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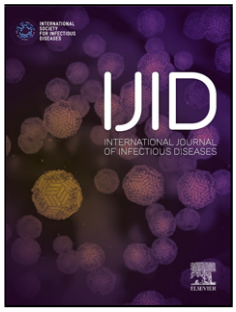

Fatal Zika virus infection in the Americas: A systematic review

Jaime A. Cardona-Ospina, Valentina Henao-SanMartin, Wilmer F. Acevedo-Mendoza, Katherinn Melissa Nasner-Posso, Dayron F. Martínez-Pulgarín, Abril Restrepo-López, Valentina Gallego-Valencia, Matthew H. Collins, Alfonso J. Rodriguez-Morales

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Systematic Review

Fatal Zika virus infection in the Americas: A systematic review[◊]

Running Head: Fatal Zika virus disease in adults – Systematic Review

Jaime A. Cardona-Ospina,[1,2,3,4,5,6] Valentina Henao-SanMartin,[1] Wilmer F. Acevedo-Mendoza,[1] Katherinn Melissa Nasner-Posso,[1] Dayron F. Martínez-Pulgarín,[1] Abril Restrepo-López,[1] Valentina Gallego-Valencia,[4,5] Matthew H. Collins,[7,*] Alfonso J. Rodriguez-Morales.[1,2,6,*]

[1]*Public Health and Infection Research Group, Faculty of Health Sciences, Universidad Tecnológica de Pereira, Pereira, Risaralda, Colombia.*

[2]*Colombian Collaborative Network on Zika and other Arboviruses (RECOLZIKA), Pereira, Risaralda, Colombia.*

[3]*Grupo de Investigación Infección e Inmunidad, Faculty of Health Sciences, Universidad Tecnológica de Pereira, Pereira, Risaralda, Colombia.*

[4]*Grupo de Investigación Biomedicina, Faculty of Medicine, Fundación Universitaria Autónoma de las Américas, Pereira, Risaralda, Colombia.*

[5]*Semillero de Investigación en Infecciones Emergentes y Medicina Tropical, Faculty of Medicine, Fundación Universitaria Autónoma de las Américas, Pereira, Risaralda, Colombia.*

[6]*Emerging Infectious Diseases and Tropical Medicine Research Group, Instituto para la Investigación en Ciencias Biomédicas – Sci-Help, Pereira, Risaralda, Colombia.*

[7]*Hope Clinic of the Emory Vaccine Center, Division of Infectious Diseases, Department of Medicine, School of Medicine, Emory University, Decatur, GA 30030, USA.*

*Corresponding Authors. Emails: matthew.collins@emory.edu, arodriguezm@utp.edu.co

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Highlights

- Severe manifestations of Zika virus (ZIKV) infection have recently been appreciated during epidemics.

- We performed a systematic review of the literature showing 51 reported deaths associated with ZIKV infection in nine countries, the majority (56.9%) was not related to Guillain-Barré syndrome.
- Cases from Dominican Republic, Brazil and Puerto Rico accounted for 67.6% of the deaths (33.3%; 18.6%, and 15.7%, respectively). ZIKV infection in the majority (64.7%) of cases was confirmed by RT-PCR, MAC-ELISA, or immunohistochemistry.

- An increasing number of fatalities have been published in the literature since the first death was reported in 2016, however, additional research is needed to elucidate factors that may mediate the pathogenesis of severe, atypical and fatal disease.

Abstract

Introduction: While death due to ZIKV infection has also been described, reports of fatal cases have been infrequent, and no systematic reviews have been published.

Methods: We performed a systematic review of the literature in four databases to assess fatal outcomes of postnatal ZIKV infection and the available evidence that links ZIKV infection.

Results: 311 articles were retrieved, 20 of them were epidemiological reports from surveillance agencies and Ministries of Health. After screening by abstract and title, 59 articles were selected for full-text assessment. Of the 59, 35 were excluded with reasons, and 24 were finally included for qualitative analysis. We found a total of 51 reported deaths associated with ZIKV infection in nine countries, the majority (56.9%) was not related to Guillain-Barré syndrome. Cases from three countries accounted for 67.6% of the deaths. ZIKV infection in the majority (64.7%) of cases was laboratory-confirmed.

Discussion: ZIKV was not considered to be a dangerous, much less a lethal pathogen until very recently. However, an increasing number of fatalities have been published in the literature since the first death was reported in 2016. Additional research is needed to elucidate factors that may mediate the pathogenesis of severe, atypical and fatal disease.

Keywords: Zika; fatal; complications; severe; epidemiology; Americas.

Introduction

Rationale

Zika virus (ZIKV) was first isolated in 1947 from the blood of a sentinel monkey during studies of sylvatic yellow fever transmission in Uganda (Azevedo et al., 2016, Martinez-Pulgarin et al., 2016, Rodriguez-Morales, 2015). During the next 60 years, ZIKV was sporadically isolated from mosquitoes and from humans in various countries in sub-Saharan Africa and Southeast Asia. Since 2007, when ZIKV was associated with an outbreak occurred on Yap island in Micronesia (Azevedo et al., 2016, Duffy et al., 2009, Lanciotti et al., 2008, Martinez-Pulgarin et al., 2016, Rodriguez-Morales, 2015), outbreaks became more common, spreading through Oceania and eventually reaching Latin America by 2013 (Faria et al., 2016a).

Zika had long been considered a benign flavivirus infection, not responsible for severe or fatal outcomes. Reports of human cases of ZIKV infection prior to 2013 indicate relatively mild clinical manifestations of a self-limited illness of 3–5 days duration, characterized by fever, myalgia, headache, conjunctivitis and rash (Azevedo et al., 2016, Duffy et al., 2009,

Lanciotti et al., 2008, Paniz-Mondolfi et al., 2016, Rodriguez-Morales, 2015). However, ZIKV transmission, including severe or fatal cases, could easily be unrecognized given the clinical similarity of Zika to other acute viral illnesses, particularly dengue fever. Moreover, basic serologic assays lack specificity in discriminating closely related flaviviruses, and ZIKV infection could be misdiagnosed as dengue, Japanese encephalitis, or others (Azevedo et al., 2016, Duffy et al., 2009, Lanciotti et al., 2008, Paniz-Mondolfi et al., 2016, Rodriguez-Morales, 2015).

