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Zika virus infection and risk of Guillain-Barré syndrome: A meta-analysis

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ABSTRACT

Objective: Findings from studies of the association between Zika virus (ZIKV) infection and Guillain-Barré syndrome (GBS) are inconsistent. I conducted a systematic review and meta-analysis to clarify the nature of this association.

Methods: I searched PubMed, Scopus, Cochrane, CINAHL, Web of Science, Scielo, and DOAJ for case report, ecological, and analytic studies with "Zika" and "Guillain-Barré syndrome" as keywords, published up to July 1stth 2018. I evaluated if ZIKV infection status influenced the diagnosis of GBS (detection bias) in case-report and analytic studies; assessed if changes in weekly number of cases of ZIKV infection during outbreaks were followed by changes in number of GBS cases 1–8 weeks later; gauged the likelihood of selection, confounding, information, sparse data, and time-dependent bias (i.e. when ZIKV infection was ascertained after GBS onset) in analytic studies; and calculated the average ZIKV-GBS odds ratio (OR) in studies without time-dependent bias.

Results: In case reports, ZIKV infection prevalence in GBS cases was 2.4 to 25 times higher than expected. Changes in the number of ZIKV-infection cases during outbreaks were not consequentially followed by changes in the number of GBS cases (OR: 1.01; 95% CI: 0.99–1.03). Major biases were likely in all but one analytic study, which showed a non-significant ZIKV-GBS association. The average ZIKV-GBS OR in studies without time-dependent bias was 1.57 (95% CI: 0.86–2.86).

Interpretation: These findings indicate the available evidence is insufficient to claim ZIKV infection causes GBS. Therefore, stakeholders may want to reconsider current ZIKV-GBS public health and patient care recommendations.

1. Introduction

Although Zika virus (ZIKV) infection is a mild disease that courses without symptoms in 60% of the cases [1], its clinical and public health importance has been heightened by a potential association with Guillain-Barré syndrome (GBS) and newborn microcephaly. ZIKV infection outbreaks occurred in most Latin American countries during 2015–2016. On April 2016 the World Health Organization (WHO) embraced the conclusions of a literature review [2], declared there was scientific consensus ZIKV infection caused GBS, and advised countries to strengthen epidemiologic surveillance and increase emergency care services capable of managing GBS cases [3].

To date the hypothesis of a ZIKV-GBS causal link rests mostly on reports of GBS cases with past or concurrent ZIKV infection [4,5], on the temporal sequence of outbreaks of both diseases [6,7], and on findings from a small number of case-control studies [8–13]. However, findings from those studies are inconsistent, and concerns have been raised about the causal nature of a ZIKV-GBS association [14–16]. In this study, I assessed the accuracy of the diagnosis of GBS and ZIKV infection in GBS case reports and whether the observed prevalence of ZIKV infection in GBS cases differed from the expected prevalence; evaluated if changes in the number of ZIKV infection cases during outbreaks were consequentially followed by changes in GBS cases weeks later; gauged the validity of case-control studies of the ZIKV-GBS association; and estimated the average effect of ZIKV infection on GBS risk. Findings from this study could further our understanding of a possible ZIKV-GBS link and inform current research, patient care, and public health policies.

2. Methods

2.1. Selection of publications and data extraction

I searched all articles with "Zika virus" and "Guillain-Barré syndrome" as keywords, published from 2014 to July 1stth 2018, using PubMed, Scopus, Cochrane, CINAHL, Web of Science, Scielo, and DOAJ, reviewed their abstracts, and selected those useful to formulate causal hypotheses (case-reports and ecological studies) or to identify

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causal effects (analytic observational studies; Appendix, item 1). Articles in English, Spanish, and Portuguese were eligible. Reviews, commentaries, and surveillance reports were excluded, as they provide no data useful for identifying a ZIKV-GBS causal link. Studies on mechanisms were also excluded, because they could provide support for a causal claim only if a difference-making relationship between the presumptive cause and the outcome has already been established [17]. The search was conducted with Endnote X6, PubMed, and database sites.

Data were extracted in two separate occasions and recorded directly in the code for the analysis. For case reports, data extracted included the cumulative risk of ZIKV infection in the country, the prevalence of ZIKV infection in GBS cases, and the number of GBS cases by diagnostic certainty according to Brighton criteria [18]; for ecological studies, the number of weekly cases of ZIKV infection and GBS; and for analytic studies, the number of participants for all combinations of exposure and outcome, and the odds ratio (OR) of GBS in ZIKV-infected individuals.

