliveira LNe, dos Santos Júnior OT. Computed tomography and magnetic resonance imaging findings in infants with microcephaly potentially related to congenital Zika virus infection. Radiol Bras. 2018;51(2):119–122. doi:10.1590/0100-3984.2016.0135.

[432] Peña F, Pimentel R, Khosla S, Mehta SD, Brito MO. Zika virus epidemic in pregnant women, Dominican republic, 2016–2017. Emerg Infect Dis. 2019;25(2):236–244. doi:10.3201/eid2502.181054.

[433] Maykin M, Pereira JP, Moreira M, Nielsen-Saines K, Gaw S. Role of amniocentesis in the diagnosis of congenital zika syndrome. Am J Obstet Gynecol. 2018;219(6):652. doi:10.1016/j.ajog.2018.10.059.

[434] Pereira JP, Nielsen-Saines K, Sperling J, Maykin MM, Damasceno L, Cardozo RF, et al. Association of Prenatal Ultrasonographic Findings With Adverse Neonatal Outcomes Among Pregnant Women With Zika Virus Infection in Brazil. JAMA Netw open. 2018;1(8):e186529. doi:10.1001/jamanetworkopen.2018.6529.

[435] Petribu NCDL, Aragao MDFV, Van Der Linden V, Parizel P, Jungmann P, Araújo L, et al. Follow-up brain imaging of 37 children with congenital Zika syndrome: Case series study. BMJ. 2017;359:j4188. doi:10.1136/bmj.j4188.

[436] Petribu NCdL, Fernandes ACV, Abath MdB, Araújo LC, de Queiroz FRS, Araújo JdM, et al. Common findings on head computed tomography in neonates with confirmed congenital Zika syndrome. Radiol Bras. 2018;51(6):366–371. doi:10.1590/0100-3984.2017.0119.

[437] Pires P, Jungmann P, Galvão JM, Hazin A, Menezes L, Ximenes R, et al. Neuroimaging findings associated with congenital Zika virus syndrome: case series at the time of first epidemic outbreak in Pernambuco State, Brazil. Child's Nerv Syst. 2018;34(5):957–963. doi:10.1007/s00381-017-3682-9.

[438] Pomar L, Vouga M, Lambert V, Pomar C, Hcini N, Jolivet A, et al. Maternal-fetal transmission and adverse perinatal outcomes in pregnant women infected with Zika virus: Prospective cohort study in French Guiana. BMJ. 2018;363:k4431. doi:10.1136/bmj.k4431.

[439] Porse CC, Messenger S, Vugia DJ, Jilek W, Salas M, Watt J, et al. Travel-associated zika cases and threat of local transmission during global outbreak, California, USA. Emerg Infect Dis. 2018;24(9):1626–1632. doi:10.3201/eid2409.180203.

[440] Prata-Barbosa A, Cleto-Yamane TL, Robaina JR, Guastavino AB, de Magalhães-Barbosa MC, Brindeiro RdM, et al. Co-infection with Zika and Chikungunya viruses associated with fetal death—A case report. Int J Infect Dis. 2018;72:25–27. doi:10.1016/j.ijid.2018.04.4320.

[441] Rabelo K, de Souza Campos Fernandes, de Souza LJ, de Souza TL, dos Santos FB, Nunes PCG, et al. Placental Histopathology and clinical presentation of severe congenital Zika syndrome in a human immunodeficiency virus-exposed uninfected infant. Front Immunol. 2017;8(DEC):1704. doi:10.3389/fimmu.2017.01704.

[442] Rabelo K, Souza LJ, Salomão NG, Oliveira ERA, Sentinelli LdP, Lacerda MS, et al. Placental inflammation and fetal injury in a rare Zika case associated with Guillain-Barré Syndrome and abortion. Front Microbiol. 2018;9(MAY):1018. doi:10.3389/fmicb.2018.01018.

[443] Rajapakse NS, Ellsworth K, Liesman RM, Ho ML, Henry N, Theel ES, et al. Unilateral phrenic nerve palsy in infants with congenital Zika syndrome. Emerg Infect Dis. 2018;24(8):1422–1427. doi:10.3201/eid2408.180057.

[444] Ramalho FS, Yamamoto AY, Da Silva LL, Figueiredo LTM, Rocha LB, Neder L, et al. Congenital Zika Virus Infection Induces Severe Spinal Cord Injury. Clin Infect Dis. 2017;65(4):687–690. doi:10.1093/cid/cix374.

[445] Ramos CL, Moreno-Carvalho OA, Nascimento-Carvalho CM. Cerebrospinal fluid aspects of neonates with or without microcephaly born to mothers with gestational Zika virus clinical symptoms. J Infect. 2018;76(6):563–569. doi:10.1016/j.jinf.2018.02.004.

[446] Raymond A, Jakus J. Cerebral Infarction and Refractory Seizures in a Neonate with Suspected Zika Virus Infection. Pediatr Infect Dis J. 2018;37(4):e112–e114. doi:10.1097/INF.0000000000001832.

[447] Regadas VC, Silva MdCe, Abud LG, Labadessa LMPL, de Oliveira RGG, Miyake CH, et al. Microcephaly caused by congenital Zika virus infection and viral detection in maternal urine during pregnancy. Rev Assoc Med Bras. 2018;64(1):11–14. doi:10.1590/1806-9282.64.01.11.

[448] Reynolds MR, Jones AM, Petersen EE, Lee EH, Rice ME, Bingham A, 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. 2017;66(13):366–373. doi:10.15585/mmwr.mm6613e1.

[449] Ribeiro BNdF, Marchiori E. Congenital Zika syndrome associated with findings of cerebellar cortical dysplasia - Broadening the spectrum of presentation of the syndrome. J Neuroradiol. 2018;doi:10.1016/j.neurad.2018.10.009.

[450] Ribeiro IG, de Andrade MR, Silva JdM, Silva ZM, Costa MAdO, Vieira MAdCeS, et al. Microcefalia no Piauí, Brasil: estudo descritivo durante a epidemia do vírus Zika, 2015-2016. Epidemiol e Serviços Saúde. 2018;27(1):e20163692. doi:10.5123/S1679-49742018000100002.

[451] Rice ME, Galang RR, Roth NM, Ellington SR, Moore CA, Valencia-Prado M, et al. Vital Signs: Zika-Associated Birth Defects and Neurodevelopmental Abnormalities Possibly Associated with Congenital Zika Virus Infection — U.S. Territories and Freely Associated States, 2018. MMWR Morb Mortal Wkly Rep. 2018;67(31):858–867. doi:10.15585/mmwr.mm6731e1.

[452] Rodó C, Suy A, Sulleiro E, Soriano-Arandes A, Antón A, García-Ruiz I, et al. In utero negativization of Zika virus in a foetus with serious central nervous system abnormalities. Clin Microbiol Infect. 2018;24(5):549.e1–549.e3. doi:10.1016/j.cmi.2017.09.022.

[453] Rodó C, Suy A, Sulleiro E, Soriano-Arandes A, Maiz N, García-Ruiz I, et al. Pregnancy outcomes after maternal Zika virus infection in a non-endemic region: prospective cohort study. Clin Microbiol Infect. 2019;25(5):633.e5–633.e9. doi:10.1016/j.cmi.2019.02.008.

[454] Rodriguez-Morales AJ, Cardona-Ospina JA, Ramirez-Jaramillo V, Gaviria JA, González-Moreno GM, Castrillón-Spitia JD, 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;25:20–25. doi:10.1016/j.tmaid.2018.06.006.

[455] Rodriguez J, Diaz M, Montalvo A, Gonzalez J. Case Series: Congenital Zika Virus Infection Associated with Epileptic Spasms. Ann Neurol. 2018;84(Supplement 22):S401–S401. doi:10.1002/ana.25305.

[456] Rosado L, Gomes MBF, Lacerda FA, Martins TP, De Medeiros M, De Toledo Lima T, et al. Cohort of infected Zika Virus pregnant women from central Brazil: Preliminary report. Int J Gynecol Obstet. 2018;143(Supplement 3):228. doi:10.1002/ijgo.12582.

[457] Rosenstierne MW, Schaltz-Buchholzer F, Bruzadelli F, Có A, Cardoso P, Jørgensen CS, et al. Zika virus IgG in infants with microcephaly, Guinea-Bissau, 2016. Emerg Infect Dis. 2018;24(5):948–950. doi:10.3201/eid2405.180153.

[458] Sanín-Blair JE, Gutiérrez-Márquez C, Herrera DA, Vossough A. Fetal Magnetic Resonance Imaging Findings in Prenatal Zika Virus Infection. Fetal Diagn Ther. 2017;42(2):153–157. doi:10.1159/000454860.

[459] Santa Rita TH, Barra RB, Peixoto GP, Mesquita PG, Barra GB. Association between suspected Zika virus disease during pregnancy and giving birth to a newborn with congenital microcephaly: A matched case-control study. BMC Res Notes. 2017;10(1):457. doi:10.1186/s13104-017-2796-1.

[460] Santana MB, Lamas CC, Athayde JG, Calvet G, Moreira J, De Lorenzo A. Congenital Zika syndrome: is the heart part of its spectrum? Clin Microbiol Infect. 2019;25(8):1043–1044. doi:10.1016/j.cmi.2019.03.020.

[461] Santos GR, Pinto CAL, Prudente RCS, Bevilacqua E, Witkin SS, Passos SD, et al. Case Report: Histopathologic Changes in Placental Tissue Associated With Vertical Transmission of Zika Virus. Int J Gynecol Pathol. 2019;doi:10.1097/PGP.0000000000000586.

[462] Santos VS, Oliveira SJG, Gurgel RQ, Lima DRR, Dos Santos CA, Martins-Filho PRS. Case report: Microcephaly in twins due to the Zika Virus. Am J Trop Med Hyg. 2017;97(1):151–154. doi:10.4269/ajtmh.16-1021.

[463] Sanz Cortes M, Rivera AM, Yepez M, Guimaraes CV, Diaz Yunes I, Zarutskie A, et al. Clinical assessment and brain findings in a cohort of mothers, fetuses and infants infected with ZIKA virus. Am J Obstet Gynecol. 2018;218(4):440.e1–440.e36. doi:10.1016/j.ajog.2018.01.012.

[464] Sassetti M, Zé-Zé L, Franco J, da Cunha J, Gomes A, Tomé A, et al. First case of confirmed congenital Zika syndrome in continental Africa. Trans R Soc Trop Med Hyg. 2018;112(10):458–462. doi:10.1093/trstmh/try074.

[465] Saukko P, Pollak S. Autopsy. Encycl Forensic Sci Second Ed. 2012;97:205–209. doi:10.1016/B978-0-12-382165-2.00185-9.

[466] Schaub B, Vouga M, Najioullah F, Gueneret M, Monthieux A, Harte C, et al. Analysis of blood from Zika virus-infected fetuses: a prospective case series. Lancet Infect Dis. 2017;17(5):520–527. doi:10.1016/S1473-3099(17)30102-0.

[467] Schaub B, Gueneret M, Jolivet E, Decatrelle V, Yazza S, Gueye H, et al. Ultrasound imaging for identification of cerebral damage in congenital Zika virus syndrome: a case series. Lancet Child Adolesc Heal. 2017;1(1):45–55. doi:10.1016/S2352-4642(17)30001-9.

[468] Schwartz KL, Chan T, Rai N, Murphy KE, Whittle W, Drebot MA, et al. Zika virus infection in a pregnant Canadian traveler with congenital fetal malformations noted by ultrasonography at 14-weeks gestation. Trop Dis Travel Med Vaccines. 2018;4(1):2. doi:10.1186/s40794-018-0062-8.

[469] Seferovic MD, Turley M, Valentine GC, Rac M, Castro ECC, Major AM, et al. Clinical Importance of Placental Testing among Suspected Cases of Congenital Zika Syndrome. Int J Mol Sci. 2019;20(3):712. doi:10.3390/ijms20030712.

[470] Shapiro-Mendoza CK, Rice ME, Galang RR, Fulton AC, VanMaldeghem K, Prado MV, et al. Pregnancy Outcomes After Maternal Zika Virus Infection During Pregnancy — U.S. Territories, January 1, 2016–April 25, 2017. MMWR Morb Mortal Wkly Rep. 2017;66(23):615–621. doi:10.15585/mmwr.mm6623e1.

[471] Shiu C, Starker R, Kwal J, Bartlett M, Crane A, Greissman S, et al. Zika virus testing and outcomes during pregnancy, Florida, USA, 2016. Emerg Infect Dis. 2018;24(1):1–8. doi:10.3201/eid2401.170979.

[472] Silva JHD, Tercas ACP, Pinheiro LCB, Franca GVA, Atanaka M, Schuler-Faccini L. Profile of congenital anomalies among live births in the municipality of Tangara da Serra, Mato Grosso, Brazil, 2006-2016. Epidemiol Serv Saude. 2018;27(3):e2018008. doi:10.5123/S1679-49742018000300017.

[473] Soares-Marangoni DdA, Tedesco NM, Nascimento AL, Almeida PRD, dos Santos Pereira CN. General movements and motor outcomes in two infants exposed to Zika virus: brief report. Dev Neurorehabil. 2019;22(1):71–74. doi:10.1080/17518423.2018.1437843.

[474] Sohan K, Cyrus CA. Ultrasonographic observations of the fetal brain in the first 100 pregnant women with Zika virus infection in Trinidad and Tobago. Int J Gynecol Obstet. 2017;139(3):278–283. doi:10.1002/ijgo.12313.

[475] Sousa AQ, Cavalcante DIM, Franco LM, Araújo FMC, Sousa ET, Valença-Junior JT, et al. Postmortem findings for 7 neonates with congenital Zika virus

infection. Emerg Infect Dis. 2017;23(7):1164–1167. doi:10.3201/eid2307.162019.

[476] Souza AI, Siqueira MTD, Ferreira ALCG, Freitas CUD, Bezerra ACV, Ribeiro AG, et al. Geography of microcephaly in the zika era: A study of newborn distribution and socio-environmental indicators in recife, brazil, 2015-2016. Public Health Rep. 2018;133(4):461–471. doi:10.1177/0033354918777256.