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An increase in cases of Guillain-Barre syndrome (GBS) and other neurological diseases, as well as cases of microcephaly and other birth defects (Martinez-Pulgarin et al., 2016, Mlakar et al., 2016, Rodriguez-Morales et al., 2019, Villamil-Gomez et al., 2016), began to be reported in 2015 as ZIKV spread rapidly from Brazil to other countries in the Americas (Faria et al., 2016b, Rodriguez-Morales et al., 2016). Notably, there were fatalities among early reported cases (Soares et al., 2016). In addition to that patients with comorbidities, such as diabetes, hypertension and sickle cell disease, among others, also have been reported to develop severe disease and fatal outcome (Arzuza-Ortega et al., 2016, Sarmiento-Ospina et al., 2016). Severe manifestations of ZIKV infection have raised many questions regarding the determinants of disease, ranging from immunologic, host or viral genetic, environmental, etc. Since the onset of the epidemic, additional reports of Zika-associated deaths have accumulated; however, a systematic effort to consolidate what has been learned from each individual reported case is lacking, as is a rigorous evaluation of causality linkage between infection and outcome.

Objectives

- To systematically review published literature on fatal outcomes of ZIKV infection in the Americas.
- To assess the available evidence that causally links ZIKV and fatal cases.
- To examine the clinical context and spectrum of disease in Zika cases that progress to death.

Methods

Protocol and registration

This protocol follows the recommendations established by the PRISMA statement (Moher et al., 2009), and it has been registered in the PROSPERO (http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42017059347).

Information sources

We conducted a systematic review using Medline/PubMed, Scopus, and LILACS, SciELO, the official webpages of Ministries of Health of countries included in the WHO list of the Americas region and of the following international surveillance agencies: the Centers for Disease Control and Prevention, CDC (https://www.cdc.gov), European Centers for Disease Control and Prevention, ECDC (https://ecdc.europa.eu/en/home), World Health Organization WHO (https://www.who.int), and Pan-American Health Organization, PAHO (https://www.paho.org/).

Search Strategy

The search terms used were these: “Zika AND Fatal”, “Zika AND Death” and “Zika AND Severe.” Article language limit was not set and there was no limit for time of publication. The official webpages of surveillance agencies and Ministries of Health were checked looking for epidemiological reports and bulletins reporting deaths associated with ZIKV infection. The searches were concluded by January 1, 2018, and search results were independently evaluated by five different researchers. We included studies in English, Spanish, Portuguese and French.

Eligibility criteria

Eligible articles were required to meet the following criteria: published peer-reviewed articles that reported cases with fatal outcome linked with ZIKV infection. Eligible study designs were case-control, cohort studies, case reports and series of cases. We also included information published in epidemiological reports and bulletins by CDC, ECDC, WHO, PAHO and National Governments through their surveillance systems or Health Ministries.

Study Selection

Results of the initial search strategy were first screened by title and abstract. The full texts of appropriate articles were examined for inclusion and exclusion criteria (Figure 1). Studies reporting antenatal deaths, cases without laboratory confirmation test, reports with incomplete information were excluded, as were studies about severe cases not associated with Zika infection. Letters to the editor and reviews that did not report severe cases related to ZIKV were also excluded. When a bulletin or article reported duplicate information from the same patient, the information of both were reports was combined in order to obtain the most complete data, but only counted as a single case.

Data collection process and data items:

Data extraction forms including information on the type of publication, the publishing institution, country, year of publication, the number of reported deaths, laboratory testing, and the cause of death were filled independently by five investigators. A sixth researcher checked the article list and data extractions to ensure there were no duplicate articles or duplicate information of the same patient and also resolved discrepancies about study inclusion.

Assessment of methodological quality and risk of bias:

For quality assessment we used the Quality Appraisal of Case Series Studies Checklist of the IHE (Institute of Health Economics (IHE), 2014). And in order to assess the causality linkage between ZIKV and the evolution to severe or fatal

outcomes, as well as the risk of bias on the definition of the exposure, and the outcome, we used the WHO Zika Causality Working Group causality framework (Krauer et al., 2017). This framework evaluates causality in ten dimensions (temporality, biological plausibility, strength of association, exclusion of alternate explanations, cessation, dose response relationship, animal experiments, analogy, specificity and consistency). The questions in each category were adapted from the previously published framework, and defined and addressed by two investigators in order to assess the linkage between ZIKV and severe and fatal cases.

Results

Study Selection and Characteristics:

A total of 311 articles were retrieved using the search strategy, and 20 of them were epidemiological reports or bulletins from surveillance agencies and Ministries of Health. After screening by abstract and title, 59 articles were selected for full-text assessment, of these 35 were excluded with reasons, and 24 were finally included for final qualitative analysis (Figure 1). The included articles included the following: thirteen epidemiological bulletins from national ministries of health that reported deaths attributable to ZIKV, eight case reports, and three morbidity and mortality weekly reports from the CDC (Table 1).

We found 50 reported deaths associated to postnatal ZIKV infection in nine countries, the 56.9% of them non related with GBS. Dominican Republic, Brazil and Puerto Rico reported the 67.6% of the deaths (33.3%; 18.6%, and 15.7% respectively). The 64.7% of the cases were confirmed either by RT-PCR, MAC-ELISA, or Immunohistochemistry (Table 1). The rest (35.3%), were not-laboratory confirmed, but diagnosed by the clinical definition of fever, rash, conjunctivitis and arthralgias in a place with previously ZIKV circulation (with at least one case confirmed by RT-PCR in the same area) (Pan American Health Organization / World Health Organization, 2017a, 2017f). Sixteen records were used to evaluate causality linkage according to the Zika Causality Group Framework (Krauer et al., 2017) (Table 2). The quality assessment of each study using the IHE instrument is summarized in Table 3.

Due to the lack of population-based studies assessing ZIKV and the risk of death, only 9 of the 20 studies examined here directly addressed the questions of the Zika Causality Group Framework (Table 2). The retrieved answer to each question is shown in Table 3. Hence, for the assessment of strength of association, cessation, and consistency, we took together the findings of the included case reports and epidemiological bulletins in order to summarize the available evidence in each dimension, as described later. For the assessment of animal experiments, we summarized the evidence derived from references of the included articles, as well as experiments in animal models published by January 1, 2018.

Causality linkage assessment by dimension:

The characteristics of individualized case reports by dimension is summarized in Table 4.

Temporality

Fifteen cases, reported in eight articles, addressed questions related with the temporality dimension (Arzuza-Ortega et al., 2016, Azevedo et al., 2016, Dirlikov et al., 2016, Sarmiento-Ospina et al., 2016, Schwartzmann et al., 2017, Sharp et al., 2016, Swaminathan et al., 2016, Zonneveld et al., 2016). In most of these, ZIKV infection was confirmed before the evolution to death, and the interval between exposure to ZIKV and occurrence of related symptoms was typical for arbovirus infections (Arzuza-Ortega et al., 2016, Azevedo et al., 2016, Dirlikov et al., 2016, MM et al., 2018, Sarmiento-Ospina et al., 2016, Schwartzmann et al., 2017, Sharp et al., 2016, Swaminathan et al., 2016, Zonneveld et al., 2016). Remarkably, one of the patients, a 73-year-old man from USA, developed the clinical picture after a three-week travel to a Zika endemic area. When he came back to his home country, a non-arboviral endemic area, he developed a febrile illness for a few days with a RT-PCR test confirmatory for ZIKV. After, he presented hypotension, dyspnea and death (Brent, 2016, Swaminathan et al., 2016, Walker, 2016). In two cases (Dirlikov et al., 2016, Soares et al., 2016), the infection was not documented before the complication, and in one of them the patient quickly developed neurological symptoms and signs leading to death (Soares et al., 2016). There were not studies addressing a time-dependent relationship between the occurrence of ZIKV cases and cases that evolve to death in a population level.