2.2. Analysis of case reports

I recorded the number of cases with laboratory confirmed ZIKV infection and by GBS diagnostic certainty (Appendix, item 2). I evaluated detection bias, i.e. whether ZIKV infection or symptoms influenced the diagnosis of GBS, by comparing the observed ($OP_{Z(+)GBS}$) and the expected ($EP_{z(+)GBS}$) prevalence of ZIKV-infected GBS cases in case reports with ≥10 cases (Appendix, item 3). The $EP_{z(+)GBS}$ was calculated assuming the country-specific risk of ZIKV infection (R_{ZIKV}) applied to GBS cases and non-cases, and that the maximum ZIKV-GBS OR ($_{MAX}OR_{Z\rightarrow GBS}$) was 8.88. This figure corresponds to the average of the largest OR reported in all published case-controls studies [8–13]. Under those assumptions $EP_{Z(+)GBS} = (R_{ZIKV} \times OR_{Z\rightarrow GBS}) \div [(R_{ZIKV} \times {}_{MAX}OR_{Z\rightarrow GBS}) + (1 - {}_{MAX}OR_{Z\rightarrow GBS})]$. I also calculated the smallest $OR_{Z\rightarrow GBS}$ that would result in the $OP_{Z(+)GBS}$, given the risk of ZIKV infection in the population (R_{ZIKV}).

2.3. Time series analysis of outbreaks

I used surveillance data from Latin America [7] and French Polynesia [13] to assess if changes in the weekly number of cases of *suspected ZIKV infection* (self-reported rash plus at least two of fever, conjunctivitis, arthralgia, myalgia, and periarticular edema) [19] were consequentially followed by changes in the number of GBS cases weeks later. The numbers of cases were extracted three times from figures in the original articles [7,13], using Engauge Digitizer 8.3 [20], and averaged for the analysis. I used Poisson autoregressive models to account for time fluctuations and auto-correlation in each time series (outbreak), and modeled the weekly numbers of cases with sine and cosine functions (Appendix, item 4) [21]. Changes in the number of ZIKV infection cases per week, one to eight weeks before, one week at a time, were used as predictors of the weekly number of GBS cases [22]. Separate analyses were conducted for each country [7] and the effects for each back-lagged week were averaged across countries using fixed effect models [23].

2.4. Evaluation of biases in observational analytical studies

For analytic studies of the ZIKV-GBS association, I evaluated the likelihood of specific types of bias. Detection bias was evaluated as described above. Selection bias was assessed taking into account who was eligible as a control, how controls were selected, and whether controls represented the population from which the cases came from.

Outcome misclassification was judged by the diagnostic certainty of GBS cases [18]. Exposure misclassification was assessed by considering how timing of ZIKV infection tests in relation to the onset of infection, and cross-reaction between antibodies for ZIKV and for other flaviviruses could bias the ZIKV-GBS association.

Confounding was evaluated by checking whether the study adjusted for factors associated to both ZIKV infection and GBS: age (reported risk ratio –RR: 1.20 per 10 years) [1,24]; male gender (reported RR = 1.78) [1,24]; diarrhea (a correlate of *C jenuni* –reported RR: 38.4, and infectious intestinal disease –reported RR: 7.3) [25,26]; and acute respiratory infection (RR:5.2) [25,26].

Time-dependent bias happens when exposure occurs after onset of the outcome, and precludes causal inferences [27]. It was determined by ascertaining when tests for ZIKV infection were conducted, and whether the infection plausibly happened before onset of GBS.

Sparse data bias was assessed by verifying if the study had few or no observations for all combinations of exposure, outcome, and matching factors, and by looking for extremely large ORs with extremely wide confidence intervals [28].

I re-estimated the ZIKV-GBS OR from Cao-Lormeau et al. [13], the seminal case-control study on the ZIKV-GBS association. To reduce the likelihood of time-dependent bias, I used ZIKV IgG level in GBS cases at the time of recruitment as the relevant exposure, instead of three months after recruitment (see their supplement Table 1) [13]. Also, to avoid selection bias, I compared cases to a random sample of the population from which they came from, instead of patients without fever [29]. Finally, I calculated the average of the largest OR reported in ZIKV-GBS association studies, for the assessment of detection bias (described above), and the average of the OR in studies without time-dependent bias, using a random-effects model [23] (Appendix, item 5). All analyses were conducted in Stata 15.1.

3. Results

Twenty four case reports, four ecological studies, and six analytical studies were included in this review (Fig. 1; Appendix, item 2).