[477] Souza WVD, Albuquerque MDFPMD, Vazquez E, Bezerra LCA, Mendes ADCG, Lyra TM, et al. Microcephaly epidemic related to the Zika virus and living conditions in Recife, Northeast Brazil. BMC Public Health. 2018;18(1):130. doi:10.1186/s12889-018-5039-z.

[478] Sowunmi S, Eckert V, Griffin A. 58th Annual Teratology Society Meeting. Birth Defects Res. 2018;110(9):667–822. doi:10.1002/bdr2.1355.

[479] Spiliopoulos D, Panchal M, Economides DL. Surveillance of pregnant women with potential exposure to Zika virus following travel. G Chir. 2019;40(1):58–65.

[480] Spiliopoulos D, Wooton G, Economides DL, Wright A. Fetal Medicine Poster Abstracts. BJOG An Int J Obstet Gynaecol. 2017;124:17–49. doi:10.1111/1471-0528.14586.

[481] Subissi L, Dub T, Besnard M, Mariteragi-Helle T, Nhan T, Lutringer-Magnin D, et al. Zika virus infection during pregnancy and effects on early childhood development, French Polynesia, 2013–2016. Emerg Infect Dis. 2018;24(10):1850–1858. doi:10.3201/eid2410.172079.

[482] Sulleiro E, Frick MA, Rodo C, Espasa M, Thorne C, Espiau M, et al. The challenge of the laboratory diagnosis in a confirmed congenital Zika virus syndrome in utero: A case report. Med. 2019;98(20):e15532. doi:10.1097/MD.0000000000015532.

[483] Sulleiro E, Rando A, Alejo I, Suy A, Gonce A, Rodo C, et al. Screening for Zika virus infection in 1057 potentially exposed pregnant women, Catalonia (northeastern Spain). Travel Med Infect Dis. 2019;29:69–71. doi:10.1016/j.tmaid.2019.03.006.

[484] Tellechea AL, Luppo V, Morales MA, Groisman B, Baricalla A, Fabbri C, et al. Surveillance of microcephaly and selected brain anomalies in Argentina: Relationship with Zika virus and other congenital infections. Birth Defects Res. 2018;110(12):1016–1026. doi:10.1002/bdr2.1347.

[485] Terzian ACB, Estofolete CF, Da Silva RA, Vaz-Oliani DCM, Oliani AH, De Mattos CCB, et al. Long-term viruria in zika virus-infected pregnant women, Brazil, 2016. Emerg Infect Dis. 2017;23(11):1891–1893. doi:10.3201/eid2311.170078.

[486] Tse C, Picon M, Rodriguez P, Gonzalez I, Starker R, Curry C. The Effects of Zika in Pregnancy. Obstet Gynecol. 2017;129:S137–S138. doi:10.1097/01.aog.0000514691.21416.35.

[487] Tsui I, Moreira MEL, Rossetto JD, Vasconcelos Z, Gaw SL, Neves LM, et al. Eye Findings in Infants With Suspected or Confirmed Antenatal Zika Virus Exposure. Pediatrics. 2018;142(4):e20181104. doi:10.1542/peds.2018-1104.

[488] Valdespino-Vazquez MY, Sevilla-Reyes EE, Lira R, Yocupicio-Monroy M, Piten-Isidro E, Boukadida C, 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(6):903–912. doi:10.1093/cid/ciy616.

[489] and Ventura CV, Bandstra ES, Fernandez MP, Cooper JM, Saigal GM, Bauer CR, et al. First Locally Acquired Congenital Zika Syndrome Case in the United States: Neonatal Clinical Manifestations. Ophthalmic Surgery, Lasers Imaging Retin. 2018;49(9):e93–e98. doi:10.3928/23258160-20180907-14.

[490] Ventura LO, Ventura CV, Dias NdC, Vilar IG, Gois AL, Arantes TE, et al. Visual impairment evaluation in 119 children with congenital Zika syndrome. J AAPOS. 2018;22(3):218–222.e1. doi:10.1016/j.jaapos.2018.01.009.

[491] Ventura LO, Ventura CV, Lawrence L, van der Linden V, van der Linden A, Gois AL, et al. Visual impairment in children with congenital Zika syndrome. J AAPOS. 2017;21(4):295–299.e2. doi:10.1016/j.jaapos.2017.04.003.

[492] Venturi G, Fortuna C, Alves RM, do Prado Paschoal AG, da Silva Junior PJ, Remoli ME, et al. Epidemiological and clinical suspicion of congenital Zika virus infection: Serological findings in mothers and children from Brazil. J Med Virol. 2019;doi:10.1002/jmv.25504.

[493] Verçosa I, Carneiro P, Verçosa R, Girão R, Ribeiro EM, Pessoa A, et al. The visual system in infants with microcephaly related to presumed congenital Zika syndrome. J AAPOS. 2017;21(4):300–304.e1. doi:10.1016/j.jaapos.2017.05.024.

[494] Villamil-Gómez WE, Guijarro E, Castellanos J, Rodríguez-Morales AJ. Congenital Zika syndrome with prolonged detection of Zika virus RNA. J Clin Virol. 2017;95:52–54. doi:10.1016/j.jcv.2017.08.010.

[495] Vissoci JRN, Rocha TAH, da Silva NC, de Sousa Queiroz RC, Thomaz EBAF, Amaral PVM, et al. Zika virus infection and microcephaly: Evidence regarding geospatial associations. PLoS Negl Trop Dis. 2018;12(4):e0006392. doi:10.1371/journal.pntd.0006392.

[496] Vouga M, Baud D, Jolivet E, Najioullah F, Monthieux A, Schaub B. Congenital Zika virus syndrome...what else? Two case reports of severe combined fetal pathologies. BMC Pregnancy Childbirth. 2018;18(1):356. doi:10.1186/s12884-018-1998-4.

[497] Walker CL, Merriam AA, Ohuma EO, Dighe MK, Gale M, Rajagopal L, et al. Femur-sparing pattern of abnormal fetal growth in pregnant women from New York City after maternal Zika virus infection. Am J Obstet Gynecol. 2018;219(2):187.e1–187.e20. doi:10.1016/j.ajog.2018.04.047.

[498] Wongsurawat T, Athipanyasilp N, Jenjaroenpun P, Jun SR, Kaewnapan B, Wassenaar TM, et al. Case of microcephaly after congenital infection with asian lineage zika virus, Thailand. Emerg Infect Dis. 2018;24(9):1758–1761. doi:10.3201/eid2409.180416.

[499] Wongsurawat T, Jenjaroenpun P, Athipanyasilp N, Kaewnapan B, Leelahakorn N, Angkasekwinai N, et al. Genome Sequences of Zika Virus Strains Recovered from Amniotic Fluid, Placenta, and Fetal Brain of a Microcephaly Patient in Thailand, 2017. Microbiol Resour Announc. 2018;7(11). doi:10.1128/mra.01020-18.

[500] Ximenes ASFC, Pires P, Werner H, Jungmann PM, Rolim Filho EL, Andrade EP, et al. Neuroimaging findings using transfontanellar ultrasound in newborns with microcephaly: a possible association with congenital Zika virus infection. J Matern Neonatal Med. 2019;32(3):493–501. doi:10.1080/14767058.2017.1384459.

[501] Yarrington CD, Hamer DH, Kuohung W, Lee-Parritz A. Congenital Zika syndrome arising from sexual transmission of Zika virus, a case report. Fertil Res Pract. 2019;5(1):1. doi:10.1186/s40738-018-0053-5.

[502] Yepez JB, Murati FA, Pettito M, Peñaranda CF, De Yepez J, Maestre G, et al. Ophthalmic manifestations of congenital Zika syndrome in Colombia and Venezuela. JAMA Ophthalmol. 2017;135(5):440–445. doi:10.1001/jamaophthalmol.2017.0561.

[503] Zacharias N, Whitty J, Noblin S, Tsakiri S, Garcia J, Covinsky M, et al. First Neonatal Demise with Travel-Associated Zika Virus Infection in the United States of America. Am J Perinatol Reports. 2017;07(02):e68–e73. doi:10.1055/s-0037-1601890.

[504] Zambrano H, Waggoner J, León K, Pinsky B, Vera K, Schettino M, et al. High incidence of Zika virus infection detected in plasma and cervical cytology specimens from pregnant women in Guayaquil, Ecuador. Am J Reprod Immunol. 2017;77(2). doi:10.1111/aji.12630.

[505] Zin AA, Tsui I, Rossetto JD, Gaw SL, Neves LM, Zin OA, et al. Visual function in infants with antenatal Zika virus exposure. J AAPOS. 2018;22(6):452–456.e1. doi:10.1016/j.jaapos.2018.07.352.

[506] Zin AA, Tsui I, Rossetto J, Vasconcelos Z, Adachi K, Valderramos S, et al. Screening criteria for ophthalmic manifestations of congenital zika virus infection. JAMA Pediatr. 2017;171(9):847–854. doi:10.1001/jamapediatrics.2017.1474.

[507] Zuanazzi D, Arts EJ, Jorge PK, Mulyar Y, Gibson R, Xiao Y, et al. Postnatal Identification of Zika Virus Peptides from Saliva. J Dent Res. 2017;96(10):1078–1084. doi:10.1177/0022034517723325.

[508] Ali A, Williams M. Zik-V outbreak and Guillain-Barre syndrome in Jamaica. Neurology. 2017;88(16).

[509] de Azevedo MB, Coutinho MSC, da Silva MA, Arduini DB, Lima JDV, Monteiro R, et al. Neurologic manifestations in emerging arboviral diseases in Rio de Janeiro city, Brazil, 2015-2016. Rev Soc Bras Med Trop. 2018;51(3):347–351. doi:10.1590/0037-8682-0327-2017.

[510] Barreira AA, Marques W, Marreco A, De Aquino Cruz C, Marques V, Sposito DLA, et al. Guilllain-barre syndrome related to Zika virus infection in Brazil. Neurology. 2017;88(16).

[511] Baskar D, Amalnath D, Mandal J, Dhodapkar R, Vanathi K. Antibodies to Zika virus, Campylobacter jejuni and gangliosides in Guillain–Barre syndrome: A prospective single-center study from southern India. Neurol India. 2019;66(5):1324. doi:10.4103/0028-3886.241402.

[512] Polara FK, Kugasia IR, Donnelly JP, Simmons J, Srinivasan M. Hypertensive encephalopathy associated vasogenic edema causing intracranial hypertension and brain death. Am J Respir Crit Care Med. 2017;195. doi:10.1164/ajrccm-conference.2017.A58.

[513] Betances N, Luciano C, Carlo J, Deliz-Roldan B, Alfonso G. Prominent distal demyelination is a feature of zika-associated guillain barre syndrome. Neurology. 2017;88(16).

[514] Boggild AK, Geduld J, Libman M, Yansouni CP, McCarthy AE, Hajek J, et al. Surveillance report of Zika virus among Canadian travellers returning from the Americas. Cmaj. 2017;189(9):E334–E340. doi:10.1503/cmaj.161241.

[515] Brenciaglia M, Noël TP, Fields PJ, Bidaisee S, Myers TE, Nelson WM, et al. Clinical, Serological, and Molecular Observations from a Case Series Study during the Asian Lineage Zika Virus Outbreak in Grenada during 2016. Can J Infect Dis Med Microbiol. 2018;2018:1–9. doi:10.1155/2018/4635647.

[516] Ferreira MLB, De Brito CAA, Moreira ÁJP, Machado MÍdM, Henriques-Souza A, Cordeiro MT, et al. Guillain–Barré syndrome, acute disseminated encephalomyelitis and encephalitis associated with Zika virus infection in Brazil: Detection of viral RNA and isolation of virus during late infection. Am J Trop Med Hyg. 2017;97(5):1405–1409. doi:10.4269/ajtmh.17-0106.

[517] Brito CAA, Henriques-Souza A, Soares CRP, Castanha PMS, Machado LC, Pereira MR, et al. Persistent detection of Zika virus RNA from an infant with severe microcephaly - a case report. BMC Infect Dis. 2018;18(1):388. doi:10.1186/s12879-018-3313-4.

[518] Cordeiro de Souza L, de Souza AA, de Almeida EEP, Honse Ribeiro L, Parada Godoy MD, Augusto Junior W, et al. Inspiratory Muscle Training with Isokinetic Device to Help Ventilatory Weaning in a Patient with Guillain-Barré Syndrome by Zika Virus. Case Reports Crit Care. 2018;2018:1–5. doi:10.1155/2018/9708451.

[519] Da Silva IRF, Frontera JA, De Filippis AMB, Do Nascimento OJM. Neurologic complications associated with the Zika virus in Brazilian adults. JAMA Neurol. 2017;74(10):1190–1198. doi:10.1001/jamaneurol.2017.1703.

[520] del Carpio-Orantes L, Peniche Moguel KG, Sánchez Díaz JS, Pola-Ramirez MdR, Mata Miranda MdP, García-Méndez S, et al. Síndrome de Guillain-Barré asociado a zika; análisis de la cohorte delegacional en la región Veracruz norte durante 2016-2017. Neurología. 2018;doi:10.1016/j.nrl.2018.05.002.

[521] del Carpio Orantes L, Juárez Rangel FJ, García-Méndez S. Incidence of Guillain-Barré syndrome at a secondary centre during the 2016 zika outbreak. Neurologia. 2017;doi:10.1016/j.nrl.2017.07.019.

[522] Dirlikov E, Major CG, Medina NA, Lugo-Robles R, Matos D, Muñoz-Jordan JL, et al. Clinical features of Guillain-Barré syndrome with vs without zika virus infection, Puerto Rico, 2016. JAMA Neurol. 2018;75(9):1089–1097. doi:10.1001/jamaneurol.2018.1058.

[523] Dirlikov E, Medina NA, Major CG, Munoz-Jordan JL, Luciano CA, Rivera-Garcia B, et al. Acute zika virus infection as a risk factor for Guillain-Barré syndrome in Puerto Rico. JAMA - J Am Med Assoc. 2017;318(15):1498–1500. doi:10.1001/jama.2017.11483.

[524] Dirlikov E, Torres JV, Martines RB, Reagan-Steiner S, Pérez GV, Rivera A, et al. Postmortem findings in patient with Guillain-Barré syndrome and zika virus infection. Emerg Infect Dis. 2018;24(1):114–117. doi:10.3201/eid2401.171331.