Biological plausibility

The reported cases support evolution to death after ZIKV infection through a disseminated viral infection. ZIKV antigens and RNA were found in tissues in three cases reported in two studies, suggesting spread of the virus to the injured organs (Azevedo et al., 2016, Schwartzmann et al., 2017). In one case the autopsy revealed features of a viral encephalitis, characterized by neuronal necrosis of basal nuclei in the brain with intense edema in the white matter, focal gliosis, neuronophagia, and perivascular hemorrhages, with an inflammatory infiltrate mainly of mononuclear cells in the cortex (Azevedo et al., 2016). On the other hand, features suggestive of a viral hepatitis were found with necrotic lesions and acidophilic bodies generally accompanied by multifocal steatosis, inflammatory infiltrates and vascular congestion in the portal space in another report (Azevedo et al., 2016). Notwithstanding, information regarding particular features of innate and adaptive immune response of the patients that can facilitate dissemination and infiltration of tissues and evolution to death in the reported cases is lacking, although animal experiments support it as will be discussed further.

Strength of Association

Since we did not find studies assessing the risk of death during acute ZIKV infection at the population level, for the assessment of this dimension, we assessed data derived from epidemiological bulletins and reports. Twelve epidemiological bulletins reported deaths associated with ZIKV infection occurring in several countries (Agence Régionale de Santé, 2016, Ministério da Saúde − Brasil, 2016, 2017, Ministerio de Salud Pública de República Dominicana, 2017, Pan American Health

Organization / World Health Organization, 2017a, 2017b, 2017c, 2017d, 2017e, 2017f, 2017g, 2017h). We calculated case fatality rates (CFR) using the total reported deaths and the laboratory confirmed cases by March 2017 when the epidemic was ending. The CFR was calculated by country using the formula (fatal cases/WHO cumulative reported cases until March 2017) x 100. We estimated a median CFR of 0.02% among countries (range 0.002% in Martinique to 0.324% in Dominican Republic). The lack of prospective cohort studies makes it difficult to properly estimate the individual risk of progression to death following ZIKV infection.

Exclusion of alternate explanations

In eleven cases from five articles, infectious diseases other than ZIKV were excluded as alternative explanations for the cause of death (Azevedo et al., 2016, Sarmiento-Ospina et al., 2016, Schwartzmann et al., 2017, Sharp et al., 2016, Zonneveld et al., 2016). The assessment of co-infections or an alternative infectious explanation for the outcome varied across different reports, as expected because of the different epidemiological backgrounds and clinical pictures. It included a wide variety of arboviral diseases like dengue, chikungunya, St. Louis encephalitis, West Nile and yellow fever, as well as other viral diseases like rotavirus infection, viral hepatitis A, B and C, HIV-1 and −2, HTLVI/II, herpes virus types 1, 2 and 6, polyomavirus, Epstein-Barr virus, cytomegalovirus, and varicella zoster (Azevedo et al., 2016, Schwartzmann et al., 2017) among others. Bacterial diseases like syphilis, leptospirosis, bacterial meningitis, bacteremia, and parasitic infections like Chagas disease, malaria, and toxoplasmosis were also assessed (Azevedo et al., 2016, Sharp et al., 2016, Swaminathan et al., 2016, Zonneveld et al., 2016).

Ten out of the sixteen cases reported at least one comorbidity (Arzuza-Ortega et al., 2016, Azevedo et al., 2016, Sarmiento-Ospina et al., 2016, Schwartzmann et al., 2017, Sharp et al., 2016, Swaminathan et al., 2016, Zonneveld et al., 2016). But in some of these, the comorbidity was not directly related to the cause of death. For example, hyperlipidemia or high blood pressure with severe thrombocytopenia (Sharp et al., 2016) or arterial hypotension with septic shock and respiratory failure (Sarmiento-Ospina et al., 2016, Zonneveld et al., 2016) (Table 4). According to the information reported in the articles, there is no evidence pointing to inappropriate treatment as the cause of death. Notably, in four cases, the researchers were able to exclude an infection other than Zika, co-morbidities and inappropriate treatment as alternate associated factors with the evolution to death (Azevedo et al., 2016, Zonneveld et al., 2016).

Cessation

Although no specific item specifically assessed this dimension, we did not find reports of ZIKV-associated deaths after the reduction of ZIKV infection cases during the first half of 2017.

Dose response relationship

For viral infections, a dose-response relationship would be present if a greater degree of viremia correlated with disease manifestations and/or death. Only four cases reported quantification of viral load (Swaminathan et al., 2016, Zonneveld et al., 2016), though some of them reported qualitative results with quantitative RT-PCR. Since we did not find assessment of ZIKV infection and its relationship with viral load at the population level, it is not possible to conclude whether higher viral copy numbers confer an increased risk of complications and death. Our search revealed cases of death with viral loads at both the high and low end of the spectrum: two cases with viral load lower than 1×10^4 copies/mL (Zonneveld et al., 2016) and two cases with viral load higher than 1×10^8 copies/mL (Swaminathan et al., 2016, Zonneveld et al., 2016). Animal experiments as well as analogy are explained in the Supplemental Material.

Specificity

Three out of the sixteen individual cases showed pathological findings specific of ZIKV infection as demonstrated by RT-PCR and immunohistochemistry in damaged tissues (Azevedo et al., 2016, Schwartzmann et al., 2017). One of them was a 36 years old man with a history of heart transplant eight months before the onset of fever and a clinical picture compatible with a viral meningoencephalitis. During treatment, immunosuppressive drugs were withdrawn except methylprednisolone, and the patient died because of cardiogenic shock due to acute cardiac allograft rejection. ZIKV antigens were detected in inflammatory cells within the central nervous system as well as in the heart, liver, and lung (Schwartzmann et al., 2017). The other patient was a 36-year-old man with erythematous lupus who presented with a clinical picture similar to severe dengue and evolved to acute respiratory failure. Intra-cardiac blood as well as fragments of brain, spleen and liver, and a pool of heart, lung and kidney were obtained at autopsy for examination. RT-qPCR for ZIKV was positive in all specimens, and ZIKV antigen were found in the brain, liver, kidney, heart and lung, mainly in apoptotic cells. Viral isolation was possible in pooled viscera (Azevedo et al., 2016). The last patient was a 20-year-old woman who presented with a febrile illness, pancytopenia, and bilateral pulmonary abscess. During the autopsy specimens of lung, kidney and liver were collected, and tested positive for ZIKV ARN with RT-qPCR and ZIKV antigens with immunohistochemistry, although viral isolation was not possible in this case (Azevedo et al., 2016). Consequently, the evidence suggest that tissue injuries could be related specifically to the presence of the virus or its components in tissues and organs demonstrated through immunological and molecular tools.