3.1. GBS case reports

Case reports included 357 GBS cases (Appendix, item 3). Only 34.4% of them were tested for infection using ZIKV-specific plaque reduction neutralizing antibodies (PRNT) or RT-PCR, and 18.2% had lab-confirmed ZIKV infection (22.8% of those tested). Brighton level of diagnostic certainty was unknown in 31.6% of the cases, 25.8% were level 1, 31.1% level 2, 10.4% level 3, and 1.1% level 4. Briefly, about 4/5 GBS cases did not have lab-confirmed ZIKV infection and 1/3 of were of unknown diagnostic certainty.

In case reports with at least 10 cases, the observed prevalence of ZIKV infection ranged from 9.5% in cases from Jamaica [30] to 83.3% in cases from French Polynesia [31] (Table 1; Appendix, item 3). The smallest $OR_{Z\rightarrow GBS}$ that would have resulted in the $OP_{Z(+)GBS}$, given the risk of ZIKV infection in the country (R_{ZIKV}), ranged from 6 in French Polynesia [31] to 364 in Colombia [32]. For the $_{MAX}OR_{Z\rightarrow GBS}$, the $OP_{z(+)GBS}$ was about 8, 11, and 25 times higher than the $EP_{Z(+)GBS}$ in case reports from Brazil [33], Puerto Rico [26], and Colombia [32], respectively.

3.2. Time series analysis of outbreaks

Three out of four ecological studies [6,7,13,34] assessed a ZIKV-GBS link by estimating the cross-correlation between numbers of cases of both diseases. Mendez et al. [34] found no significant correlation in Colombia. In contrast, Paploski et al. [6] and dos Santos et al. [7] concluded ZIKV infection outbreaks have caused GBS outbreaks in seven Latin American countries. The fourth study [13] concluded an outbreak of ZIKV infection caused an outbreak of GBS in French Polynesia, based only on their temporal sequence. However, in none of these studies were changes in the weekly number of cases of *suspected ZIKV infection* [19] followed by changes in the number of GBS cases one to eight weeks later, once time effects were accounted for through time series analyses [16] (Fig. 2 and Appendix Fig. 3, item 4). The largest OR corresponded to a lagged exposure of eight weeks (OR = 1.01; 95%

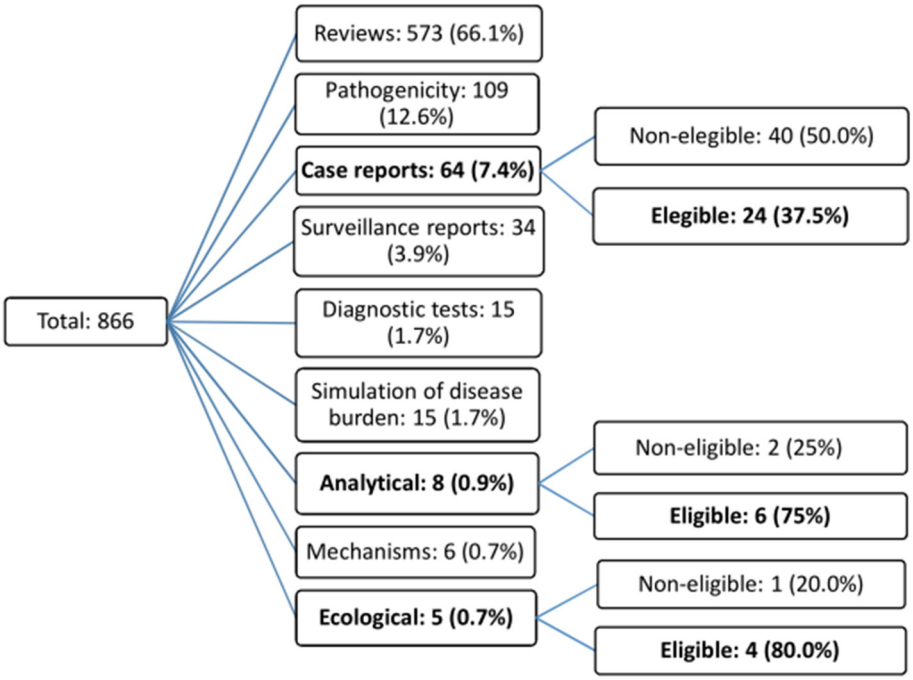

Fig. 1. Flowchart of study selection.

confidence interval -CI: 0.99–1.03).

3.3. Analytic observational studies

Six analytic studies were found. They included 602 GBS cases [8–13]. At least two major biases were likely in all but one study [12] (Table 2). Only two studies, including 17% of all cases, failed to report diagnostic certainty [11,13]. Within the group with known diagnostic certainty, 87% were Brighton levels 1 and 2.