[525] Dourado ME, Fernandes U, Vital AL, Ramos E, Urbano JC, Sena A, et al. 2017 Peripheral Nerve Society Meeting July 8-12, 2017 Sitges, Barcelona, Spain. J Peripher Nerv Syst. 2017;22(3):226–414. doi:10.1111/jns.12225.

[526] Francois R, Berkowitz A. Zika-associated atypical Guillain-Barre variants in rural Haiti. Neurology. 2017;88(16).

[527] GeurtsvanKessel CH, Islam Z, Islam MB, Kamga S, Papri N, van de Vijver DAMC, et al. Zika virus and Guillain–Barré syndrome in Bangladesh. Ann Clin Transl Neurol. 2018;5(5):606–615. doi:10.1002/acn3.556.

[528] Gibelin N, Romero J, Gregoire V, Kaidomar M. Miller Fisher syndrome associated with a Zika virus infection. Eur J Neurol. 2018;25(2):e20–e21. doi:10.1111/ene.13514.

[529] Gonzalez-Escobar G, Valadere AM, Adams R, Polson-Edwards K, Hinds AQJ, Misir A, et al. Prolonged Zika virus viremia in a patient with Guillain-Barré syndrome in Trinidad and Tobago. Rev Panam Salud Publica. 2018;41:e136.

[530] Ikejezie J, Shapiro CN, Kim J, Chiu M, Almiron M, Ugarte C, et al. Zika Virus Transmission — Region of the Americas, May 15, 2015–December 15, 2016. MMWR Morb Mortal Wkly Rep. 2017;66(12):329–334. doi:10.15585/mmwr.mm6612a4.

[531] Jones M. Multidisciplinary approach to Guillain-Barre Syndrome and neurologic injuries after zika virus. J Spinal Cord Med. 2018;41(5):588. doi:10.1080/10790268.2018.1498265.

[532] Leonhard SE, Munts AG, Van Der Eijk AA, Jacobs BC. Acute-onset chronic inflammatory demyelinating polyneuropathy after Zika virus infection. J Neurol Neurosurg Psychiatry. 2018;89(10):1118–1119. doi:10.1136/jnnp-2017-317346.

[533] Lynch RM, Mantus G, Encinales L, Pacheco N, Li G, Porras A, et al. Augmented zika and dengue neutralizing antibodies are associated with guillain-Barré syndrome. J Infect Dis.

2019;219(1):26–30. doi:10.1093/infdis/jiy466.

[534] Malta JMAS, Vargas A, Leite PLe, Percio J, Coelho GE, Ferraro AHA, et al. Síndrome de Guillain-Barré e outras manifestações neurológicas possivelmente relacionadas à infecção pelo vírus Zika em municípios da Bahia, 2015. Epidemiol e Serviços Saúde. 2017;26(01):09–18. doi:10.5123/S1679-49742017000100002.

[535] Mancera-Páez O, Román GC, Pardo-Turriago R, Rodríguez Y, Anaya JM. Concurrent Guillain-Barré syndrome, transverse myelitis and encephalitis post-Zika: A case report and review of the pathogenic role of multiple arboviral immunity. J Neurol Sci. 2018;395:47–53. doi:10.1016/j.jns.2018.09.028.

[536] Mehta R, Soares CN, Medialdea-Carrera R, Ellul M, da Silva MTT, Rosala-Hallas A, 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(2):e0006212. doi:10.1371/journal.pntd.0006212.

[537] Miller E, Becker Z, Shalev D, Lee CT, Cioroiu C, Thakur K. Probable Zika virus-associated Guillain-Barré syndrome: Challenges with clinico-laboratory diagnosis. J Neurol Sci. 2017;375:367–370. doi:10.1016/j.jns.2017.02.029.

[538] Muñoz LS, Barreras P, Lizarazo J, Jimenez-Arango JA, Gonzalez-Manrique G, Zea-Vera AF, et al. Neuroviruses Emerging in the Americas Study(NEAS): The Colombian experience during the 2016 outbreak of Zika virus infection. Neurology. 2017;88(16).

[539] da Nóbrega MEB, Araújo ELdL, Wada MY, Leite PLe, Dimech GS, Pércio J. Surto de síndrome de Guillain-Barré possivelmente relacionado à infecção prévia pelo vírus Zika, Região Metropolitana do Recife, Pernambuco, Brasil, 2015. Epidemiol e Serviços Saúde. 2018;27(2):e2017039. doi:10.5123/s1679-49742018000200016.

[540] Raboni SM, Bonfim C, Almeida BM, Zanluca C, Koishi AC, Rodrigues PRVP, et al. Flavivirus cross-reactivity in serological tests and Guillain-Barré syndrome in a hematopoietic stem cell transplant patient: A case report. Transpl Infect Dis. 2017;19(4). doi:10.1111/tid.12700.

[541] Resiere D, Fergé JL, Fabre J, Raad M, Aitsatou S, Inamo J, et al. Cardiovascular complications in patients with Zika virus-induced Guillain-Barré syndrome. J Clin Virol. 2018;98:8–9. doi:10.1016/j.jcv.2017.11.002.

[542] Rivera-Concepcion JR, Betancourt JP, Cerra J, Reyes E. The Zika Virus: An Association to Guillain-Barre Syndrome in the United States - A Case Report. P R Heal Sci J. 2018;37(Special Issue):S93–s95.

[543] Rozé B, Najioullah F, Fergé JL, Dorléans F, Apetse K, Barnay JL, et al. Guillain-Barré Syndrome Associated with Zika Virus Infection in Martinique in 2016: A Prospective Study. Clin Infect Dis. 2017;65(9):1462–1468. doi:10.1093/cid/cix588.

[544] Salinas JL, Walteros DM, Styczynski A, Garzón F, Quijada H, Bravo E, et al. Zika virus disease-associated Guillain-Barré syndrome—Barranquilla, Colombia 2015–2016. J Neurol Sci. 2017;381:272–277. doi:10.1016/j.jns.2017.09.001.

[545] Santos P, Nogueira F, Modenezi L, Andorinho AC, C Pupe, Silva I, et al. Movement disorder development later after acute disseminated encephalomyelitis associated with Zika virus: Case report. Neurology. 2017;not found(not found):not found.

[546] Sebastián UU, Ricardo AVA, Alvarez BC, Cubides A, Luna AF, Arroyo-Parejo M, et al. Zika virus-induced neurological critical illness in Latin America: Severe Guillain-Barre Syndrome and encephalitis. J Crit Care. 2017;42:275–281. doi:10.1016/j.jcrc.2017.07.038.

[547] Simon O, Acket B, Forfait C, Girault D, Gourinat AC, Millon P, et al. Zika virus outbreak in New Caledonia and Guillain-Barré syndrome: a case-control study. J Neurovirol. 2018;24(3):362–368. doi:10.1007/s13365-018-0621-9.

[548] Siwarit R. Guillain-Barre syndrome in the Context of Zika Virus Infection:the First Report Case in Thailand. Buddhachinaraj Medical Journal. 2017;34(2):Sep 14.

[549] Soto-Hernandez JL, de Leon Rosales S, Vargas Canas ES, Cardenas G, Carrillo Loza K, Diaz-Quinonez JA, et al. Guillain-Barre Syndrome Associated With Zika Virus Infection: A Prospective Case Series From Mexico. Front Neurol. 2019;10:435. doi:10.3389/fneur.2019.00435.

[550] Styczynski AR, Malta JMAS, Krow-Lucal ER, Percio J, Nóbrega ME, Vargas A, et al. Increased rates of Guillain-Barré syndrome associated with Zika virus outbreak in the Salvador metropolitan area, Brazil. PLoS Negl Trop Dis. 2017;11(8):e0005869. doi:10.1371/journal.pntd.0005869.

[551] Tantillo G, Sclar G, Vasa C, Shin S, Sivak M. Zika associated Guillain-Barre syndrome in the United States (P2. 327). Neurology. 2017;88(16 Supplement):P2–327.

[552] Tolosa N, Tinker SC, Pacheco O, Valencia D, Botero DS, Tong VT, et al. Zika Virus Disease in Children in Colombia, August 2015 to May 2016. Paediatr Perinat Epidemiol. 2017;31(6):537–545. doi:10.1111/ppe.12391.

[553] Uncini A, González-Bravo DC, Acosta-Ampudia YY, Ojeda EC, Rodríguez Y, Monsalve DM, et al. Clinical and nerve conduction features in Guillain—Barré syndrome associated with Zika virus infection in Cúcuta, Colombia. Eur J Neurol. 2018;25(4):644–650. doi:10.1111/ene.13552.

[554] Vieira MAdCES, Costa CHN, Linhares AdC, Borba AdS, Henriques DF, da Silva EVP, et al. Potential role of dengue virus, chikungunya virus and Zika virus in neurological diseases. Mem Inst Oswaldo Cruz. 2018;113(11):e170538. doi:10.1590/0074-02760170538.

[555] Vieira MAdCeS, Cruz ACR, Barros ANM, Costa DL, da Silva EVP, Batista FMdA, et al. Guillain-barré syndrome and dengue-like disease in 2015: Temporal relationship in piauí state and implications on zika virus surveillance. Rev Inst Med Trop Sao Paulo. 2017;59:e22. doi:10.1590/S1678-9946201759022.

[556] Villamil-Gomez WE, Sánchez-Herrera ÁR, Hernandez H, Hernández-Iriarte J, Díaz-Ricardo K, Castellanos J, et al. Guillain-Barré syndrome during the Zika virus outbreak in Sucre, Colombia, 2016. Travel Med Infect Dis. 2017;16:62–63. doi:10.1016/j.tmaid.2017.03.012.

[557] Wright JK, Castellani L, Lecce C, Khatib A, Bonta M, Boggild AK. Zika Virus-Associated Aseptic Meningitis and Guillain–Barre Syndrome in a Traveler Returning from Latin America: a Case Report and Mini-Review. Curr Infect Dis Rep. 2019;21(1):3. doi:10.1007/s11908-019-0661-1.

[558] Wu Y, Cui X, Wu N, Song R, Yang W, Zhang W, et al. A unique case of human Zika virus infection in association with severe liver injury and coagulation disorders. Sci Rep. 2017;7(1):11393. doi:10.1038/s41598-017-11568-4.

[559] Zambrano LI, Fuentes-Barahona IC, Soto-Fernandez RJ, Zuniga C, da Silva JC, Rodriguez-Morales AJ. Guillain-Barre syndrome associated with Zika virus infection in Honduras, 2016-2017. Int J Infect Dis. 2019;84:136–137. doi:10.1016/j.ijid.2019.05.008.

[560] Costa Monteiro LM, De Oliveira Cruz GN, Fontes JM, Saad Salles TRD, Bastos Boechat MC, Monteiro AC, et al. Neurogenic bladder findings in patients with congenital zika syndrome: A novel condition. PLoS One. 2018;13(3):e0193514. doi:10.1371/journal.pone.0193514.

[561] Riley D. 02B. Case Reports in an Evidence-based World. Glob Adv Heal Med. 2013;2(1_suppl):gahmj.2013.097C. doi:10.7453/gahmj.2013.097cp.s02b.

[562] Kopec JA, Esdaile JM. Bias in case-control studies. A review. J Epidemiol Community Health. 1990;44(3):179–86.

[563] Lipton P. Inference to the Best Explanation. Routledge; 2003.

[564] Grubaugh ND, Ishtiaq F, Setoh YX, Ko AI. Misperceived Risks of Zika-related Microcephaly in India. Trends Microbiol. 2019;27(5):381–383. doi:10.1016/j.tim.2019.02.004.

[565] Nutt C, Adams P. Zika in Africa-the invisible epidemic? Lancet (London, England). 2017;389(10079):1595–1596. doi:10.1016/S0140-6736(17)31051-6.

[566] Petersen EE, Meaney-Delman D, Neblett-Fanfair R, Havers F, Oduyebo T, Hills SL, et al. Update: Interim Guidance for Preconception Counseling and Prevention of Sexual Transmission of Zika Virus for Persons with Possible Zika Virus Exposure — United States, September 2016. MMWR Morb Mortal Wkly Rep. 2016;65(39):1077–1081. doi:10.15585/mmwr.mm6539e1.

[567] Chambers T. Flavivirus Genome Organization, Expression, And Replication. Annu Rev Microbiol. 1990;44(1):649–688. doi:10.1146/annurev.micro.44.1.649.

[568] Moreira J, Peixoto TM, Siqueira AM, Lamas CC. Sexually acquired Zika virus: a systematic review. Clinical Microbiology and Infection. 2017;23(5):296–305. doi:10.1016/j.cmi.2016.12.027.

[569] Hastings AK, Fikrig E. Zika virus and sexual transmission: A new route of transmission for mosquito-borne flaviviruses. Yale J Biol Med. 2017;90(2):325–330.

[570] Kuno G, Chang GJ, Tsuchiya KR, Karabatsos N, Cropp CB. Phylogeny of the genus Flavivirus. J Virol. 1998;72(1):73–83.

[571] Barbosa CM, Di Paola N, Cunha MP, Rodrigues-Jesus MJ, Araujo DB, Silveira VB, et al. Yellow fever virus RNA in urine and semen of convalescent patient, Brazil. Emerg Infect Dis. 2018;24(1):176–178. doi:10.3201/eid2401.171310.

[572] Salam AP, Horby PW. The breadth of viruses in human semen. Emerg Infect Dis. 2017;23(11):1922–1924. doi:10.3201/eid2311.171049.

[573] Giesecke J. Modern infectious disease epidemiology: Third edition. CRC Press; 2017.

[574] Calvet GA, Kara EO, Giozza SP, Bôtto-Menezes CHA, Gaillard P, de Oliveira Franca RF, et al. Study on the persistence of Zika virus (ZIKV) in body fluids of patients with ZIKV infection in Brazil. BMC Infect Dis. 2018;18(1):49. doi:10.1186/s12879-018-2965-4.

[575] Gómez G, Calle ML, Oller R, Langohr K. Tutorial on methods for interval-censored data and their implementation in R. Stat Modelling. 2009;9(4):259–297. doi:10.1177/1471082X0900900402.

[576] Gu X, Shapiro D, Hughes D Michael, Balasubramanian R. Stratified Weibull Regression Model for Interval-Censored Data. R J. 2019;6(1):31. doi:10.32614/rj-2014-003.