Consistency

We found ZIKV infection-related deaths across different countries in the Americas region and across a wide range of ages and different gender (Table 2). However, the reports were limited to case reports, case series and epidemiological bulletins, and in some of these, the ZIKV linage was not necessarily determined. We did not find population-based studies assessing the risk of death across different populations, but the retrieved reports were found across nine different countries in the Americas.

Discussion

The existing literature on clinical outcomes in ZIKV infection includes several reports of severe manifestations as well as fatal cases. (Rajahram et al., 2019, Rodriguez-Morales et al., 2019). We found 51 deaths reported during the 2016-2017 epidemic, with a mean CFR of 0.02% in the Americas. The assessment of the causality link through the WHO Zika Causality Working Group framework, allowed to establish that these clinical varieties of the disease occur in a period of time similar to other arboviruses, with a clinical picture analog to other arboviruses, and through mechanisms that are possibly similar to those found in lethal animal models implicating a disseminated viral disease (Arzuza-Ortega et al., 2016, Azevedo et al., 2016, Rodriguez-Morales et al., 2019, Soares et al., 2016, Swaminathan et al., 2016). Those lethal cases have been reported in different territories in the Americas and the pathological changes have been proved to be specifically related to ZIKV infection by immunohistochemistry and RT-PCR (Sarmiento-Ospina et al., 2016).

The role of comorbidities, age, and viral load could not be definitively established. An argument against ZIKV infection *per se* being causative in lethal cases is that most deaths occurred either in elderly patients or with significant medical comorbidities (Sarmiento-Ospina et al., 2016). However, cases of healthy young patients evolving to death have been also reported (Azevedo et al., 2016, Sarmiento-Ospina et al., 2016). Of note in these cases, the time of the evolution and the clinical picture were similar to other arboviruses, particularly dengue, and alternate explanations like other infections, comorbidities and treatment errors were excluded. However, because no population level studies have been conducted assessing comorbidities, age and viral load as risks factors, the weight of these variables in the evolution to death could not be established.

The role of the immune response is still incompletely understood. ZIKV infection is lethal in animal models deficient in certain innate immune responses. Features of the adaptive immune response have also been associated with protection from ZIKV-induced neurologic disease, viral replication (Aliota et al., 2016, Dowall et al., 2016, Hassert et al., 2018, Kawiecki et al., 2017, Lazear et al., 2016, Lazear et al., 2013, Rossi et al., 2016) and viral tissue burden. However, ZIKV is also able to disseminate to multiple tissues even in immunocompetent non-human primates, which is likely a necessary step along the evolution to death in fatal Zika cases. May be, for example asymptomatic humans shed live virus in semen for example, but this is not necessarily enough evidence to conclude that dissemination is a defining feature of fatal cases. We can assume that zika goes to a lot of tissues in asymptomatic and self-limiting cases as well and then, this is not the difference in the associated outcome. The role of specific features of the immune response, like interferon polymorphisms, in the evolution to disseminated and complicated infections by ZIKV is unknown but have proven to be important in other viral infections (Lazear et al., 2019, Lindqvist et al., 2016).

Limitations

Although individual cases have been reported, and epidemiological data from epidemiological bulletins were used for estimation of CFR, the lack of population-based studies properly conducted for assessment of our outcome of interest limits

the strength of the evidence for definitive conclusions. Founded on an individual basis assessment of the reported cases Certain dimensions of the WHO Zika Causality Working Group framework were assessed by compiling data from individual case reports or case series; however, other elements like consistency, cessation, and dose response relationship were addressed based on observations derived from epidemiological bulletins – though these are not the most robust source of data for answering population level questions. This systematic review highlights the need for case control and cohort studies for thoroughly assessing ZIKV infection as cause of death and for understanding common features Zika cases with adverse outcomes in order to inform optimal clinical management.

Conclusions

The results of this systematic review suggest that ZIKV infection can evolve to severe forms of disease, including death. However, severe outcomes appear to be rare, and it would be difficult to firmly and totally establish attribution to ZIKV infection (ie exclude other contributing causes) in the absence of large population-based studies. Certain comorbidities could increase the risk of severe ZIKV infection, but it should also be considered whether previous infection by heterologous flaviviruses such as DENV contribute to the pathogenesis of severe Zika. Clinicians should be aware that ZIKV infection can present with a clinical picture similar to dengue and other arboviruses, including with thrombocytopenia and bleeding, shock or severe neurological disease including encephalitis, although these severe manifestations have very rarely been reported. Most arbovirus infections are subclinical, and symptomatic illness usually manifests in three primary clinical syndromes: 1) a systemic febrile illness, 2) neuroinvasive disease, or 3) hemorrhagic fever. ZIKV infection manifests mainly as a systemic febrile illness and neuroinvasive disease. Thrombocytopenia and bleeding in ZIKV infection may be due to a nonspecific systemic inflammatory response in rare cases rather than a hemorrhagic fever syndrome as seen with other flavivirus infections such as DENV and yellow fever.

Author Contributions. AJRM and JACO formulated the research questions, designed the study, developed the preliminary search strategy, and drafted the manuscript. WFAM, KMNP and DFMP refined the search strategy by conducting iterative database queries and incorporating novel search terms. WFAM, KMNP and DFMP searched and collected the articles. JACO and VGV conducted the quality assessment. All authors critically reviewed the manuscript for important intellectual content. All authors have read and approved the final version of the manuscript.

Conflicts of interest. All authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential.

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Figure 1. Study selection and characteristics.