Styczynski et al. [8] studied 41 cases (Brighton level 1 and 2) and 85 randomly selected age-matched neighborhood controls from Salvador, Bahia, Brazil. GBS cases occurred from January 1st to August 31st 2015, and were retrospectively identified through a surveillance system. Controls were selected and blood samples were collected in cases and controls from January 16th to February 5th 2016. No participant had recent or prior lab-confirmed ZIKV infection, but GBS cases grossly over-reported ZIKV infection symptoms when interviewed, as compared to symptoms recorded in their clinical charts (Appendix Table 3; item 7). In that study the smallest $OR_{Z \rightarrow GBS}$ that would have resulted in the $OP_{Z(+)GBS}$ was 164, and the ratio of observed to expected prevalence ($OP_{Z(+)GBS}/EP_{z(+)GBS}$), for $_{MAX}OR_{Z \rightarrow GBS}$ = 8.88, was about 10 (Table 1). Suspected ZIKV infection was strongly associated with GBS (OR: 13.83; 95% CI: 4.93–38.78).

Salinas et al. [9] compared 40 GBS cases (72% Brighton level 3) to 79 age-matched neighborhood controls from Barranquilla, Colombia. GBS cases occurring from October 2105 to March 2016, during an outbreak of ZIKV infection, were recruited in April 2016, almost 80% of them within two months after the onset of surveillance for *suspected Zika virus-associated GBS*. Blood samples were collected nearly three months after GBS onset. Procedures to select controls and exposure definitions followed those in Styczynksi et al. [8] In that study the smallest $OR_{Z \rightarrow GBS}$ that would have resulted in the $OP_{Z(+)GBS}$ was 182, and the $OP_{Z(+)GBS}/EP_{Z(+)GBS}$ ratio was about 16 (Table 1). Lab-confirmed recent ZIKV infection was not significantly associated with GBS (OR: 1.7; 95% CI: 0.7–3.8), but suspected infection tripled the risk of GBS (OR: 3.0; 95% CI: 1.1–8.6).

Table 1
Observed and expected prevalence of Zika virus infection (ZIKV-I) in cases of Guillain-Barre syndrome (GBS) included in case reports and case-control studies of the association between ZIKV-I and risk of GBS.

Study type/Country	Author (year)	Risk of ZIKV-I in popula-tion (/1000)	Observed preva-lence of ZIKV-I in cases (%)	Expected preva-lence of ZIKV-I in cases (%)	Minimum risk ratio[a]	Observed/Ex-pected risk ratio[b]
Case reports						
French Polynesia [31]	Watrin (2016)	490.0	83.3	89.5	6	0.91
Brazil [33]	Nóbrega (2018)	6.31	40.9	5.3	110	7.67
Colombia [32]	Parra (2016)	1.83	40.0	1.6	364	24.97
Puerto Rico [26]	Dirlikov (2018)	7.19	66.3	6.0	272	10.97
Jamaica [30]	Williams (2016)	4.6	9.52	3.9	23	2.42
Case-control studies						
Puerto Rico [11]	Dirlikov (2017)	7.19	69.0	6.0	307	11.42
Brazil [8]	Styczynski (2017)	6.31	51.0	5.3	164	9.55
Colombia [9]	Salinas (2017)	1.83	25.0	1.6	182	15.61
New Caledonia [10]	Simon (2018)	41.0	33.0	27.5	12	1.20
Bangladesh [12]	GeurtsvanKessel (2018)	???	4.3	???	1	????

[a] Smallest increase in the risk of GBS among ZIKV-infected individuals that would be necessary to result in the observed prevalence of ZIKV-infection in GBS cases.
[b] Number of times that the observed prevalence of ZIKV-infection in GBS cases is higher than the expected prevalence, based on the observed risk of ZIKV infection in the corresponding country.