[577] National Institutes of Health. NIH - Quality Assessment Tool for Case Series Studies; 2014. [Online]. Available from: https://www.nhlbi.nih.gov/health-pro/guidelines/in-develop/cardiovascular-risk-reduction/tools/case_series.

[578] National Institute for Health and Care Excellence. Guideline Development Methods: Information for National Collaborating Centres and Guideline Developers; 2006. [Online]. Available from: http://www.nice.org.uk.

[579] Chen KY, Ma B, Wang YN, Chen CH, Zhao YQ, Zheng JX, et al. SYRCLE's risk of bias tool for animal studies. Chinese J Evidence-Based Med. 2014;14(10):1281–1285. doi:10.7507/1672-2531.20140206.

[580] Jaime Caro J, Eddy DM, Kan H, Kaltz C, Patel B, Eldessouki R, et al. Questionnaire to assess relevance and credibility of modeling studies for informing health care decision making: An ISPOR-AMCP-NPC good practice task force report. Value Heal. 2014;17(2):174–182. doi:10.1016/j.jval.2014.01.003.

[581] Balshem H, Helfand M, Schünemann HJ, Oxman AD, Kunz R, Brozek J, et al. GRADE guidelines: 3. Rating the quality of evidence. J Clin Epidemiol. 2011;64(4):401–6. doi:10.1016/j.jclinepi.2010.07.015.

[582] Egger M, Johnson L, Althaus C, Schöni A, Salanti G, Low N, et al. Developing WHO guidelines: Time to formally include evidence from mathematical modelling studies. F1000Research. 2018;6:1584. doi:10.12688/f1000research.12367.2.

[583] Guyatt GH, Oxman AD, Sultan S, Glasziou P, Akl EA, Alonso-Coello P, et al. GRADE guidelines: 9. Rating up the quality of evidence. J Clin Epidemiol. 2011;64(12):1311–1316. doi:10.1016/j.jclinepi.2011.06.004.

[584] Aid M, Abbink P, Larocca RA, Boyd M, Nityanandam R, Nanayakkara O, et al. Zika Virus Persistence in the Central Nervous System and Lymph Nodes of Rhesus Monkeys. Cell. 2017;169(4):610–620.e14. doi:10.1016/j.cell.2017.04.008.

[585] Bagasra O, Addanki KC, Goodwin GR, Hughes BW, Pandey P, McLean E. Cellular Targets and Receptor of Sexual Transmission of Zika Virus. Appl Immunohistochem Mol Morphol. 2017;25(10):679–686. doi:10.1097/PAI.0000000000000580.

[586] Carroll T, Lo M, Lanteri M, Dutra J, Zarbock K, Silveira P, et al. Zika virus preferentially replicates in the female reproductive tract after vaginal inoculation of rhesus macaques. PLoS Pathog. 2017;13(7):e1006537. doi:10.1371/journal.ppat.1006537.

[587] Chiu CY, Sánchez-San Martín C, Bouquet J, Li T, Yagi S, Tamhankar M, et al. Experimental Zika Virus Inoculation in a New World Monkey Model Reproduces Key Features of the Human Infection. Sci Rep. 2017;7(1):17126. doi:10.1038/s41598-017-17067-w.

[588] Coffey LL, Pesavento PA, Keesler RI, Singapuri A, Watanabe J, Watanabe R, et al. Zika virus tissue and blood compartmentalization in acute infection of rhesus macaques. PLoS One. 2017;12(1):e0171148. doi:10.1371/journal.pone.0171148.

[589] Deng YQ, Zhang NN, Li XF, Wang YQ, Tian M, Qiu YF, et al. Intranasal infection and contact transmission of Zika virus in Guinea pigs. Nat Commun. 2017;8(1):1648. doi:10.1038/s41467-017-01923-4.

[590] Dudley DM, Aliota MT, Mohr EL, Weiler AM, Lehrer G, Weisgrau KL, et al. Natural history of Asian lineage Zika virus infection in macaques 1 2. bioRxiv. 2016;doi:10.1101/046334.

[591] Duggal NK, McDonald EM, Ritter JM, Brault AC. Sexual transmission of Zika virus enhances in utero transmission in a mouse model. Sci Rep. 2018;8(1):4510. doi:10.1038/s41598-018-22840-6.

[592] Duggal NK, Ritter JM, Pestorius SE, Zaki SR, Davis BS, Chang GJJ, et al. Frequent Zika Virus Sexual Transmission and Prolonged Viral RNA Shedding in an Immunodeficient Mouse Model. Cell Rep. 2017;18(7):1751–1760. doi:10.1016/j.celrep.2017.01.056.

[593] Govero J, Esakky P, Scheaffer SM, Fernandez E, Drury A, Platt DJ, et al. Zika virus infection damages the testes in mice. Nature. 2016;540(7633):438–442. doi:10.1038/nature20556.

[594] Griffin BD, Muthumani K, Warner BM, Majer A, Hagan M, Audet J, et al. DNA vaccination protects mice against

Zika virus-induced damage to the testes. Nat Commun. 2017;8:15743. doi:10.1038/ncomms15743.

[595] Haddow AD, Nalca A, Rossi FD, Miller LJ, Wiley MR, Perez-Sautu U, et al. High infection rates for adult macaques after intravaginal or intrarectal inoculation with zika virus. Emerg Infect Dis. 2017;23(8):1274–1281. doi:10.3201/eid2308.170036.

[596] Hastings AK, Yockey LJ, Jagger BW, Hwang J, Uraki R, Gaitsch HF, et al. TAM Receptors Are Not Required for Zika Virus Infection in Mice. Cell Rep. 2017;19(3):558–568. doi:10.1016/j.celrep.2017.03.058.

[597] Julander JG, Siddharthan V, Evans J, Taylor R, Tolbert K, Apuli C, et al. Efficacy of the broad-spectrum antiviral compound BCX4430 against Zika virus in cell culture and in a mouse model. Antiviral Res. 2017;137:14–22. doi:10.1016/j.antiviral.2016.11.003.

[598] Khan S, Woodruff EM, Trapecar M, Fontaine KA, Ezaki A, Borbet TC, et al. Dampened antiviral immunity to intravaginal exposure to RNA viral pathogens allows enhanced viral replication. J Exp Med. 2016;213(13):2913–2929. doi:10.1084/jem.20161289.

[599] Koide F, Goebel S, Snyder B, Walters KB, Gast A, Hagelin K, et al. Development of a zika virus infection model in cynomolgus macaques. Front Microbiol. 2016;7(DEC):2028. doi:10.3389/fmicb.2016.02028.

[600] Lazear HM, Govero J, Smith AM, Platt DJ, Fernandez E, Miner JJ, et al. A Mouse Model of Zika Virus Pathogenesis. Cell Host Microbe. 2016;19(5):720–730. doi:10.1016/j.chom.2016.03.010.

[601] Ma W, Li S, Ma S, Jia L, Zhang F, Zhang Y, et al. Zika Virus Causes Testis Damage and Leads to Male Infertility in Mice. Cell. 2016;167(6):1511–1524.e10. doi:10.1016/j.cell.2016.11.016.

[602] Martínez LE, Jr GG, Contreras D, Gong D, Sun R. Pathogenesis of Zika Virus Infection via Rectal Route. bioRxiv. 2017; p. 1–23. doi:10.1101/128876.

[603] McDonald EM, Duggal NK, Brault AC. Pathogenesis and sexual transmission of Spondweni and Zika viruses. PLoS Negl Trop Dis. 2017;11(10):e0005990. doi:10.1371/journal.pntd.0005990.

[604] Newman CM, Dudley DM, Aliota MT, Weiler AM, Barry GL, Mohns MS, et al. Oropharyngeal mucosal transmission of Zika virus in rhesus macaques. Nat Commun. 2017;8(1):169. doi:10.1038/s41467-017-00246-8.

[605] Osuna CE, Lim SY, Deleage C, Griffin BD, Stein D, Schroeder LT, et al. Corrigendum: Zika viral dynamics and shedding in rhesus and cynomolgus macaques. Nat Med. 2017;23(2):264. doi:10.1038/nm0217-264b.

[606] Cyr DR. Scientific Abstracts. J Diagnostic Med Sonogr. 1989;5(4):198–200. doi:10.1177/875647938900500416.

[607] Rossi SL, Tesh RB, Azar SR, Muruato AE, Hanley KA, Auguste AJ, et al. Characterization of a novel murine model to study zika virus. Am J Trop Med Hyg. 2016;94(6):1362–1369. doi:10.4269/ajtmh.16-0111.

[608] Scott JM, Lebratti TJ, Richner JM, Jiang X, Fernandez E, Zhao H, et al. Cellular and Humoral Immunity Protect against Vaginal Zika Virus Infection in Mice. J Virol. 2018;92(7). doi:10.1128/jvi.00038-18.

[609] Sheng ZY, Gao N, Wang ZY, Cui XY, Zhou DS, Fan DY, et al. Sertoli Cells Are Susceptible to ZIKV Infection in Mouse Testis. Front Cell Infect Microbiol. 2017;7:272. doi:10.3389/fcimb.2017.00272.

[610] Siemann DN, Strange DP, Maharaj PN, Shi PY, Verma S. Zika Virus Infects Human Sertoli Cells and Modulates the Integrity of the In Vitro Blood-Testis Barrier Model . J Virol. 2017;91(22). doi:10.1128/jvi.00623-17.

[611] Simanjuntak Y, Liang JJ, Chen SY, Li JK, Lee YL, Wu HC, et al. Ebselen alleviates testicular pathology in mice with Zika virus infection and prevents its sexual transmission. PLoS Pathog. 2018;14(2):e1006854. doi:10.1371/journal.ppat.1006854.

[612] Spencer JL, Lahon A, Tran LL, Arya RP, Kneubehl AR, Vogt MB, et al. Replication of Zika virus in human prostate cells: A potential source of sexually transmitted virus. J Infect Dis. 2018;217(4):538–547. doi:10.1093/infdis/jix436.

[613] Tang WW, Young MP, Mamidi A, Regla-Nava JA, Kim K, Shresta S. A Mouse Model of Zika Virus Sexual Transmission and Vaginal Viral Replication. Cell Rep. 2016;17(12):3091–3098. doi:10.1016/j.celrep.2016.11.070.

[614] Uraki R, Hwang J, Jurado KA, Householder S, Yockey LJ, Hastings AK, et al. Zika virus causes testicular atrophy. Sci Adv. 2017;3(2):e1602899. doi:10.1126/sciadv.1602899.

[615] Winkler CW, Woods TA, Rosenke R, Scott DP, Best SM, Peterson KE. Sexual and Vertical Transmission of Zika Virus in anti-interferon receptor-treated Rag1-deficient mice. Sci Rep. 2017;7(1):7176. doi:10.1038/s41598-017-07099-7.

[616] Xu K, Song Y, Dai L, Zhang Y, Lu X, Xie Y, et al. Recombinant Chimpanzee Adenovirus Vaccine AdC7-M/E Protects against Zika Virus Infection and Testis Damage. J Virol. 2018;92(6). doi:10.1128/jvi.01722-17.

[617] Kumar A, Jovel J, Lopez-Orozco J, Limonta D, Airo AM, Hou S, et al. Human sertoli cells support high levels of zika virus replication and persistence. Sci Rep. 2018;8(1):5477. doi:10.1038/s41598-018-23899-x.

[618] Clancy CS, Van Wettere AJ, Siddharthan V, Morrey JD, Julander JG. Comparative Histopathologic Lesions of the Male Reproductive Tract during Acute Infection of Zika Virus in AG129 and Ifnar —/— Mice. Am J Pathol. 2018;188(4):904–915. doi:10.1016/j.ajpath.2017.12.019.

[619] Mead PS, Duggal NK, Hook SA, Delorey M, Fischer M, Olzenak McGuire D, et al. Zika Virus Shedding in Semen of Symptomatic Infected Men. N Engl J Med. 2018;378(15):1377–1385. doi:10.1056/NEJMoa1711038.

[620] Armstrong P, Hennessey M, Adams M, Cherry C, Chiu S, Harrist A, et al. Travel-Associated Zika Virus Disease Cases Among U.S. Residents — United States, January 2015–February 2016. MMWR Morb Mortal Wkly Rep. 2016;65(11):286–289. doi:10.15585/mmwr.mm6511e1.

[621] Arsuaga M, Bujalance SG, Díaz-Menéndez M, Vázquez A, Arribas JR. Probable sexual transmission of Zika virus from a vasectomised man. Lancet Infect Dis. 2016;16(10):1107. doi:10.1016/S1473-3099(16)30320-6.

[622] Atkinson B, Thorburn F, Petridou C, Bailey D, Hewson R, Simpson AJH, et al. Presence and persistence of zika virus RNA in semen, United Kingdom, 2016. Emerg Infect Dis. 2017;23(4):611–615. doi:10.3201/eid2304.161692.

[623] Barzon L, Pacenti M, Berto A, Sinigaglia A, Franchin E, Lavezzo E, et al. Isolation of infectious zika virus from saliva and prolonged viral RNA shedding in a traveller returning from the dominican republic to Italy, January 2016. Eurosurveillance. 2016;21(10):30159. doi:10.2807/1560-7917.

[624] Barzon L, Pacenti M, Franchin E, Lavezzo E, Trevisan M, Sgarabotto D, et al. Infection dynamics in a traveller with persistent shedding of Zika virus RNA in semen for six months after returning from Haiti to Italy, January 2016. Eurosurveillance. 2016;21(32):0. doi:10.2807/1560-7917.ES.2016.21.32.30316.

[625] Barzon L, Percivalle E, Pacenti M, Rovida F, Zavattoni M, Del Bravo P, et al. Virus and Antibody Dynamics in Travelers with Acute Zika Virus Infection. Clin Infect Dis. 2018;66(8):1173–1180. doi:10.1093/cid/cix967.

[626] Biava M, Caglioti C, Castilletti C, Bordi L, Carletti F, Colavita F, et al. Persistence of ZIKV-RNA in the cellular fraction of semen is accompanied by a surrogate-marker

of viral replication. Diagnostic implications for sexual transmission. New Microbiol. 2018;41(1):30–33.