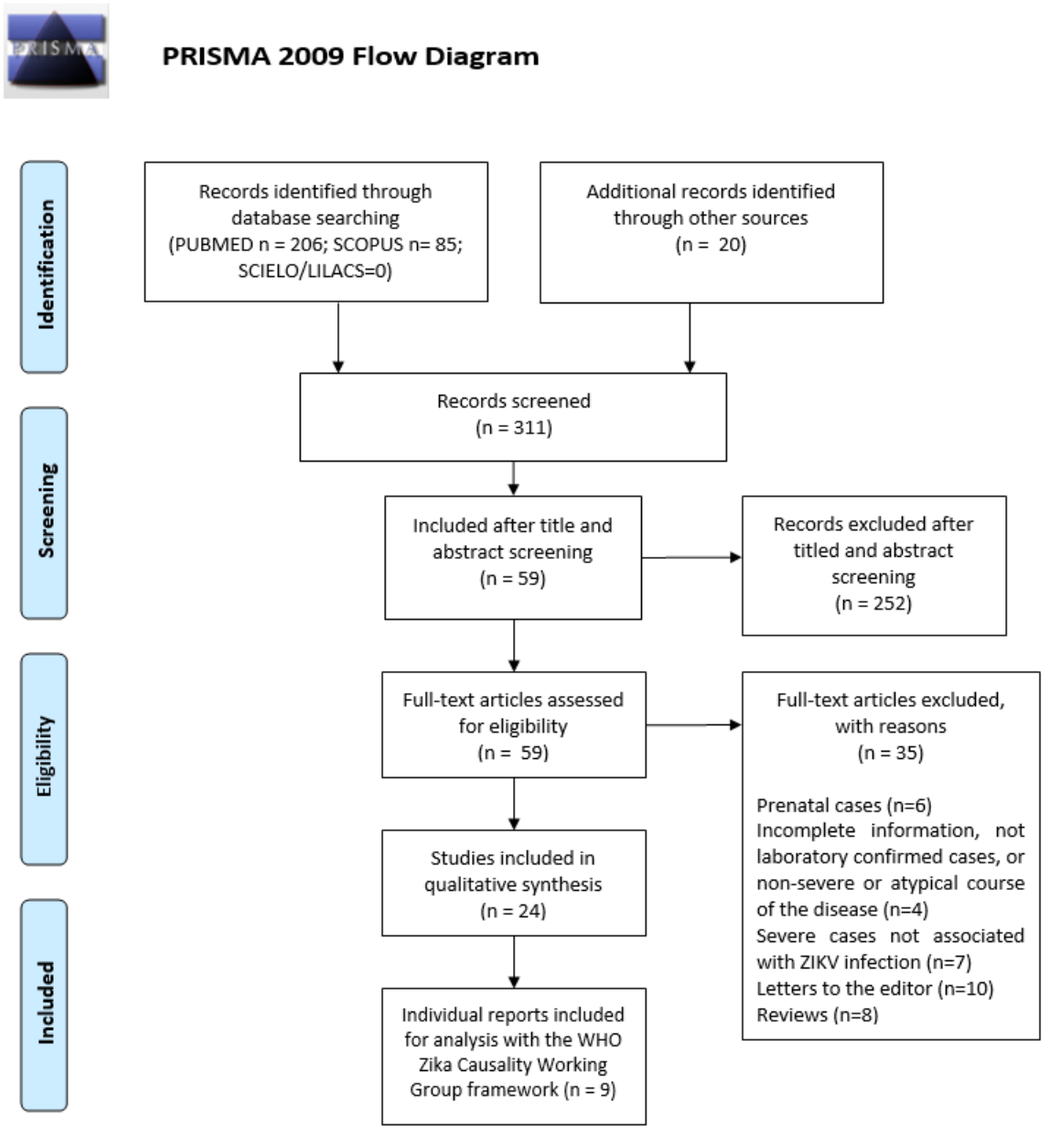

Table 1. Characteristics of the studies.

Studies	Type of publication	Institution	Country	Total of Zika Cases (Country)	Reported Deaths	Infection Assessment	n (Total Deaths)	GBS related deaths	Non GBS related deaths	CFR (Country)	Year
Walker WL, et al. 2016	MMWR	CDC	USA	4969	1	RT-PCR, MAC-ELISA, PNRT	1	0	1	0.020	2016
Brent C, et al. 2016	MMWR	CDC			1						
PAHO/WHO, 2017	Epidemiologic Bulletin	WHO			1						
Swaminathan S, et al. 2016	Case report	University of Utah School of Medicine			1						
PAHO/WHO, 2017	Epidemiologic Bulletin	WHO	Puerto Rico	39339	8	RT-PCR	8	2	6	0.020	2016
Dirlikov E, et al. 2016	MMWR	CDC			1	MAC-ELISA					
Sharp TM, et al. 2016	Case report	CDC			1	RT-PCR, ELISA					
PAHO/WHO, 2017	Epidemiologic Bulletin	WHO	Brazil	346475	11	RT-PCR	11	0	11	0.003	2015-2016
Ministério da Saúde – Brasil, 2015	Epidemiologic Bulletin	Secretaria de Vigilância em Saúde − Ministério da Saúde									
Ministério da Saúde – Brasil, 2016	Epidemiologic Bulletin	Secretaria de Vigilância em Saúde − Ministério da Saúde									
Soares CN, et al. 2016	Case report	Hospital Federal dos Servidores do Estado			1	RT-PCR, ELISA					
Azevedo RS, et al. 2016	Case report	Instituto Evandro Chagas			3	RT-PCR, Immunohisto chemistry					

Studies	Type of publication	Institution	Country	Total of Zika Cases (Country)	Reported Deaths	Infection Assessment	n (Total Deaths)	GBS related deaths	Non GBS related deaths	CFR (Country)	Year
Schwartzmann PV, et al. 2017	Case report	Clinic Hospital of Ribeirão Preto			1	RT-PCR					
PAHO/WHO, 2017	Epidemiologic Bulletin	WHO	Suriname	3490	4	RT-PCR/ELISA	4	0	4	0.115	2016-2017
Zonneveld R, et al. 2016	Case report	Academic Hospital Paramaribo			3	RT-PCR					
Sarmiento-Ospina A, et al. 2016	Case report	Hospital del Tolima	Colombia	107206	4	RT-PCR	5	0	5	0.005	2015
Arzuza-Ortega L, et al. 2016	Case report	Empresa Social del Estado Hospital de Malambo			1	RT-PCR					
Ministerio de Salud Pública – República Dominicana, 2017	Epidemiologic Bulletin	WHO	Dominican Republic	5241	17	Unknown	17	17	0	0.324	2016
Ministerio de Salud Pública – República Dominicana, 2017	Epidemiologic Bulletin	Ministerio de Salud Pública			17	Unknown					
PAHO/WHO, 2017	Epidemiologic Bulletin	WHO	Guadeloupe	31227	3	RT-PCR	3	1	2	0.010	2016-2017
Agence Régionale de Santé, 2017	Epidemiologic Bulletin	Agence Régionale de Santé			2	RT-PCR					
PAHO/WHO, 2017	Epidemiologic Bulletin	WHO	Martinique	36701	1	RT-PCR	1	1	0	0.003	2016
Agence Régionale, 2016	Epidemiologic Bulletin	Agence Régionale de Santé			1	RT-PCR					
PAHO/WHO, 2017	Epidemiologic Bulletin	WHO	Bolivia	1029	1	Unknown	1	1	0	0.097	2016

Table 2. References framework for assessment of Zika deaths.