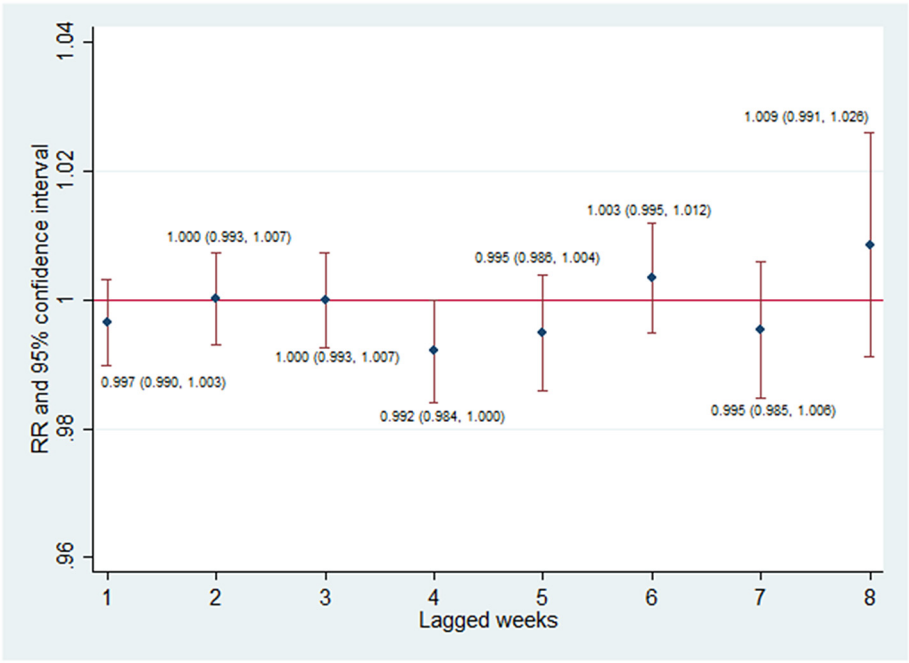

Fig. 2. Risk ratio (RR) of Guillain-Barre syndrome for a change of 100 cases in the lagged number of cases of Zika virus infections per week in seven Latin American countries.

Simon et al. [10] conducted a study in 15 cases of GBS (Brighton level 1 and 2) prospectively recruited at a New Caledonian hospital, from January through December 2014, during an outbreak of ZIKV infection. Two geographic residence, age, and sex-matched controls were retrospectively selected for each case, from non-febrile patients from another hospital. The timing of control selection and evaluation, in relation to the corresponding time for the matching case, was not reported. The smallest $OR_{Z \rightarrow GBS}$ that would have resulted in the $OP_{Z(+)GBS}$ was 12, and the $OP_{Z(+)GBS}/EP_{Z(+)GBS}$ ratio was 1.20 (Table 1). Recent flavivirus infection was defined as a positive RT-PCR or a positive anti-ZIKV or anti-dengue IgM, and prior ZIKV infection as a positive anti-ZIKV IgM or IgG. No cases of recent ZIKV infection were confirmed, but five cases and one control were ZIKV IgM or IgG positive, and also dengue IgG positive (unmatched OR: 14.5; 95% CI: 1.51–139.53).

Dirlikov et al. [11] recruited 39 GBS cases reported through surveillance in Puerto Rico, from April through December 2016, within one month of symptoms onset. GBS diagnosis was confirmed through clinical chart review, but Brighton level of certainty was not reported. Cases were age-matched to 78 community controls recruited within one week of case recruitment, from a randomly selected residence within one kilometer radius from the case's residence. Serum, urine, and saliva samples were collected at the time of recruitment. In this study the smallest $OR_{Z \rightarrow GBS}$ that would have resulted in the $OP_{Z(+)GBS}$ was 307, and the $OP_{Z(+)GBS}/EP_{Z(+)GBS}$ ratio was about 11 (Table 1). *Recent ZIKV infection* was defined as a positive RT-PCR in any body fluid and *ZIKV infection* as a positive RT-PCR or IgM test. *Recent ZIKV infection* and *ZIKV infection* increased the risk of GBS 16.0 times (95% CI: 2.1 to 120.6) and 36.0 times (95% CI: 4.9–262.5), respectively.

GeurtsvanKessel et al. [12] conducted a prospective case-control

Table 2
Smallest cell size, odds ratio and likelihood of different biases in analytic studies of the association between Zika virus infection (ZIKV) and Guillain-Barré syndrome (GBS).

Study	Smallest cell[a]	Odds ratio (95% CI)	Detection bias	Selection bias	Time-dep bias[b]	Misclass. ZIKV[c]	Misclass. GBS[d]	Confoun-ding bias	Sparse data bias
Styczynski [8]	6 ExCo	13.8 (4.9–38.8)	Likely	Unlikely	Likely	Likely	Unlikely	Likely	Likely
Salinas [9]	19 ExCa	1.7 (0.7–3.8)	Likely	Unlikely	Likely	Unlikely	Unlikely	Likely	Unlikely[e]
Simon [10]	1 NExco	14.5 (1.51–139.5)	?	Likely	?	Likely	Unlikely	Likely	Certain
Dirlikov [11]	3 ExCo	16.0 (2.1–120.6)	Likely	Unlikely	Unlikely	Unlikely	Unlikely	Likely	Certain
Cao-Lormeau [13]	1 NExCa	59.7 (10.4– + ∞)	Likely	Likely	Likely	Likely[f]	Likely	Likely	Certain
GeurtsvanKessel [12]	13 ExCo	2.23 (0.77–6.53)	Unlikely	Unlikely	Unlikely	Unlikely	Unlikely	Unlikely	Unlikely

[a] Smallest number of participants in a cell of a 2 × 2 table of exposure and outcome; ExCo = Exposed controls; ExCa = Exposed cases; NExCo = Non-exposed controls; NExCa = Non-exposed cases. All studies had at least 24 strata, with the exception of Cao-Lormeau et al.'s study, that had at least 10 strata, and GeurtsvanKessel et al.'s study, that was single-matched.