[627] Bonaldo MC, Ribeiro IP, Lima NS, dos Santos AAC, Menezes LSR, da Cruz SOD, et al. Isolation of Infective Zika Virus from Urine and Saliva of Patients in Brazil. PLoS Negl Trop Dis. 2016;10(6):e0004816. doi:10.1371/journal.pntd.0004816.

[628] Borrego BV, Kosanic K, de Ory F, Merino Fernández FJ, Rodríguez BG. Zika virus infection acquired through sexual contact: First documented case of local transmission in Spain. Emergencias. 2017;29(4):290–291.

[629] Brooks RB, Carlos MP, Myers RA, White MG, Bobo-Lenoci T, Aplan D, et al. Likely Sexual Transmission of Zika Virus from a Man with No Symptoms of Infection — Maryland, 2016. MMWR Morb Mortal Wkly Rep. 2016;65(34):915–916. doi:10.15585/mmwr.mm6534e2.

[630] Cassuto NG, Marras G, Jacomo V, Bouret D. Persistence of Zika virus in gradient sperm preparation. J Gynecol Obstet Hum Reprod. 2018;47(5):211–212. doi:10.1016/j.jogoh.2018.02.004.

[631] D'Ortenzio E, Matheron S, de Lamballerie X, Hubert B, Piorkowski G, Maquart M, et al. Evidence of Sexual Transmission of Zika Virus. N Engl J Med. 2016;374(22):2195–2198. doi:10.1056/NEJMc1604449.

[632] Davidson A, Slavinski S, Komoto K, Rakeman J, Weiss D. Suspected Female-to-Male Sexual Transmission of Zika Virus — New York City, 2016. MMWR Morb Mortal Wkly Rep. 2016;65(28):716–717. doi:10.15585/mmwr.mm6528e2.

[633] de Laval F, Matheus S, Labrousse T, Enfissi A, Rousset D, Briolant S. Kinetics of Zika Viral Load in Semen. N Engl J Med. 2017;377(7):697–699. doi:10.1056/NEJMc1612600.

[634] Deckard DT, Chung WM, Brooks JT, Smith JC, Woldai S, Hennessey M, et al. Male-to-Male Sexual Transmission of Zika Virus — Texas, January 2016. MMWR Morb Mortal Wkly Rep. 2016;65(14):372–374. doi:10.15585/mmwr.mm6514a3.

[635] Desclaux A, de Lamballerie X, Leparc-Goffart I, Vilain-Parcé A, Coatleven F, Fleury H, et al. Probable Sexually Transmitted Zika Virus Infection in a Pregnant Woman. N Engl J Med. 2018;378(15):1458–1460. doi:10.1056/nejmc1710453.

[636] Duijster JW, Brandwagt DAH, Timen A, van der Eijk AA, Vennema H, Te Wierik MJM. [Zika virus and sexual transmission; when and for how long does one need to use a condom?]. Ned Tijdschr Geneeskd. 2017;161(0):D1622.

[637] Fonseca K, Meatherall B, Zarra D, Drebot M, MacDonald J, Pabbaraju K, et al. Case report: First case of Zika virus infection in a returning Canadian traveler. Am J Trop Med Hyg. 2014;91(5):1035–1038. doi:10.4269/ajtmh.14-0151.

[638] Frank C, Cadar D, Schlaphof A, Neddersen N, Günther S, Schmidt-Chanasit J, et al. Sexual transmission of Zika virus in Germany, April 2016. Eurosurveillance. 2016;21(23):0. doi:10.2807/1560-7917.ES.2016.21.23.30252.

[639] Fréour T, Mirallié S, Hubert B, Splingart C, Barrière P, Maquart M, et al. Sexual transmission of Zika virus in an entirely asymptomatic couple returning from a Zika epidemic area, France, April 2016. Eurosurveillance. 2016;21(23):0. doi:10.2807/1560-7917.ES.2016.21.23.30254.

[640] Froeschl G, Huber K, von Sonnenburg F, Nothdurft HD, Bretzel G, Hoelscher M, et al. Long-term kinetics of Zika virus RNA and antibodies in body fluids of a vasectomized traveller returning from Martinique: A case report. BMC Infect Dis. 2017;17(1):55. doi:10.1186/s12879-016-2123-9.

[641] García-Bujalance S, Gutiérrez-Arroyo A, De la Calle F, Díaz-Menéndez M, Arribas JR, García-Rodríguez J, et al. Persistence and infectivity of Zika virus in semen after returning from endemic areas: Report of 5 cases. J Clin Virol. 2017;96:110–115. doi:10.1016/j.jcv.2017.10.006.

[642] Gaskell KM, Houlihan C, Nastouli E, Checkley AM. Persistent zika virus detection in semen in a traveler returning to the United Kingdom from Brazil, 2016. Emerg Infect Dis. 2017;23(1):137–139. doi:10.3201/eid2301.161300.

[643] Grossi PA, Percivalle E, Campanini G, Sarasini A, Premoli M, Zavattoni M, et al. An autochthonous sexually transmitted Zika virus infection in Italy 2016. New Microbiol. 2018;41(1):80–82.

[644] Gulland A. First case of Zika virus spread through sexual contact is detected in UK. BMJ. 2016;355:i6500. doi:10.1136/bmj.i6500.

[645] Harrower J, Kiedrzynski T, Baker S, Upton A, Rahnama F, Sherwood J, et al. Sexual Transmission of Zika Virus and Persistence in Semen, New Zealand, 2016. Emerg Infect Dis. 2016;22(10):1855–1857. doi:10.3201/eid2210.160951.

[646] Hearn PT, Atkinson B, Hewson R. Identification of the first case of imported Zika Fever to the UK: A novel sample type for diagnostic purposes and support for a potential non-vectorborne route of transmission. Am J Trop Med Hyg. 2014;91(5 SUPPL. 1):62–63. doi:10.4269/ajtmh.abstract2017.

[647] Hills SL, Russell K, Hennessey M, Williams C, Oster AM, Fischer M, et al. Transmission of Zika Virus Through Sexual Contact with Travelers to Areas of Ongoing Transmission — Continental United States, 2016. MMWR Morb Mortal Wkly Rep. 2016;65(8):215–216. doi:10.15585/mmwr.mm6508e2.

[648] Huits R, De Smet B, Ariën KK, Van Esbroeck M, Bottieau E, Cnops L. Zika virus in semen: a prospective cohort study of symptomatic travellers returning to Belgium. Bull World Health Organ. 2017;95(12):802–809. doi:10.2471/BLT.17.181370.

[649] Huits RM, De Smet B, Ariën KK, Van Esbroeck M, de Jong BC, Bottieau E, et al. Kinetics of Zika virus persistence in semen. Bull World Health Organ. 2016;doi:10.2471/blt.16.181370.

[650] Iovine NM, Lednicky J, Cherabuddi K, Crooke H, White SK, Loeb JC, 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(1):72–75. doi:10.1093/cid/ciw667.

[651] Jang HC, Park WB, Kim UJ, Chun JY, Choi SJ, Choe PG, et al. First imported case of Zika virus infection into Korea. J Korean Med Sci. 2016;31(7):1173–1177. doi:10.3346/jkms.2016.31.7.1173.

[652] Jia H, Zhang M, Chen M, Yang Z, Li J, Huang G, et al. Zika virus infection in travelers returning from countries with local transmission, Guangdong, China, 2016. Travel Med Infect Dis. 2018;21:56–61. doi:10.1016/j.tmaid.2017.11.012.

[653] Joguet G, Mansuy JM, Matusali G, Hamdi S, Walschaerts M, Pavili L, et al. Effect of acute Zika virus infection on sperm and virus clearance in body fluids: a prospective observational study. Lancet Infect Dis. 2017;17(11):1200–1208. doi:10.1016/S1473-3099(17)30444-9.

[654] Mansuy JM, Dutertre M, Mengelle C, Fourcade C, Marchou B, Delobel P, et al. Zika virus: high infectious viral load in semen, a new sexually transmitted pathogen? Lancet Infect Dis. 2016;16(4):405. doi:10.1016/s1473-3099(16)00138-9.

[655] Mansuy JM, Pasquier C, Daudin M, Chapuy-Regaud S, Moinard N, Chevreau C, et al. Zika virus in semen of a patient returning from a non-epidemic area. Lancet Infect Dis. 2016;16(8):894–895. doi:10.1016/s1473-3099(16)30153-0.

[656] Mansuy JM, Suberbielle E, Chapuy-Regaud S, Mengelle C, Bujan L, Marchou B, et al. Zika virus in semen and spermatozoa. Lancet Infect Dis. 2016;16(10):1106–1107. doi:10.1016/S1473-3099(16)30336-X.

[657] Maria AT, Maquart M, Makinson A, Flusin O, Segondy M, Leparc-Goffart I, et al. Zika virus infections in three travellers returning from South America and the Caribbean respectively, to Montpellier, France, December 2015 to January 2016. Eurosurveillance. 2016;21(6). doi:10.2807/1560-7917.ES.2016.21.6.30131.

[658] Matheron S, D'Ortenzio E, Leparc-Goffart I, Hubert B, de Lamballerie X, Yazdanpanah Y. Long-Lasting Persistence of Zika Virus in Semen. Clin Infect Dis. 2016;63(9):ciw509. doi:10.1093/cid/ciw509.

[659] Murray KO, Gorchakov R, Carlson AR, Berry R, Lai L, Natrajan M, et al. Prolonged detection of zika virus in vaginal secretions and whole blood. Emerg Infect Dis. 2017;23(1):99–101. doi:10.3201/eid2301.161394.

[660] Nicastri E, Castilletti C, Balestra P, Galgani S, Ippolito G. Zika Virus Infection in the Central Nervous System and Female Genital Tract. Emerg Infect Dis. 2016;22(12):2228–2230. doi:10.3201/eid2212.161280.

[661] Nicastri E, Liuzzi G, Castilletti C, Capobianchi MR, Ippolito G, Iannetta M. Persistent detection of Zika virus RNA in semen for six months after symptom onset in a traveller returning from Haiti to Italy, February 2016. Eurosurveillance. 2016;21(32):1–4. doi:10.2807/1560-7917.es.2016.21.32.30314.

[662] Oliveira Souto I, Alejo-Cancho I, Gascón Brustenga J, Peiró Mestres A, Muñoz Gutiérrez J, Martínez Yoldi MJ. Persistence of Zika virus in semen 93 days after the onset of symptoms. Enferm Infecc Microbiol Clin. 2018;36(1):21–23. doi:10.1016/j.eimc.2016.10.009.

[663] Percivalle E, Zavattoni M, Fausto F, Rovida F. Zika virus isolation from semen. New Microbiol. 2017;40(3):197–198.

[664] Prisant N, Breurec S, Moriniere C, Bujan L, Joguet G. Zika virus genital tract shedding in infected women of childbearing age. Clin Infect Dis. 2017;64(1):107–109. doi:10.1093/cid/ciw669.

[665] Visseaux B, Mortier E, Houhou-Fidouh N, Brichler S, Collin G, Larrouy L, et al. Zika virus in the female genital tract. Lancet Infect Dis. 2016;16(11):1220. doi:10.1016/s1473-3099(16)30387-5.

[666] Reusken C, Pas S, Geurtsvankessel C, Mögling R, van Kampen J, Langerak T, et al. Longitudinal follow-up of Zika virus RNA in semen of a traveller returning from Barbados to the Netherlands with Zika virus disease, march 2016. Eurosurveillance. 2016;21(23). doi:10.2807/1560-7917.ES.2016.21.23.30251.

[667] Russell K, Hills SL, Oster AM, Porse CC, Danyluk G, Cone M, et al. Male-to-female sexual transmission of zika virus-United States, January-April 2016. Clin Infect Dis. 2017;64(2):211–213. doi:10.1093/cid/ciw692.

[668] Sánchez-Montalvá A, Pou D, Sulleiro E, Salvador F, Bocanegra C, Treviño B, et al. Zika virus dynamics in body fluids and risk of sexual transmission in a non-endemic area. Trop Med Int Heal. 2018;23(1):92–100. doi:10.1111/tmi.13019.

[669] Sun J, Wu D, Zhong H, Guan D, Zhang H, Tan Q, et al. Returning ex-patriot Chinese to Guangdong, China, increase the risk for local transmission of Zika virus. J Infect. 2017;75(4):356–367. doi:10.1016/j.jinf.2017.07.001.

[670] Torres JR, Martínez N, Moros Z. Microhematospermia in acute Zika virus infection. Int J Infect Dis. 2016;51:127. doi:10.1016/j.ijid.2016.08.025.

[671] Turmel JM, Abgueguen P, Hubert B, Vandamme YM, Maquart M, Le Guillou-Guillemette H, et al. Late sexual transmission of Zika virus related to persistence in the semen. Lancet. 2016;387(10037):2501. doi:10.1016/s0140-6736(16)30775-9.

[672] Venturi G, Zammarchi L, Fortuna C, Remoli ME, Benedetti E, Fiorentini C, et al. An autochthonous case of zika due to possible sexual transmission, Florence, Italy, 2014. Eurosurveillance. 2016;21(8):1–4. doi:10.2807/1560-7917.ES.2016.21.8.30148.

[673] Visseaux B, Mortier E, Houhou-Fidouh N, Brichler S, Collin G, Larrouy L, et al. Zika virus in the female genital tract. Lancet Infect Dis. 2016;16(11):1220. doi:10.1016/s1473-3099(16)30387-5.

[674] World Health Organization. Zika situation report. Zika virus, Microcephaly and Guillain-Barré syndrome - 21 April 2016; 2016. [Online]. Available from: http://www.who.int/emergencies/zika-virus/situation-report/21-april-2016/en/.

[675] Wu D, Sun J, Zhong H, Guan D, Zhang H, Tan Q, et al. A family cluster of imported ZIKV cases: Viremia period may be longer than previously reported. J Infect. 2016;73(3):300–303. doi:10.1016/j.jinf.2016.06.008.

[676] Zheng N, Liu Y, Chen Q, Chen M, Zheng C, Yang Z, et al. Clinical manifestations of 10 cases of imported human zika virus disease in jiangmen city of China. Natl Med J China. 2016;96(45):3666–3668. doi:10.3760/cma.j.issn.0376-2491.2016.45.012.