	Dimension	Number of linked cases	Linked Items
1	**Temporality**		
1.1	Does ZIKV infection precede the evolution to death in individuals?	14	(Arzuza-Ortega et al., 2016, Azevedo et al., 2016, Sarmiento-Ospina et al., 2016, Schwartzmann et al., 2017, Swaminathan et al., 2016, Zonneveld et al., 2016)
1.2	Is there a consistent time- dependent relationship between the occurrence of ZIKV cases and cases that evolve to death in a population- level?	0	
1.3	Is the interval between exposure to ZIKV and occurrence of related symptoms typical for post-infectious death?	15	(Arzuza-Ortega et al., 2016, Azevedo et al., 2016, Dirlikov et al., 2016, Sarmiento-Ospina et al., 2016, Schwartzmann et al., 2017, Sharp et al., 2016, Swaminathan et al., 2016, Zonneveld et al., 2016)
2	**Biological Plausibility**		
2.1	Are there biologically plausible mechanisms that explain how ZIKV can evolve to death?	-	
3	**Strength of Association**		
3.1	How strong is the association between ZIKV infection and death at the individual level?	-	
3.2	How strong is the association between ZIKV infection and death at the population level?	-	
4	**Exclusion of Alternate Explanations**		
4.1	Have other explanations/confounders of the association between ZIKV infection and death been excluded, such as other infections?	11	(Azevedo et al., 2016, Sarmiento-Ospina et al., 2016, Schwartzmann et al., 2017, Sharp et al., 2016, Zonneveld et al., 2016)
4.2	Have other explanations/confounders of the association between ZIKV infection and fatal cases, such as underlying systemic disease?	4	(Azevedo et al., 2016, Sarmiento-Ospina et al., 2016, Zonneveld et al., 2016)
4.3	Have other explanations/confounders of the association between ZIKV infection and death been excluded, such as inappropriate treatment?	13	(Arzuza-Ortega et al., 2016, Azevedo et al., 2016, Dirlikov et al., 2016, Schwartzmann et al., 2017, Soares et al., 2016, Zonneveld et al., 2016)
5	**Cessation**		
5.1	Does the intentional prevention/removal/elimination of ZIKV infection in individuals, e.g. by insect repellents, lead to a reduction in fatal cases?	-	

	Dimension	Number of linked cases	Linked Items
5.2	Does the intentional removal/elimination/prevention of ZIKV at population-level, e.g. by vector control, lead to a reduction in fatal cases?	-	
5.3	Does a natural removal/elimination/prevention of ZIKV at population-level, e.g. increase in immune individuals or decrease in vector abundance lead to a reduction in fatal cases?	-	
6	**Dose/Response relationship**		
6.1	Are the risk of death and the clinical severity of ZIKV infection associated with higher viral titres or viral load in biological samples?	1	(Zonneveld et al., 2016)
7	**Animal experiments**		
7.1	Do animal experiments support the association of ZIKV infection and fatal cases?	-	
8	**Analogy**		
8.1	Do other flaviviruses or arboviruses cause death and by similar mechanism(s)?	16	(Arzuza-Ortega et al., 2016, Azevedo et al., 2016, Dirlikov et al., 2016, Sarmiento-Ospina et al., 2016, Schwartzmann et al., 2017, Swaminathan et al., 2016)
9	**Specificity**		
9.1	Are there pathological findings in fatal cases specific for ZIKV infection?	2	(Azevedo et al., 2016, Schwartzmann et al., 2017)
10	**Consistency**		
10.1	Is the association between ZIKV infection and fatal cases consistently found across different geographical regions?	-	
10.2	Is the association between ZIKV infection and fatal cases consistently found across different populations/subpopulations?	-	
10.3	Is the association between ZIKV infection and fatal cases consistently found across different ZIKV lineages/strains?	-	
10.4	Is the association between ZIKV infection and fatal cases consistently found across different study designs?	-	

Table 3. Quality assessment of the studies.

	Quality Criteria																			
Case	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Swaminathan S, Schlaberg R, Lewis J, Hanson KE, Couturier MR. Fatal Zika Virus Infection with Secondary Nonsexual Transmission. N Engl J Med. 2016;375(19):1907-9	Yes	Yes	No	Yes	Partial	Partial	Yes	Partial	No	Yes	Unclear	Yes	Unclear	No	Yes	No	Yes	Yes	Yes	Yes
Dirlikov E, Major CG, Mayshack M, et al. Guillain-Barré Syndrome During Ongoing Zika Virus Transmission — Puerto Rico, January 1–July 31, 2016. MMWR Morb Mortal Wkly Rep 2016;65:910–914	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Partial	Partial	No	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Soares CN, Brasil P, Carrera RM, Sequeira P, de Filippis AB, Borges VA, et al. Fatal encephalitis associated with Zika virus infection in an adult. J Clin Virol. 2016;83:63-5	Yes	Yes	No	No	Yes	Yes	No	Yes	Yes	Yes	No	Yes	Yes	No	Yes	No	No	Yes	Yes	Yes
Azevedo RS, Araujo MT, Martins Filho AJ, Oliveira CS, Nunes BT, Cruz AC, et al. Zika virus epidemic in Brazil. I. Fatal disease in adults: Clinical and laboratorial aspects. J Clin Virol. 2016;85:56-64	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes
Schwartzmann PV, Ramalho LN, Neder L, Vilar FC, Ayub-Ferreira SM, Romeiro MF, et al. Zika Virus Meningoencephalitis in an Immunocompromised Patient. Mayo Clin Proc. 2017;92(3):460-6.	Yes	Yes	No	Yes	Yes	Partial	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	yes
Sharp TM, Munoz-Jordan J, Perez-Padilla J, Bello-Pagan MI, Rivera A, Pastula DM, et al. Zika Virus Infection Associated With Severe Thrombocytopenia. Clin Infect Dis. 2016;63(9):1198-201.	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes
Zonneveld R, Roosblad J, Staveren JW, Wilschut JC, Vreden SG, Codrington J. Three atypical lethal cases associated with	Yes	Yes	Yes	No	Yes	Partial	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Unclear	No	Yes	Yes	Yes	Yes