[b] Time-dep: Time-dependent bias.

[c] Misclass. ZIKV: Misclassification of ZIKV infection status.

[d] Misclass. GBS: Misclassification of GBS case status.

[e] This corresponds to anti-ZIKV IgM. However, for the analysis of suspected and probable ZIKV infection there were only 10 and 6 exposed cases and 8 and 3 exposed controls, respectively.

[f] ZIKV and dengue virus IgG cross-reactivity was only evaluated in GBS cases, using a blood sample collected about three months after GBS onset. Only one case had neutralizing antibodies ≥4 times higher for dengue than for ZIKV. Neutralizing antibodies were not measured in the blood sample collected in cases and controls at the time of diagnosis of the GBS case.

study in Bangladesh, a country with endemic transmission of Asian ZIKV. Cases ($n = 418$) and household healthy matched controls (n = 418) were recruited from 2011 through 2015, as part of an international study on clinical and biological predictors of GBS course [35]. Cases were followed for one year to exclude the possibility of other diagnoses, and 91% were Brighton level 1 and 2. Cases were recruited within two weeks of onset of weakness and diagnosis was confirmed following standard criteria [36]. Blood samples were collected in both cases and controls upon enrollment of the case. An in-house ZIKV neutralization test titer ≥32 was considered as evidence of infection. There was no statistically significant association between ZIKV infection and GBS (OR: 2.23; 95% CI: 0.77–6.53). The study was not conducted in the context of a ZIKV-infection outbreak, and was the only one without major design flaws.

Cao-Lormeau et al. [13] recruited 42 cases of GBS, identified during a ZIKV infection outbreak in French Polynesia, between November 2013 and February 2014 [13]. Average characteristics of the cases, but not their distribution by Brighton's diagnostic certainty, were reported in two papers [13,31]. Controls ($n = 98$) were age, sex, and residence-matched patients with a non-febrile illness [13]. In cases, blood samples were collected at admission, and three, eight, and 12 weeks later. In controls, only one blood sample was collected, within 16 days from admission of the matching case [13]. RT-PCR was used for diagnosis of ZIKV acute infection in cases, but not in controls. IgM, IgG, and neutralizing antibodies were measured in cases and controls. None of the cases were RT-PCR positive. The smallest $OR_{Z \rightarrow GBS}$ that would have resulted in the $OP_{Z(+)GBS}$ was 6, and the $OP_{Z(+)GBS}/EP_{Z(+)GBS}$ ratio was about 1 (Table 1). All but one case had levels of neutralizing antibodies for dengue virus higher than those for ZIKV.

When combining ZIKV IgM and IgG test results, ZIKV infection increased the risk of GBS 59.7 times (95% CI: 10.4– + ∞) [13]. However, this comparison was based on tests conducted in blood samples collected about three months after admission in GBS cases, and within 16 days from the day of recruitment of the matched case in controls. I re-estimated the ZIKV-GBS association avoiding selection and time-dependent bias and found an OR of (24/18) ÷ (98/98) = 1.33 (95% CI: 0.65–2.78; $p = 0.40$) [14].

The average OR in studies where a causal ZIKV-GBS association was plausibly identifiable, i.e. those without time-dependent bias [11–13], was 2.57 (95% CI: 0.87–7.62; Appendix, item 5). When Dirlikov et al.'s [11] study was excluded, because it was clearly affected by sparse data bias, the average OR decreased to 1.57 (95% CI: 0.86–2.86; Table 3).

4. Discussion

4.1. Main findings

Published case reports, ecological, and analytical studies provided little support for a ZIKV-GBS association. About 1/3 GBS cases included in case reports were of unknown diagnostic certainty and 4/5 had unconfirmed ZIKV infection. Even if ZIKV infection increased the risk of GBS about nine times, the prevalence of suspected ZIKV infection in GBS cases was two to 25 times higher than expected, suggesting strong detection bias. In spite of the time sequence of outbreaks, changes in the number of cases of ZIKV infection were not consequentially followed by changes in the number of GBS cases weeks later. Detection, selection, misclassification, confounding, time-dependent, or sparse data bias were likely in all [8–11,13] but one [12] analytic study. The latter showed a null ZIKV-GBS association. Finally, the association between ZIKV infection and GBS was not significant when ORs from studies with time-dependent bias were excluded.