[677] Althouse GC, Rossow K. The potential risk of infectious disease dissemination via artificial insemination in swine. Reprod Domest Anim. 2011;46(SUPPL. 2):64–67. doi:10.1111/j.1439-0531.2011.01863.x.

[678] Armah HB, Wang G, Omalu BI, Tesh RB, Gyure KA, Chute DJ, et al. Systemic distribution of West Nile virus infection: Postmortem immunohistochemical study of six cases. Brain Pathol. 2007;17(4):354–362. doi:10.1111/j.1750-3639.2007.00080.x.

[679] Burke SA, Wen LI, King NJC. Routes of inoculation and the immune response to a resolving genital flavivirus infection in a novel murine model. Immunol Cell Biol. 2004;82(2):174–183. doi:10.1046/j.0818-9641.2004.01239.x.

[680] Fujita N, Izaki S, Oda K, Kimura E, Hotta S. Research on dengue in tissue culture. V. Production of anti dengue antibodies in tissue cultures from monkeys inoculated with an attenuated type I dengue virus strain. Kobe J Med Sci. 1972;18(3):143–152.

[681] Gerlinskaya LA, Bakhvalova VN, Morozova OV, Tsekhanovskaya NA, Matveeva VA, Moshkin MP. Sexual transmission of tick-borne encephalitis virus in laboratory mice. Bull Exp Biol Med. 1997;123(3):283–284. doi:10.1007/BF02445427.

[682] Guérin B, Pozzi N. Viruses in boar semen: Detection and clinical as well as epidemiological consequences regarding disease transmission by artificial insemination. Theriogenology. 2005;63(2 SPEC. ISS.):556–572. doi:10.1016/j.theriogenology.2004.09.030.

[683] Habu A, Murakami Y, Ogasa A, Fujisaki Y. [Disorder of spermatogenesis and viral discharge into semen in boars infected with Japanese encephalitis virus (author's transl)]. Uirusu. 1977;27(1):21–6.

[684] Iannetta M, Lalle E, Musso M, Carletti F, D'Abramo A, Chinello P, et al. Persistent detection of dengue virus RNA in vaginal secretion of a woman returning from Sri Lanka to Italy, April 2017. Eurosurveillance. 2017;22(34). doi:10.2807/1560-7917.ES.2017.22.34.30600.

[685] Kelley RE, Berger JR, Kelley BP. West Nile Virus Meningo-Encephalitis: Possible Sexual Transmission. J La State Med Soc. 2016;168(1):21–2.

[686] Liu WJ, Zhu M, Pei JJ, Dong XY, Liu W, Zhao MQ, et al. Molecular phylogenetic and positive selection

analysis of Japanese encephalitis virus strains isolated from pigs in China. Virus Res. 2013;178(2):547–552. doi:10.1016/j.virusres.2013.09.002.

[687] Mizuno Y, Kotaki A, Harada F, Tajima S, Kurane I, Takasaki T. Confirmation of dengue virus infection by detection of dengue virus type 1 genome in urine and saliva but not in plasma. Trans R Soc Trop Med Hyg. 2007;101(7):738–739. doi:10.1016/j.trstmh.2007.02.007.

[688] Prabandari SA, Iskandriati D, Saepuloh U, Arifin E, Pamungkas J. Tissues lesions associated with primary infection of dengue-3 virus in pigtailed macaques (macaca nemestrina). Exp Anim. 2015;64:S27.

[689] Rasinhas AdC, da Silva MAN, Caldas GC, Jácome FC, Leonardo R, dos Santos FB, et al. First detection of dengue virus in the saliva of immunocompetent murine model. Mem Inst Oswaldo Cruz. 2018;113(4):e170208. doi:10.1590/0074-02760170208.

[690] Sriprapun M, Laosakul C, Krajiw S, Arunyingmongkol K, Siriyasatien P, Kulwichit W. Symposia and Oral Presentations. Clin Microbiol Infect. 2012;18:1–113. doi:10.1111/j.1469-0691.2012.03801.x.

[691] Torres JR, Liprandi F, Goncalvez AP. Acute Parotitis Due to Dengue Virus. Clin Infect Dis. 2002;31(5):e28–e29. doi:10.1086/317454.

[692] Müller JA, Harms M, Schubert A, Jansen S, Michel D, Mertens T, et al. Inactivation and Environmental Stability of Zika Virus. Emerg Infect Dis. 2016;22(9):1685–1687. doi:10.3201/eid2209.160664.

[693] Vink MA, Bootsma MCJ, Wallinga J. Serial intervals of respiratory infectious diseases: A systematic review and analysis. Am J Epidemiol. 2014;180(9):865–875. doi:10.1093/aje/kwu209.

[694] Majumder MS, Cohn E, Fish D, Brownstein JS. Estimating a feasible serial interval range for Zika fever. Bull World Health Organ. 2016;doi:10.2471/blt.16.171009.

[695] Coelho FC, Durovni B, Saraceni V, Lemos C, Codeco CT, Camargo S, et al. Higher incidence of Zika in adult women than adult men in Rio de Janeiro suggests a significant contribution of sexual transmission from men to women. Int J Infect Dis. 2016;51:128–132. doi:10.1016/j.ijid.2016.08.023.

[696] Dejnirattisai W, Supasa P, Wongwiwat W, Rouvinski A, Barba-Spaeth G, Duangchinda T, et al. Dengue virus sero-cross-reactivity drives antibody-dependent enhancement of infection with zika virus. Nat Immunol. 2016;17(9):1102–8. doi:10.1038/ni.3515.

[697] Aubry M, Teissier A, Huart M, Merceron S, Vanhomwegen J, Roche C, et al. Zika Virus Seroprevalence, French Polynesia, 2014–2015. Emerg Infect Dis. 2017;23(4):669–672.

[698] Gallian P, Cabié A, Richard P, Paturel L, Charrel RN, Pastorino B, et al. Zika virus in asymptomatic blood donors in Martinique. Blood. 2017;129(2):263–266. doi:10.1182/blood-2016-09-737981.

[699] Wikan N, Smith DR. Zika virus: History of a newly emerging arbovirus. Lancet Infect Dis. 2016;16(7):e119–e126. doi:10.1016/S1473-3099(16)30010-X.

[700] Kohl A, Gatherer D. Zika virus: a previously slow pandemic spreads rapidly through the Americas. J Gen Virol. 2015;97(2):269–273. doi:10.1099/jgv.0.000381.

[701] Cao-Lormeau VM, Roche C, Teissier A, Robin E, Berry AL, Mallet HP, et al. Zika Virus, French Polynesia, South Pacific, 2013. Emerg Infect Dis. 2014;20(6):1085.

[702] Faria NR, Do Socorro Da Silva Azevedo R, Kraemer MUG, Souza R, Cunha MS, Hill SC, et al. Zika virus in the Americas: Early epidemiological and genetic findings. Science. 2016;352(6283):345–349. doi:10.1126/science.aaf5036.

[703] Johansson MA, Mier-Y-Teran-Romero L, Reefhuis J, Gilboa SM, Hills SL. Zika and the risk of Microcephaly. Obstet Gynecol Surv. 2016;71(11):635–636. doi:10.1097/OGX.0000000000000386.

[704] Abbink P, Stephenson KE, Barouch DH. Zika virus vaccines. Nat Rev Microbiol. 2018;16(10):594–600. doi:10.1038/s41579-018-0039-7.

[705] World Health Organization. WHO Vaccine Pipeline Tracker; 2019. [Online]. Available from: https://www.who.int/immunization/research/vaccine_pipeline_tracker_spreadsheet/en/.

[706] Cohen J. Steep drop in Zika cases undermines vaccine trial. Science. 2018;361(6407):1055–1056. doi:10.1126/science.361.6407.1055.

[707] Balmaseda A, Stettler K, Medialdea-Carrera R, Collado D, Jin X, Zambrana JV, et al. Antibody-based assay discriminates Zika virus infection from other flaviviruses. Proc Natl Acad Sci. 2017;114(31):8384–8389. doi:10.1073/pnas.1704984114.

[708] Saba Villarroel PM, Nurtop E, Pastorino B, Roca Y, Drexler JF, Gallian P, et al. Zika virus epidemiology in Bolivia: A seroprevalence study in volunteer blood donors. PLoS Negl Trop Dis. 2018;12(3):e0006239. doi:10.1371/journal.pntd.0006239.

[709] Worldbank. World development indicators; 2013. [Online]. Available from: https://data.worldbank.org/indicator/SP.DYN.TFRT.IN?locations=NI.

[710] World Health Organization. WHO | By Category | Life Tables by Country - Nicaragua; 2019. [Online]. Available from: http://apps.who.int/gho/data/?theme=main&vid=61180.

[711] Carpenter B, Gelman A, Hoffman MD, Lee D, Goodrich B, Betancourt M, et al. Stan : A Probabilistic Programming Language. J Stat Softw. 2017;76(1). doi:10.18637/jss.v076.i01.

[712] Guan Y. Variance stabilizing transformations of Poisson, binomial and negative binomial distributions. Stat Probab Lett. 2009;79(14):1621–1629. doi:10.1016/j.spl.2009.04.010.

[713] Roche B, Drake JM, Rohani P. An Agent-Based Model to study the epidemiological and evolutionary dynamics of Influenza viruses. BMC Bioinformatics. 2011;12(1):87. doi:10.1186/1471-2105-12-87.

[714] World Bank. World Development Indicators; 2019. [Online]. Available from: https://databank.worldbank.org/data/reports.aspx?source=2&series=SP.POP.TOTL.MA.IN&country=NIC.

[715] Vyse AJ, Gay NJ, White JM, Ramsay ME, Brown DWG, Cohen BJ, et al. Evolution of surveillance of measles, mumps, and rubella in England and Wales: Providing the platform for evidence-based vaccination policy. Epidemiol Rev. 2002;24(2):125–136. doi:10.1093/epirev/mxf002.

[716] Roche B, Drake JM, Rohani P. An Agent-Based Model to study the epidemiological and evolutionary dynamics of Influenza viruses. BMC Bioinformatics. 2011;12(1). doi:10.1186/1471-2105-12-87.

[717] Tuite AR, Thomas-Bachli A, Acosta H, Bhatia D, Huber C, Petrasek K, et al. Infectious disease implications of large-scale migration of Venezuelan nationals. J Travel Med. 2018;25(1). doi:10.1093/jtm/tay077.

[718] Durham DP, Fitzpatrick MC, Ndeffo-Mbah ML, Parpia AS, Michael NL, Galvani AP. Evaluating vaccination strategies for zika virus in the Americas. Ann Intern Med. 2018;168(9):621–630. doi:10.7326/M17-0641.

[719] Bartsch SM, Asti L, Cox SN, Durham DP, Randall S, Hotez PJ, et al. What Is the Value of Different Zika Vaccination

Strategies to Prevent and Mitigate Zika Outbreaks? J Infect Dis. 2018;doi:10.1093/infdis/jiy688.

[720] Valega-Mackenzie W, Ríos-Soto KR. Can Vaccination Save a Zika Virus Epidemic? Bull Math Biol. 2018;80(3):598–625. doi:10.1007/s11538-018-0393-7.

[721] Bellan SE, Eggo RM, Gsell PS, Kucharski AJ, Dean NE, Donohue R, et al. An online decision tree for vaccine efficacy trial design during infectious disease epidemics: The InterVax-Tool. Vaccine. 2019;37(31):4376–4381. doi:10.1016/j.vaccine.2019.06.019.

[722] Vandenbroucke JP, Broadbent A, Pearce N. Causality and causal inference in epidemiology: The need for a pluralistic approach. Int J Epidemiol. 2016;45(6):1776–1786. doi:10.1093/ije/dyv341.

[723] Maxcy KF. Papers of Wade Hampton Frost, M.D.— A Contribution to Epidemiological Method. New York: The Commonwealth Fund; 1941.

[724] Ioannidis JPA, Fanelli D, Dunne DD, Goodman SN. Meta-research: Evaluation and Improvement of Research Methods and Practices. PLoS Biol. 2015;13(10):1–7. doi:10.1371/journal.pbio.1002264.

[725] Schooler JW. Metascience could rescue the 'replication crisis'. Nature. 2014;515(7525):9. doi:10.1038/515009a.

[726] Rubin DB. The design versus the analysis of observational studies for causal effects: Parallels with the design of randomized trials. Stat Med. 2007;26(1):20–36. doi:10.1002/sim.2739.

[727] Mynatt CR, Doherty ME, Tweney RD. Confirmation Bias in a Simulated Research Environment: An Experimental Study of Scientific Inference. Q J Exp Psychol. 1977;29(1):85–95. doi:10.1080/00335577430000053.

[728] Fischhoff B, Beyth-Marom R. Hypothesis Evaluation From a Bayesian Perspective. Psychol Rev. 1983;90(3):239–260. doi:10.1037/0033-295X.90.3.239.

[729] Popper K. The logic of scientific discovery. Routledge; 2005.

[730] Kikuti M, Tauro LB, Moreira PSS, Campos GS, Paploski IAD, Weaver SC, et al. Diagnostic performance of commercial IgM and IgG enzyme-linked immunoassays (ELISAs) for diagnosis of Zika virus infection. Virol J. 2018;15(1). doi:10.1186/s12985-018-1015-6.

[731] Andrews JC, Schunemann HJ, Oxman AD, Pottie K, Meerpohl JJ, Coello PA, et al. GRADE guidelines: 15. Going from evidence to recommendation-determinants of a recommendation's direction and strength. J Clin Epidemiol. 2013;66(7):726–35. doi:10.1016/j.jclinepi.2013.02.003.

[732] Thézé J, Li T, du Plessis L, Bouquet J, Kraemer MUG, Somasekar S, et al. Genomic Epidemiology Reconstructs the Introduction and Spread of Zika Virus in Central America and Mexico. Cell Host Microbe. 2018;23(6):855–864.e7. doi:10.1016/j.chom.2018.04.017.

[733] Ralph Edwards I. Causality Assessment in Pharmacovigilance: Still a Challenge. Drug Saf. 2017;40(5):365–372. doi:10.1007/s40264-017-0509-2.