	Quality Criteria																			
Case	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
acute Zika virus infection in Suriname. IDCases. 2016;5:49-53.																				
Sarmiento-Ospina A, Vasquez-Serna H, Jimenez-Canizales CE, Villamil-Gomez WE, Rodriguez-Morales AJ. Zika virus associated deaths in Colombia. Lancet Infect Dis. 2016;16(5):523-4	Partial	Yes	No	Yes	Yes	Partial	Yes	Yes	No	Yes	Yes	Partial	Unclear	No	Yes	No	Yes	No	Yes	Yes
Arzuza-Ortega L, Polo A, Perez-Tatis G, Lopez-Garcia H, Parra E, Pardo-Herrera LC, et al. Fatal Sickle Cell Disease and Zika Virus Infection in Girl from Colombia. Emerg Infect Dis. 2016;22(5):925-7	Partial	Yes	Yes	No	Yes	Partial	No	Yes	Partial	Yes	No	Yes	Yes	No	Yes	No	No	Yes	Yes	Yes

1: Was the hypothesis/aim/objective of the study clearly stated? 2: Was the study conducted prospectively? 3: Were the cases collected in more than one centre? 4: Were patients recruited consecutively? 5: Were the characteristics of the patients included in the study described? 6: Were the eligibility criteria (i.e. inclusion and exclusion criteria) for entry into the study clearly stated? 7: Did patients enter the study at a similar point in the disease? 8: Was the intervention of interest clearly described? 9: Were additional interventions (co-interventions) clearly described? 10: Were relevant outcome measures established a priori? 11: Were outcome assessors blinded to the intervention that patients received? 12: Were the relevant outcomes measured using appropriate objective/subjective methods? 13: Were the relevant outcome measures made before and after the intervention? 14: Were the statistical tests used to assess the relevant outcomes appropriate? 15: Was follow-up long enough for important events and outcomes to occur? 16: Were losses to follow-up reported? 17: Did the study provided estimates of random variability in the data analysis of relevant outcomes? 18: Were the adverse events reported? 19: Were the conclusions of the study supported by results? 20: Were both competing interests and sources of support for the study reported?

Table 4. Characteristics of individualized cases of Zika deaths.

Case	Age (y)	Gender	Comorbilities	Cause of death	Causality Dimension																			
					1.Temporality			2.Biological Plausibility	3.Strength of Association		4.Exclusion of Alternate Explanations			5.Cessation			6.Dose/ Response relationship	7.Animal experiments	8.Analogy	9.Specificity	10.Consistency			
					1.1	1.2	1.3	2.1	3.1	3.2	4.1	4.2	4.3	5.1	5.2	5.3	6.1	7.1	8.1	9.1	10.1	10.2	10.3	10.4
Swaminathan S, Schlaberg R, Lewis J, Hanson KE, Couturier MR. Fatal Zika Virus Infection with Secondary Nonsexual Transmission. N Engl J Med. 2016;375(19):1907-9	73	M	YES	Respiratory and Renal failure, Hepatitis, and Shock	YES	-	YES	-	-	-	NO	NO	NO	-	-	-	YES	-	YES	NO	-	-	-	-
Dirlikov E, Major CG, Mayshack M, et al. Guillain-Barré Syndrome During Ongoing Zika Virus Transmission — Puerto Rico, January 1–July 31, 2016. MMWR Morb Mortal Wkly Rep 2016;65:910–914	N/A	N/A	N/A	GBS related Septic Shock	NO	-	YES	-	-	-	NO	NO	YES	-	-	-	NO	-	YES	NO	-	-	-	-
Soares CN, Brasil P, Carrera RM, Sequeira P, de Filippis AB, Borges VA, et al. Fatal encephalitis associated with Zika virus infection in an adult. J Clin Virol. 2016;83:63-5	47	F	NO	Respiratory failure associated to a neurologic syndrome	NO	-	NO	-	-	-	NO	NO	YES	-	-	-	NO	-	YES	NO	-	-	-	-
Azevedo RS, Araujo MT, Martins Filho AJ, Oliveira CS, Nunes BT, Cruz AC, et al. Zika virus epidemic in Brazil. I. Fatal disease in adults: Clinical and laboratorial aspects. J Clin Virol. 2016;85:56-64	36	M	YES	Respiratory failure	YES	-	YES	YES	-	-	YES	NO	NO	-	-	-	NO	-	YES	YES	-	-	-	-
	16	F	NO	Severe thrombocytopenia related with jaundice and severe hemorrhage	YES	-	YES	YES	-	-	YES	YES	YES	-	-	-	NO	-	YES	NO	-	-	-	-

Case	Age (y)	Gender	Comorbilities	Cause of death	Causality Dimension																			
					1.Temporality			2.Biological Plausibility	3.Strength of Association		4.Exclusion of Alternate Explanations			5.Cessation			6.Dose/ Response relationship	7.Animal experiments	8.Analogy	9.Specificity	10.Consistency			
					1.1	1.2	1.3	2.1	3.1	3.2	4.1	4.2	4.3	5.1	5.2	5.3	6.1	7.1	8.1	9.1	10.1	10.2	10.3	10.4
	20	F	NO	Pancytopenia with haemorrhage and pulmonary abscess	YES	-	YES	YES	-	-	YES	YES	YES	-	-	-	NO	-	YES	YES	-	-	-	-
Schwartzmann PV, Ramalho LN, Neder L, Vilar FC, Ayub-Ferreira SM, Romeiro MF, et al. Zika Virus Meningoencephalitis in an Immunocompromised Patient. Mayo Clin Proc. 2017;92(3):460-6.	36	M	YES	Encephalitis, lymphopenia and cardiogenic shock due to acute cardiac allograft rejection	YES	-	YES	-	-	-	YES	NO	YES	-	-	-	NO	-	YES	YES	-	-	-	-
Sharp TM, Munoz-Jordan J, Perez-Padilla J, Bello-Pagan MI, Rivera A, Pastula DM, et al. Zika Virus Infection Associated With Severe Thrombocytopenia. Clin Infect Dis. 2016;63(9):1198-201.	72	M	YES	Severe thrombocytopenia related with severe hemorrhage	YES	-	YES	-	-	-	YES	NO	NO	-	-	-	NO	-	YES	NO	-	-	-	-
Zonneveld R, Roosblad J, Staveren JW, Wilschut JC, Vreden SG, Codrington J. Three atypical lethal cases associated with acute Zika virus infection in Suriname. IDCases. 2016;5:49-53.	61	M	YES	Septic Shock	YES	-	YES	-	-	-	YES	YES	YES	-	-	-	YES	-	YES	NO	-	-	-	-
	64	M	YES	Thrombocytopenia and septic Shock	YES	-	YES	-	-	-	YES	NO	YES	-	-	-	NO	-	YES	NO	-	-	-	-
	59	M	YES	Septic Shock	YES	-	YES	-	-	-	YES	YES	YES	-	-	-	NO	-	YES	NO	-	-	-	-
Sarmiento-Ospina A, Vasquez-Serna H, Jimenez-Canizales CE, Villamil-Gomez WE, Rodriguez-Morales AJ. Zika virus	2	F	NO	Respiratory failure and shock	YES	-	YES	-	-	-	YES	NO	YES	-	-	-	NO	-	YES	NO	-	-	-	-