Table 3
Average odds ratios (OR) and 95% confidence intervals (95% CI) for the effect of Zika virus infection on Guillain-Barré syndrome, based on studies without time-dependent bias.

Study	OR (95% CI)	% Weight	Pooled OR if study is excluded (95% CI)
Cao-Lormeau [13]	1.33 (0.65–2.78)	44.74	4.91 (0.74–32.61)
Dirlikof [11]	16.00 (2.10–120.60)	18.99	1.57 (0.86–2.86)
GeurtsvanKessel [12]	2.23 (0.77–6.53)	36.28	3.83 (0.34–42.60)
Combined OR	2.57 (0.87–7.62)	100.00	

Heterogeneity test $p = 0.020$.
Variation in OR attributable to heterogeneity = 69.4%.
Test of OR = 1, $p = 0.15$.

4.2. Case reports

The higher than expected prevalence of suspected ZIKV infection in GBS cases suggests patients with peripheral neuropathy may have being more likely diagnosed as GBS cases if they had symptoms of *suspected ZIKV infection* [19], than if they did not. Large numbers of false positive GBS cases could have resulted from the labeling of GBS as a complication of ZIKV infection, starting in December 2013 [37], and from the call of public health agencies to search for so called *ZIKV-associated GBS cases,* two months later [19,38,39]. At that time, no evidence of a ZIKV-GBS association was available.

Claims of a ZIKV-GBS link were made in several GBS case reports [4,5,31]. Unfortunately, exposure-disease associations cannot be measured in case reports, because disease status does not vary, and identifying causes is impossible without evidence of association [17]. However, a prevalence of ZIKV infection in GBS cases higher than in the general population could suggest an association, under two conditions. First, the cases in the series should represent all cases in the population. Unfortunately, this condition is unlikely met, because cases with both conditions are the ones of interest for inclusion in case reports. Second, a recent or current history of ZIKV infection should not influence the diagnosis of GBS (i.e., there should be no detection bias). However, deeming GBS as a complication of ZIKV infection and selectively searching for *ZIKV-associated GBS cases* through surveillance systems, made this assumption unlikely true.

Although GBS is a rare disease, finding numerous ZIKV-infected GBS cases during the course of a ZIKV-infection outbreak should be expected, as this depends on the cumulative risk of infection, as well as on efforts to preferentially find and report ZIKV-infected GBS-cases. For instance, even if ZIKV and GBS were not associated, half of all GBS cases in French Polynesia, as well as half the cases of other diseases, should have been infected with ZIKV, because 50% of the whole population got infected [29]. Briefly, the high prevalence of ZIKV infection in GBS cases included in GBS case reports was likely due to selection and detection bias, and does not support a hypothetical ZIKV-GBS association.

4.3. Ecological studies

The lack of consequential changes in the number of GBS cases after changes in the number of ZIKV infection cases during an outbreak suggests the temporal sequence of the outbreaks [6,7,13] was likely due to shared time-varying factors, such as concurrent enhancement of surveillance of both diseases [22]. Indeed, in most instances the peak in GBS occurred before or at the same time as the peak in ZIKV infection cases [7,32,40], instead of 1–4 weeks later, expected if ZIKV caused GBS by an immune-mediated mechanism, like other infections [41]. Moreover, small increases in GBS incidence limited to Suriname (27%), El Salvador (33%), and Venezuela (67%), as compared to a global incidence of 0.4–4.0/100,000 [42], could be a consequence of enhanced surveillance and detection bias.

Unfortunately, ZIKV-GBS associations could have been weakened by errors in the diagnosis and in the ascertainment of the time of onset of both conditions. Moreover, findings from the time series analysis may not accurately reflect individual-level ZIKV-GBS associations, due to the

possibility of ecological bias [43].

4.4. Analytic studies

Findings of a strong statistically significant association between ZIKV infection and risk of GBS in three [10,11,13] out of six case-control studies [8–13] were likely due to major biases.

4.4.1. Detection bias

All but two analytic studies [12,13] identified GBS cases through surveillance during ZIKV infection outbreaks. Like in case reports, detection bias likely resulted in an overestimate of the prevalence of ZIKV infection in the case group. Indeed, the high prevalence of ZIKV infection in GBS cases was not consistent with the risk of ZIKV infection in the population, previous experience with ZIKV in Africa and Asia [44], or reports of two to five-fold increases in GBS incidence in Brazil, Colombia, and Puerto Rico, during ZIKV infection outbreaks [26,32,45].