[734] Rossi D, Faucz FR, Melo A, Pezzuto P, de Azevedo GS, Schamber-Reis BLF, et al. Variations in maternal adenylate cyclase genes are associated with congenital Zika syndrome in a cohort from Northeast, Brazil. J Intern Med. 2019;285(2):215–222. doi:10.1111/joim.12829.

[735] Souza-Neto JA, Powell JR, Bonizzoni M. Aedes aegypti vector competence studies: A review. Infect Genet Evol. 2019;67:191–209. doi:10.1016/j.meegid.2018.11.009.

[736] Shoukat A, Vilches T, Moghadas SM. Cost-effectiveness of a potential Zika vaccine candidate: A case study for Colombia. BMC Med. 2018;16(1). doi:10.1186/s12916-018-1091-x.

[737] Sanchez Clemente N, Rodrigues M, Pascalicchio AP, Gazeta RE, Vedovello D, Brickley EB, et al. Cohort profile: the Jundiaí Zika cohort (JZC), a pregnancy and birth cohort in São Paulo state, Brazil. BMJ Open. 2019;9(8):e027947. doi:10.1136/bmjopen-2018-027947.

[738] Lebov JF, Arias JF, Balmaseda A, Britt W, Cordero JF, Galvão LA, et al. International prospective observational cohort study of Zika in infants and pregnancy (ZIP study): study protocol. BMC Pregnancy Childbirth. 2019;19(1):282. doi:10.1186/s12884-019-2430-4.

[739] Marmot M. Social determinants of health inequalities. Lancet. 2005;365(9464):1099–1104. doi:10.1016/S0140-6736(05)71146-6.

[740] Quinn SC, Kumar S. Health inequalities and infectious disease epidemics: A challenge for global health security. Biosecurity and Bioterrorism. 2014;12(5):263–273. doi:10.1089/bsp.2014.0032.

[741] Ngonghala CN, Pluciński MM, Murray MB, Farmer PE, Barrett CB, Keenan DC, et al. Poverty, Disease, and the Ecology of Complex Systems. PLoS Biol. 2014;12(4). doi:10.1371/journal.pbio.1001827.

[742] Hui SL, Walter SD. Estimating the Error Rates of Diagnostic Tests. Biometrics. 1980;36(1):167. doi:10.2307/2530508.

[743] Wilder-Smith A, Wei Y, Araújo TVBD, Vankerkhove M, Turchi Martelli CM, Turchi MD, 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. 2019;9(6). doi:10.1136/bmjopen-2018-026092.

[744] Cousien A, Abel S, Monthieux A, Andronico A, Calmont I, Cervantes M, et al. Assessing Zika Virus Transmission within Households during an Outbreak in Martinique, 2015-2016. Am J Epidemiol. 2019;doi:10.1093/aje/kwz091.

[745] World Health Organization. WHO R&D Blueprint - List of Blueprint priority diseases; 2018. [Online]. Available from: https://www.who.int/blueprint/priority-diseases/en/.

[746] Gostin LO, Friedman EA. A retrospective and prospective analysis of the west African Ebola virus disease epidemic: Robust national health systems at the foundation and an empowered WHO at the apex. Lancet. 2015;385(9980):1902–1909. doi:10.1016/S0140-6736(15)60644-4.

[747] Enemark C, Selgelid M. Ethics and Security Aspects of Infectious Disease Control: Introduction. Farnham, UK: Ashgate Publishing Ltd; 2012.

[748] Kraemer MUG, Reiner RC, Brady OJ, Messina JP, Gilbert M, Pigott DM, et al. Past and future spread of the arbovirus vectors Aedes aegypti and Aedes albopictus. Nat Microbiol. 2019;4(5):854–863. doi:10.1038/s41564-019-0376-y.

[749] Althaus CL, Low N. How Relevant Is Sexual Transmission of Zika Virus? PLOS Med. 2016;13(10):e1002157. doi:10.1371/journal.pmed.1002157.

[750] Counotte M, Maxwell L, Kim C, Broutet N, Low N. O14.6 Sexual transmission of flaviviruses – a living systematic review. Sex Transm Infect. 2017;93:A33.2–A34. doi:10.1136/sextrans-2017-053264.83.

[751] Perera-Lecoin M, Meertens L, Carnec X, Amara A. Flavivirus entry receptors: An update. Viruses. 2013;6(1):69–88. doi:10.3390/v6010069.

[752] World Health Organization. WHO Sexually Transmitted Infections (STIs) Fact Sheet. 2017; 2017. [Online]. Available from: https://www.who.int/en/news-room/fact-sheets/detail/sexually-transmitted-infections-(stis).

[753] Bowen A. Shigellosis 2016; 2016. [Online]. Available

from: https://wwwnc.cdc.gov/travel/yellowbook/2016/infectiousdiseases-related-to-travel/shigellosis.

[754] Mildvan D, Gelb AM, William D. Venereal transmission of enteric pathogens in male homosexuals. Two case reports. JAMA. 1977;238(13):1387–1389.

[755] William DC, Felman YM, Marr JS, Shookhoff HB. Sexually transmitted enteric pathogens in male homosexual population. N Y State J Med. 1977;77(13):2050–2052.

[756] Dritz SK, Back AF. Letter: Shigella enteritis venereally transmitted. N Engl J Med. 1974;291(22):1194.

[757] Bader M, Pedersen AH, Williams R, Spearman J, Anderson H. Venereal transmission of shigellosis in Seattle-King county. Sex Transm Dis. 1977;4(3):89–91.

[758] Gilbart VL, Simms I, Jenkins C, Furegato M, Gobin M, Oliver I, et al. Sex, drugs and smart phone applications: findings from semistructured interviews with men who have sex with men diagnosed with Shigella flexneri 3a in England and Wales. Sex Transm Infect. 2015;91(8):598–602. doi:10.1136/sextrans-2015-052014.

[759] Aragon TJ, Vugia DJ, Shallow S, Samuel MC, Reingold A, Angulo FJ, et al. Case-control study of shigellosis in San Francisco: the role of sexual transmission and HIV infection. Clin Infect Dis. 2007;44(3):327–334. doi:10.1086/510593.

[760] Tauxe RV, McDonald RC, Hargrett-Bean N, Blake PA. The persistence of Shigella flexneri in the United States: increasing role of adult males. Am J Public Health. 1988;78(11):1432–1435. doi:10.2105/ajph.78.11.1432.

[761] Simms I, Field N, Jenkins C, Childs T, Gilbart VL, Dallman TJ, et al. Intensified shigellosis epidemic associated with sexual transmission in men who have sex with men–Shigella flexneri and S. sonnei in England, 2004 to end of February 2015. Euro Surveill. 2015;20(15). doi:10.2807/1560-7917.es2015.20.15.21097.

[762] Centers for Disease Control and Prevention. FoodNet Fast; 2017. [Online]. Available from: https://wwwn.cdc.gov/foodnetfast/.

[763] Gaudreau C, Ratnayake R, Pilon PA, Gagnon S, Roger M, Levesque S. Ciprofloxacin-resistant Shigella sonnei among men who have sex with men, Canada, 2010. Emerg Infect Dis. 2011;17(9):1747–1750. doi:10.3201/eid1709.102034.

[764] Gaudreau C, Barkati S, Leduc JM, Pilon PA, Favreau J, Bekal S. Shigella spp. with reduced azithromycin susceptibility, Quebec, Canada, 2012-2013. Emerg Infect Dis. 2014;20(5):854–856. doi:10.3201/eid2005.130966.

[765] Baker KS, Dallman TJ, Ashton PM, Day M, Hughes G, Crook PD, et al. Intercontinental dissemination of azithromycin-resistant shigellosis through sexual transmission: a cross-sectional study. Lancet Infect Dis. 2015;15(8):913–921. doi:10.1016/s1473-3099(15)00002-x.

[766] Bowen A, Hurd J, Hoover C, Khachadourian Y, Traphagen E, Harvey E, et al. Importation and domestic transmission of Shigella sonnei resistant to ciprofloxacin - United States, May 2014-February 2015. MMWR Morb Mortal Wkly Rep. 2015;64(12):318–320.

[767] Chiou CS, Izumiya H, Kawamura M, Liao YS, Su YS, Wu HH, et al. The worldwide spread of ciprofloxacin-resistant Shigella sonnei among HIV-infected men who have sex with men, Taiwan. Clin Microbiol Infect. 2016;22(4):383.e11–383.e16. doi:10.1016/j.cmi.2015.12.021.

[768] Heiman KE, Karlsson M, Grass J, Howie B, Kirkcaldy RD, Mahon B, et al. Notes from the field: Shigella with decreased susceptibility to azithromycin among men who have sex with men - United States, 2002-2013. MMWR Morb Mortal Wkly Rep. 2014;63(6):132–133.

[769] Lane CR, Sutton B, Valcanis M, Kirk M, Walker C, Lalor K, et al. Travel Destinations and Sexual Behavior as Indicators of Antibiotic Resistant Shigella Strains–Victoria, Australia. Clin Infect Dis. 2016;62(6):722–729. doi:10.1093/cid/civ1018.

[770] Mook P, McCormick J, Bains M, Cowley LA, Chattaway MA, Jenkins C, et al. ESBL-Producing and Macrolide-Resistant Shigella sonnei Infections among Men Who Have Sex with Men, England, 2015. Emerg Infect Dis. 2016;22(11):1948–1952. doi:10.3201/eid2211.160653.

[771] Centers for Disease Control and Prevention. Pneumocystis pneumonia–Los Angeles. MMWR Morb Mortal Wkly Rep. 1981;30(21):250–252.

[772] Rambaut A, Posada D, Crandall KA, Holmes EC. The causes and consequences of HIV evolution. Nat Rev Genet. 2004;5(1):52–61. doi:10.1038/nrg1246.

[773] Carpenter CM, Charles R. Isolation of Meningococcus from the Genitourinary Tract of Seven Patients. American Journal of Public Health and the Nations Health. 1942;32(6):640–643. doi:10.2105/ajph.32.6.640.

[774] Tully JG, Cole RM, Taylor-Robinson D, Rose DL. a Newly Discovered Mycoplasma in the Human Urogenital Tract. The Lancet. 1981;317(8233):1288–1291. doi:10.1016/S0140-6736(81)92461-2.

[775] Most H. Manhattan: "a tropic isle"? Am J Trop Med Hyg. 1968;17(3):333–354. doi:10.4269/ajtmh.1968.17.333.

[776] Schmerin MJ, Gelston A, Jones TC. Amebiasis. An increasing problem among homosexuals in New York City. JAMA. 1977;238(13):1386–1387. doi:10.1001/jama.238.13.1386.

[777] Barre-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). 1983. Rev Invest Clin. 2004;56(2):126–129.

[778] World Health Organization. Emergencies: The history of Zika virus.; 2017. [Online]. Available from: http://www.who.int/emergencies/zika-virus/timeline/en/.

[779] Brown WJ, Kraus SJ, Arko RJ. Chimpanzee urethral meningococci. Br J Vener Dis. 1973;49(1):88. doi:10.1136/sti.49.1.88.

[780] Talbot MD, Collins BN. Presumed sexual transmission of meningococci. J Infect. 1981;3(3):273–276.

[781] Wilson AP, Wolff J, Atia W. Acute urethritis due to Neisseria meningitidis group A acquired by orogenital contact: case report. Genitourin Med. 1989;65(2):122–123. doi:10.1136/sti.65.2.122.

[782] Taha MK, Claus H, Lappann M, Veyrier FJ, Otto A, Becher D, et al. Evolutionary Events Associated with an Outbreak of Meningococcal Disease in Men Who Have Sex with Men. PLoS One. 2016;11(5):e0154047. doi:10.1371/journal.pone.0154047.

[783] Marcus U, Vogel U, Schubert A, Claus H, Baetzing-Feigenbaum J, Hellenbrand W, et al. A cluster of invasive meningococcal disease in young men who have sex with men in Berlin, October 2012 to May 2013. Euro Surveill. 2013;18(28). doi:10.2807/1560-7917.es2013.18.28.20523.

[784] Simon MS, Weiss D, Gulick RM. Invasive meningococcal disease in men who have sex with men. Ann Intern Med. 2013;159(4):300–301. doi:10.7326/0003-4819-159-4-201308200-00674.

[785] Aubert L, Taha M, Boo N, Le Strat Y, Deghmane AE, Sanna A, et al. Serogroup C invasive meningococcal disease among men who have sex with men and in gay-oriented social venues in the Paris region: July 2013 to December 2014. Euro Surveill. 2015;20(3). doi:10.2807/1560-7917.es2015.20.3.21016.

[786] Kratz MM, Weiss D, Ridpath A, Zucker JR, Geevarughese

A, Rakeman J, et al. Community-Based Outbreak of Neisseria meningitidis Serogroup C Infection in Men who Have Sex with Men, New York City, New York, USA, 2010-2013. Emerg Infect Dis. 2015;21(8):1379–1386. doi:10.3201/eid2108.141837.

[787] Kamiya H, MacNeil J, Blain A, Patel M, Martin S, Weiss D, et al. Meningococcal disease among men who have sex with men - United States, January 2012-June 2015. MMWR Morb Mortal Wkly Rep. 2015;64(44):1256–1257. doi:10.15585/mmwr.mm6444a6.

[788] Nanduri S, Foo C, Ngo V, Jarashow C, Civen R, Schwartz B, et al. Outbreak of Serogroup C Meningococcal Disease Primarily Affecting Men Who Have Sex with Men - Southern California, 2016. MMWR Morb Mortal Wkly Rep. 2016;65(35):939–940. doi:10.15585/mmwr.mm6535e1.

[789] Janda WM, Bohnoff M, Morello JA, Lerner SA. Prevalence and site-pathogen studies of Neisseria meningitidis and N gonorrhoeae in homosexual men. JAMA. 1980;244(18):2060–2064.

[790] Salit IE, Frasch CE. Seroepidemiologic aspects of Neisseria meningitidis in homosexual men. Can Med Assoc J. 1982;126(1):38–41.

[791] World Health Organization. Ebola outbreak 2014–2015; 2017. [Online]. Available from: http://www.who.int/csr/disease/ebola/en/.

[792] Thorson A, Formenty P, Lofthouse C, Broutet N. Systematic review of the literature on viral persistence and sexual transmission from recovered Ebola survivors: evidence and recommendations. BMJ Open. 2016;6(1):e008859. doi:10.1136/bmjopen-2015-008859.