Case	Age (y)	Gender	Comorbilities	Cause of death	Causality Dimension																		
					1.Temporality			2.Biological Plausibility	3.Strength of Association		4.Exclusion of Alternate Explanations			5.Cessation			6.Dose/ Response relationship	7.Animal experiments	8.Analogy	9.Specificity	10.Consistency			
					1.1	1.2	1.3	2.1	3.1	3.2	4.1	4.2	4.3	5.1	5.2	5.3	6.1	7.1	8.1	9.1	10.1	10.2	10.3	10.4
associated deaths in Colombia. Lancet Infect Dis. 2016;16(5):523-4	30	F	NO	Severe thrombocytopenia, severe hemorrhage, respiratory failure, seizures and shock	YES	-	YES	-	-	-	YES	NO	YES	-	-	-	NO	-	YES	NO	-	-	-	-
	61	M	YES	Respiratory failure, acute myocardial infarction, shock	YES	-	YES	-	-	-	NO	NO	YES	-	-	-	NO	-	YES	NO	-	-	-	-
	72	F	YES	Respiratory failure and shock	YES	-	YES	-	-	-	YES	NO	YES	-	-	-	NO	-	YES	NO	-	-	-	-
Arzuza-Ortega L, Polo A, Perez-Tatis G, Lopez-Garcia H, Parra E, Pardo-Herrera LC, et al. Fatal Sickle Cell Disease and Zika Virus Infection in Girl from Colombia. Emerg Infect Dis. 2016;22(5):925-7	15	F	YES	Respiratory failure	YES	-	YES	-	-	-	NO	NO	YES	-	-	-	NO	-	YES	NO	-	-	-	-
Surveillance du virus Zika aux Antilles Guyane, Situation épidémiologique, Point épidémiologique du 22 décembre 2016 - N° 42 / 2016	N/A	N/A	N/A	GBS related	N/A	-	N/A	-	-	-	N/A	N/A	N/A	-	-	-	N/A	-	N/A	N/A	-	-	-	-

Case	Age (y)	Gender	Comorbilities	Cause of death	Causality Dimension																		
					1.Temporality			2.Biological Plausibility	3.Strength of Association		4.Exclusion of Alternate Explanations			5.Cessation			6.Dose/ Response relationship	7.Animal experiments	8.Analogy	9.Specificity	10.Consistency			
					1.1	1.2	1.3	2.1	3.1	3.2	4.1	4.2	4.3	5.1	5.2	5.3	6.1	7.1	8.1	9.1	10.1	10.2	10.3	10.4
Pan American Health Organization / World Health Organization. Zika – Epidemiological Report Bolivia. March 2017. Washington, D.C.: PAHO/WHO; 2017	N/A	N/A	N/A	GBS related	N/A	-	N/A	-	-	-	N/A	N/A	N/A	-	-	-	N/A	-	N/A	N/A	-	-	-	-

N/A:Not available/Not applicable. GBS:Guillain-Barré syndrome. PAHO:Pan-American Health Organization. WHO:World Health Organization. M:Male. F:Female.

Supplemental Materials.

Animal experiments.

Animal models in mice have shown lethal infection with ZIKV. Mice deficient in type I interferon receptors like the A129 (Dowall et al., 2016, Rossi et al., 2016) and C57BL/6J mice(Aliota et al., 2016), in type I and II interferon receptors like the AG129 (Aliota et al., 2016, Rossi et al., 2016), the IRF3/7 double knockout mice (Kawiecki et al., 2017, Lazear et al., 2013), the IRF3/5/7 triple knockout mice, and *lfnar1* knockout mice (Lazear et al., 2016) evolve to death after six to eight days of infection(Aliota et al., 2016, Dowall et al., 2016, Hassert et al., 2018). The time to death in these models is similar to that of other mouse models for arboviruses (Bereczky et al., 2010, Dowall et al., 2012). Notably, the pathogenesis involves a disseminated viral infection affecting tissues including heart, kidney, lung, spleen, liver, and brain (Rossi et al., 2016) with the development of neurological disease (Aliota et al., 2016, Dowall et al., 2016, Lazear et al., 2016). The brain shows severe pathology with prominent neutrophil infiltration adjacent to the choroid plexus, meninges, and small vessels in the cortex (Aliota et al., 2016), and encephalomyelitis with lymphocytic perivascular cuffing, gliosis and neuronal necrosis (Kawiecki et al., 2017).

On the other hand, animal models in immunocompetent non-human primates show prolonged viral spread but mild to moderate disease (Dudley et al., 2016, Li et al., 2016), with viral dissemination to a wide variety of organs and tissues including central nervous system, spleen, and liver (Li et al., 2016). Hence, animal models support broad tissue tropism in ZIKV infection, which may be a characteristic of ZIKV related to the possible mechanisms of pathogenesis of lethal human infection.

Analogy

We found similar clinical features and complications to those caused by other flaviviruses in all the reported cases. Most of them evolved from the febrile illness to shock, respiratory failure or severe thrombocytopenia with hemorrhage to death (Arzuza-Ortega et al., 2016, Azevedo et al., 2016, Sarmiento-Ospina et al., 2016, Sharp et al., 2016, Swaminathan et al., 2016, Zonneveld et al., 2016). In other cases, the patients developed a neurologic syndrome with associated complications (Dirlikov et al., 2016, Schwartzmann et al., 2017, Soares et al., 2016). Severe thrombocytopenia and vascular leak are well-known clinical feature of severe dengue, correlated with endothelial dysfunction that can evolve to severe bleeding and death (Hapsari Putri et al., 2018, Mourao et al., 2007). Although the thrombocytopenia of dengue is usually accompanied by leukopenia in the period between day 3 and day 8 following the onset of illness, pancytopenia has been also reported (Ellis et al., 2016, Jain

and Singh, 2008). Shock (Lovera et al., 2016), and respiratory failure (Kumar et al., 2013, Tso et al., 2016) can also be a consequence of infection with other flaviviruses, as well as neurological syndromes like encephalitis or GBS, which have been previously reported even for dengue (Baheti et al., 2018, Dalugama et al., 2018, Rajapakse et al., 2018). Hence, the clinical picture of severe ZIKV infection shares some features with that of other flaviviruses, e.g. yellow fever (Kallas et al., 2019). May be Zika deaths are not all that analogous to either yellow fever (e.g. die of liver failure and/or hemorrhage) or dengue (e.g. die of endothelial dysfunction, vascular leak, shock and/or hemorrhage). Neither of these patterns seem to emerge among most of the Zika fatal cases.