4.4.2. Selection bias

The exclusion of controls with fever in two studies [10,13] likely decreased the prevalence of ZIKV infection in this group, resulting in selection bias. Indeed, the prevalence of confirmed infection in patients with ZIKV infection symptoms is about 15 times higher than in patients without symptoms (Appendix Table 2; item 7). Also, selection bias was likely in studies conducted during a ZIKV outbreak that did not recruited controls around the time of onset of their matching cases [8–10,13], due to the changing risk of ZIKV infection.

4.4.3. Outcome misclassification

Although most GBS cases in case-control studies (87%) had Brighton levels of certainty 1 and 2, detection bias likely increased the GBS false positive rate. Also, the absence of classic feature of GBS [15] suggest at least 52% of the cases in the seminal case-control study had at best a level 4 diagnostic certainty.

4.4.4. Exposure misclassification

The symptoms-based definition of suspected ZIKV infection [19] has a maximum sensitivity of 38%, the proportion of ZIKV-infected symptomatic cases [1,46]. This low sensitivity could explain the lack of a ZIKV-GBS association in some studies, if it were similar in cases and controls. However, at least in one study [8], GBS cases over-reported symptoms of ZIKV infection. This could have led to higher sensitivity in cases, and to the 14-fold increase in GBS risk in individuals with suspected ZIKV infection in that study [8].

Unfortunately, lab tests to detect ZIKV infection are compromised by the timing of the tests in relation to the onset of infection, and by cross-reaction between antibodies for ZIKV and for other flaviviruses [47]. Errors due to test timing and cross-reactions should be non-differential and would weaken a ZIKV-GBS association. Two studies [11,12] minimized timing errors by testing cases early in the course of GBS and controls shortly after diagnosis of the matching case, and only one limited cross-reaction errors by using RT-PCR [11].

4.4.5. Confounding

No study adjusted for potential confounders such as diarrhea or acute respiratory infection. However, confounding by these factors may have been less important in GeurtsvanKessel et al.'s [12] than in other studies, because the risk of those infections may have been similar in GBS cases and controls from the same family and household.

4.4.6. Time-dependent bias

ZIKV-GBS associations could be interpreted as causal only in two studies that tested for ZIKV infection in cases at a time that plausibly preceded the onset of GBS [11,12] and in the re-estimate of the ZIKV-GBS association in Cao-Lormeau et al.'s [13]. Due to its inaccuracy [46], the symptoms-based definition of ZIKV infection provided only weak evidence of infection preceding GBS onset, particularly if symptoms of ZIKV infection were reported months after occurrence of both diseases.

4.4.7. Sparse data bias

The extremely large OR, with excessively wide confidence intervals, found in some studies [10,11,13] strongly suggests sparse data bias [28]. Conditional logistic regression, grossly overestimated the OR in those studies, because the number of individuals in at least one cell of the 2×2 table defined by ZIKV infection and GBS status was zero or close to zero [28].

The average OR from studies without time-dependent bias resulted in a non-significant ZIKV-GBS association, but was not exempt from other biases in the source studies. Nevertheless, no association was found in GeurtsvanKessel et al.'s study [12] which included 70% of all cases in analytical studies ($n = 418$), and avoided major biases. Moreover, a null association is consistent with the lack of reports of a ZIKV-GBS link in Asia, a region where ZIKV has been circulating for decades [44].

4.5. Conclusions

Case-control studies are useful to quickly identify causes of an outbreak, but may be particularly prone to biases in this setting, because the need to take action may prevail over the need to accurately identify causes, and may promote anchoring and case-building [48]. Moreover, for diseases with insidious onset, like ZIKV infection and GBS, the temporal sequence between exposure and outcome may be harder to ascertain. Defining and selecting cases and controls independently of exposure status, and vice versa, as well as selecting controls around the time of onset of the cases are key to prevent major flaws in these studies. In addition, blinded data analyses [49] and sensitivity analyses [50] should be used to avoid unconscious biases stemming from first impressions about the causes of the outbreak.

Considering the scarce evidence of a ZIKV-GBS association, public health agencies may want to reassess their recommendations for continuing surveillance and preparation for GBS outbreaks [3]. Policies based on conjectures of a ZIKV-GBS link may have limited benefits, could lead to misdiagnosis and mismanagement of patients with polyneuropathies, misallocation of scarce health resources, significant economic losses, and potentially troublesome lack of trust in public health agencies.

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Data sharing

The list of studies included in this review, datasets, statistical code, and technical details are available in the Appendix.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jns.2019.06.019.

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