[793] Christie A, Davies-Wayne GJ, Cordier-Lassalle T, Blackley DJ, Laney AS, Williams DE, et al. Possible sexual transmission of Ebola virus - Liberia, 2015. MMWR Morb Mortal Wkly Rep. 2015;64(17):479–481.

[794] Mate SE, Kugelman JR, Nyenswah TG, Ladner JT, Wiley MR, Cordier-Lassalle T, et al. Molecular Evidence of Sexual Transmission of Ebola Virus. N Engl J Med. 2015;373(25):2448–2454. doi:10.1056/NEJMoa1509773.

[795] Rowe AK, Bertolli J, Khan AS, Mukunu R, Muyembe-Tamfum JJ, Bressler D, et al. Clinical, virologic, and immunologic follow-up of convalescent Ebola hemorrhagic fever patients and their household contacts, Kikwit, Democratic Republic of the Congo. Commission de Lutte contre les Epidemies a Kikwit. J Infect Dis. 1999;179 Suppl:S28–35. doi:10.1086/514318.

[796] Bausch DG, Towner JS, Dowell SF, Kaducu F, Lukwiya M, Sanchez A, et al. Assessment of the risk of Ebola virus transmission from bodily fluids and fomites. J Infect Dis. 2007;196 Suppl:S142–7. doi:10.1086/520545.

[797] Soka MJ, Choi MJ, Baller A, White S, Rogers E, Purpura LJ, et al. Prevention of sexual transmission of Ebola in Liberia through a national semen testing and counselling programme for survivors: an analysis of Ebola virus RNA results and behavioural data. Lancet Glob Health. 2016;4(10):e736–43. doi:10.1016/s2214-109x(16)30175-9.

[798] Sissoko D, Duraffour S, Kerber R, Kolie JS, Beavogui AH, Camara AM, et al. Persistence and clearance of Ebola virus RNA from seminal fluid of Ebola virus disease survivors: a longitudinal analysis and modelling study. Lancet Glob Health. 2017;5(1):e80–e88. doi:10.1016/s2214-109x(16)30243-1.

[799] World Health Organization. Clinical care for survivors of Ebola virus disease: Interim guidance; 2016. [Online]. Available from: https://www.who.int/csr/resources/publications/ebola/guidance-survivors/en/.

[800] Vetter P, Kaiser L, Schibler M, Ciglenecki I, Bausch DG. Sequelae of Ebola virus disease: the emergency within the emergency. Lancet Infect Dis. 2016;16(6):e82–e91. doi:10.1016/s1473-3099(16)00077-3.

Curriculum vitae & list of publications

Michel Counotte

Veterinary Epidemiologist (PhD)

Maienstrasse 31
Zürich 8050
Switzerland
+41 77 909 8783
michel.counotte@ispm.unibe.ch
orcid.org/0000-0003-1039-6873

Research interests

Epidemiology, zoonotic diseases, systematic reviews and evidence synthesis, infectious disease modeling, burden of disease, One-Health, machine learning, statistical methods, guideline development, veterinary public health, science communication

Education

2016–2020 **PhD**, *University of Bern*, CH, *PhD Epidemiology*.
PhD Thesis: Zika virus: causality, open science and risk of emerging infectious diseases.

2014–2016 **Msc.**, *University of Utrecht*, NL, *Msc. Epidemiology*.
Msc. Epi Thesis: The burden of zoonoses in Kyrgyzstan.

2003–2009 **Msc.**, *University of Ghent*, BE, *Msc. Veterinary Medicine*.
Msc. Vet Thesis: Small scale prediction of Fasciola Hepatica in cattle.

2002–2003 **Propaedeutic dipl.**, *University of Twente*, NL, *Mechanical Engineering*.

PhD thesis

Title *Zika virus: causality, open science and risk of emerging infectious diseases*

Result 6/6

Supervisors Prof. Nicola Low, Prof. Julian Higgins

Description In my PhD project, I investigated the causal relation between Zika virus infection and adverse outcomes, the risk of sexual transmission of Zika virus and the future risk of Zika virus outbreaks. I used mathematical and statistical models to make sense of the imperfect data collected during the Zika epidemic.

Work experience

2020–Now **Postdoctoral researcher**, *University of Bern, Institute of Social and Preventive Medicine*, CH.
Projects: Emerging outbreaks, Brucellosis/Q-fever/Rift-valley fever in Chad, European MSM Internet Survey

2016–2020 **Research assistant/PhD Student**, *University of Bern, Institute of Social and Preventive Medicine*, Bern, CH.
WHO/SNF funded project on different aspect on the 2015-2017 Zika virus epidemic

2015–2016 **Research assistant**, *University of Zürich, VetSuisse faculty*, Zürich, CH.
Participation in teaching and epidemiological consultancies

2010–2015 **Veterinarian**, *Dierenkliniek Sleeuwijk*, Sleeuwijk, NL.
Small Animal veterinarian. Main interest: medical imaging and reproductive health. Training of undergraduate veterinarians.

2009–2010 **Veterinarian**, *Arts4Dieren*, Haaksbergen, NL.
Small Animal veterinarian.

Highlighted courses

Fall 2019 Workshop on Infectious Disease Surveillance at the Institute of Social and Preventive Medicine (University of Bern)

Fall 2019 GIS for Public Health, Basel, Switzerland

Summer 2018 One health summer school, Copenhagen, Denmark

Winter 2018 Causal Inference in Observational Epidemiology, Wengen, Switzerland

Summer 2016 Mathematical Modeling of infectious disease, Utrecht, The Netherlands

Summar 2009 BTC belgian fund training on developmental work, Brussels, Belgium

Presentations

March 2019 Institute research seminar, Bern, Switzerland: Talk. The future risk of Zika virus in Managua, Nicaragua

January 2018 GCB graduate school symposium, Bern, Switzerland: Poster presentation. The sexual transmission of ZIKV

October 2018 Midterm presentation PhD, Bern, Switzerland: The Zika virus outbreak - a journey through the evidence

November 2018 SSPH Social Science for Public Health, Neuchâtel, Switzerland. Talk. Science flash talk: `https://tinyurl.com/y3uggbgd`

June 2018 International Symposium on Zika Virus Research, Marseille, France: Poster presentation. The sexual transmission of ZIKV

May 2018 Geneva, WHO expert meeting: Talk. Sexual transmission of ZIKV

August 2017 5th National Gathering of Swiss Medical Librarians - Medical librarianship in the data age: transforming research data into clinical insight, Bern, Switzerland: Talk. Living systematic reviews

July 2017 STI & HIV World Congress, Rio de Janeiro, Brazil: Talk. A living systematic review on the sexual transmission of Zika virus and other flaviviruses

August 2016 Association of Institutions for Tropical Veterinary Medicine (AITVM) and the Society of Tropical Veterinary Medicine (STVM) Joint meeting, Berlin, Germany: Poster presentation. The Burden of Zoonoses in Kyrgyzstan

Organizational skills

2018 Co-lead on sexual transmission framework for Zika WHO meeting

2013-2014 Chairman of the dutch platform for young veterinarians (KNMvD), NL

2007 Co-organiser Symposium for veterinary students IVSA Belgium, BE

2000-2002 Coach at rowing club ZRZV Zwolle, NL

Computer skills

R, RStudio, RMarkdown, Latex, Git (`http://github.com/ZikaProject/`), SQL, *Phyton*, html, Shiny

Languages

Dutch	Native speaker
English	Excellent command
German	Basic command

Interests

Running, Hiking, Cooking

Publications

Michel Jacques Counotte, Kaspar W. Meili, and Nicola Low. Emergence of evidence about causality: case study from the Zika virus outbreak. *[Manuscript in preparation]*, 2020.

Michel J. Counotte, Christian L. Althaus, Nicola Low, and Julien Riou. Impact of age-specific immunity on the timing and burden of the next Zika virus outbreak. *PLoS Negl. Trop. Dis.*, 13 (12):e0007978, 2019a. ISSN 19352735. doi: 10.1371/journal.pntd.0007978.

Michel Jacques Counotte, Kaspar Walter Meili, Katayoun Taghavi, Guilherme Calvet, James Sejvar, and Nicola Low. Zika virus infection as a cause of congenital brain abnormalities and Guillain-Barré syndrome: A living systematic review [version 1; peer review: 2 approved]. *F1000Research*, 8: 1433, 2019b. ISSN 1759796X. doi: 10.12688/f1000research.19918.1.

Michel Jacques Counotte, Caron Rahn Kim, Jingying Wang, Kyle Bernstein, Carolyn D Deal, Nathalie Jeanne Nicole Broutet, and Nicola Low. Sexual transmission of zika virus and other flaviviruses: a living systematic review. *PLoS medicine*, 15(7):e1002611, 2018a.

Caron R. Kim, Michel Counotte, Kyle Bernstein, Carolyn Deal, Philippe Mayaud, Nicola Low, and Nathalie Broutet. Investigating the sexual transmission of Zika virus. *Lancet Glob. Heal.*, 6(1): e24–e25, 2018. ISSN 2214109X. doi: 10.1016/S2214-109X(17)30419-9.

Michel Jacques Counotte, Dianne Egli-Gany, Maurane Riesen, Million Abraha, Teegwendé Valérie Porgo, Jingying Wang, and Nicola Low. Zika virus infection as a cause of congenital brain abnormalities and Guillain-Barré syndrome: From systematic review to living systematic review. *F1000Research*, 7(196):196, 2018b. doi: 10.12688/f1000research.13704.1.

Kyle Bernstein, Virginia B Bowen, Caron R Kim, Michel J Counotte, Robert D Kirkcaldy, Edna Kara, Gail Bolan, Nicola Low, and Nathalie Broutet. Re-emerging and newly recognized sexually transmitted infections: Can prior experiences shed light on future identification and control? *PLoS medicine*, 14(12):e1002474, 2017.

Alessandro Delaude, Sabrina Rodriguez-Campos, Anou Dreyfus, Michel Jacques Counotte, Thierry Francey, Ariane Schweighauser, Sophie Lettry, and Simone Schuller. Canine leptospirosis in switzerland—a prospective cross-sectional study examining seroprevalence, risk factors and urinary shedding of pathogenic leptospires. *Preventive veterinary medicine*, 141:48–60, 2017.

Michel J Counotte, Gulnara Minbaeva, Jumagul Usubalieva, Kubanychbek Abdykerimov, and Paul R Torgerson. The burden of zoonoses in kyrgyzstan: a systematic review. *PLoS neglected tropical diseases*, 10(7):e0004831, 2016.

Johannes Charlier, Sita Carolien Bennema, Yannick Caron, Michel Counotte, Els Ducheyne, Guy Hendrickx, and Jozef Vercruysse. Towards assessing fine-scale indicators for the spatial transmission risk of fasciola hepatica in cattle. *Geospatial Health*, pages 239–245, 2011.

Acknowledgements

I am grateful to all of those with whom I have had the pleasure to work during my PhD and other related projects.

I would like to thank Prof. Dr. Nicola Low for her supervision and encouragement during my PhD, you taught me to communicate science in a clear and concise way and helped me become a better epidemiologist. Prof. Dr. Julian Higgins for being my external supervisor and Prof. Dr. Frank Stüber for being my mentor at the Graduate School for Cellular and Biomedical Sciences (GCB). I would like to thank Dr. Gabriela Paz-Bailey for being my external examiner for my PhD thesis.

I would like to thank the organizations that provided funding for my PhD studies: The Swiss National Science Foundation (SNF) and the World Health Organization (WHO).

Much of the work presented in this thesis could not have been conducted without the help of many collaborators at different institutes and organizations: At the WHO: Dr. Nathalie Broutet, Dr. Caron Kim, Dr. Edna Kara, at the United States Centers for Disease Control and Prevention (CDC): Dr. Kyle Bernstein and Dr. James Sejvar, at the National Institute of Health (NIH): Dr. Carolyn Deal, at Fundação Oswaldo Cruz, Brazil: Dr. Guilherme Calvet and at Laval University, Canada: Dr. Valerie Porgo.

I also had the pleasure to collaborate with great colleagues and friends within our own institute: Kaspar, Hung, Maurane, Million, Dianne, Diana, Jingying, Kati, Julien and Christian. Thank you for your contributions to either the living systematic reviews or the mathematical modelling work. A special thanks to Kaspar Meili and Hung Nguyen. You both helped tremendously with realizing, automating and maintaining the living systematic reviews. And thanks to the Veronika and Julien for proofreading parts of my thesis.

I would like to thank all the colleagues at the ISPM, CTU and BIHAM that have made it a great place to work. Claudia, Nicole, Veronika, Joost, Anthony, Fardo, and all the others that have been near me during this PhD with their sense, but more important, nonsense. Outside the institute and the project presented here, I had the pleasure to work with Ranya and Salome, who offered some welcome distraction in the veterinary epidemiology.

Natuurlijk ben ik ook mijn vrienden en familie dankbaar. Alle vrienden van de prietpraat: Op de belangrijke momenten zijn we samen. En natuurlijk dank ik alle andere vrienden en kennissen die mijn leven kleur geven.

Henk, Elly, Stephanie en Pieter, bedankt voor jullie gastvrijheid en gezelligheid.

Ik dank mijn ouders voor hun onvoorwaardelijke steun op alle vlakken. Daantje, het is dat we in Zwitserland geen paranimpfen hebben, maar anders was jij dat natuurlijk!

Su, ik ben dankbaar dat jij er bent en blij dat we vier jaar geleden aan ons 'Zwitserland' avontuur begonnen zijn. Ik ben benieuwd en enthousiast over wat de toekomst ons gaat brengen!

The cover art is kindly provided by Prof. David S. Goodsell.

Declaration of originality

Last name, first name: Counotte, Michel Jacques

Matriculation number: 17–128–377

I hereby declare that this thesis represents my original work and that I have used no other sources except as noted by citations.
All data, tables, figures and text citations which have been reproduced from any other source, including the internet, have been explicitly acknowledged as such.
I am aware that in case of non-compliance, the Senate is entitled to withdraw the doctorate degree awarded to me on the basis of the present thesis, in accordance with the "Statut der Universität Bern (Universitätsstatut; UniSt)", Art. 69, of 7 June 2011.

Place, date

Bern, 31.10.2019

Signature