Infection-related microcephaly after the 2015 and 2016 Zika virus outbreaks in Brazil: a surveillance-based analysis

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Summary

Background On Nov 11, 2015, the Brazilian Ministry of Health declared a Public Health Emergency of National Concern in response to an increased number of microcephaly cases, possibly related to previous Zika virus outbreaks. We describe the course of the dual epidemics of the Zika virus infection during pregnancy and microcephaly in Brazil up to Nov 12, 2016, the first anniversary of this declaration.

Methods We used secondary data for Zika virus and microcephaly cases obtained through the Brazilian Ministry of Health's surveillance systems from Jan 1, 2015, to Nov 12, 2016. We deemed possible Zika virus infections during pregnancy as all suspected cases of Zika virus disease and all initially suspected, but later discarded, cases of dengue and chikungunya fever. We defined confirmed infection-related microcephaly in liveborn infants as the presence of a head circumference of at least 2 SDs below the mean for their age and sex, accompanied by diagnostic imaging consistent with an infectious cause, or laboratory, clinical, or epidemiological results positive for Zika virus or STORCH (infectious agents known to cause congenital infection, mainly syphilis, toxoplasmosis, cytomegalovirus, and *herpes simplex* virus). We excluded cases of congenital anomalies or death without microcephaly. We analyse the spatial clustering of these diseases in Brazil to obtain the kernel density estimation.

Findings Two distinct waves of possible Zika virus infection extended across all Brazilian regions in 2015 and 2016. 1 673 272 notified cases were reported, of which 41 473 (2·5%) were in pregnant women. During this period, 1950 cases of infection-related microcephaly were confirmed. Most cases (1373 [70·4%]) occurred in the northeast region after the first wave of Zika virus infection, with peak monthly occurrence estimated at 49·9 cases per 10 000 livebirths. After a major, well documented second wave of Zika virus infection in all regions of Brazil from September, 2015, to September, 2016, occurrence of microcephaly was much lower than that following the first wave of Zika virus infection, reaching epidemic levels in all but the south of Brazil, with estimated monthly peaks varying from 3·2 cases to 15 cases per 10 000 livebirths.

Interpretation The distribution of infection-related microcephaly after Zika virus outbreaks has varied across time and Brazilian regions. Reasons for these apparent differences remain to be elucidated.

Funding None.

Introduction

Zika virus is an emerging infectious disease first isolated in 1947 in the Zika Forest of Uganda.[1] Before 2007, Zika virus was rarely reported in human beings and was not a major public health concern.[1] However, new clinical findings after outbreaks in the Federated States of Micronesia (2007), French Polynesia (2013), and Brazil (2015–16) have changed this view.[1–3] Although understanding of the natural history and spectrum of Zika virus infections is incomplete, the acute illness can range from mild to severe, and chronic complications affect physical and mental domains with lifelong implications.[4–6] The virus is transmitted both indirectly (via vector, blood transfusion, or organ transplant) or directly (via sexual or mother-to-child transmission).[1,3,7]

In the Americas, Zika virus could have been introduced as early as 2013.[8] Human infections in Haiti in 2014 have been well documented.[9] In Brazil, clusters of an unknown exanthematic disease were observed in several states of the northeast region in July, 2014, and state health authorities officially reported outbreaks in February, 2015.[10,11] On April 29, 2015, Zika virus was first identified from a similar outbreak in the Brazilian state of Bahia (the largest and most southern state).[12] On Oct 22, 2015, the Secretary of Health of Pernambuco (a state in northeast Brazil) reported to the Ministry of Health (MoH) an unexpected increase in the prevalence of microcephaly, possibly related to Zika virus infection during pregnancy.[11]

On Nov 11, 2015, the Brazilian MoH declared a Public Health Emergency of National Concern.[13] On the basis of evidence for a potential association between microcephaly and other neurological disorders and Zika virus infection provided by Brazil, France, the USA, and El Salvador, WHO declared a Public Health Emergency of International Concern on Feb 1, 2016.[14] As of Nov 10, 2016, 58 countries and territories had reported Zika virus outbreaks since 2015, of which 23 (41%) reported 2227 potentially associated cases of either microcephaly

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Research in context

Evidence before this study

Zika virus has now been convincingly identified as a cause of microcephaly and other congenital abnormalities. The striking increase in microcephaly seen in the northeast of Brazil after the initial Zika virus outbreaks raised national and international concern of the future risk ensuing from uncontrollable Zika virus outbreaks around the world. Most of what has been reported so far regarding Zika virus-related microcephaly in Brazil has focused on the restricted period of the initial outbreak in the northeast region.

Added value of this study

Brazilian national surveillance databases provide a description of the Zika virus epidemic in Brazil and in specific regions from its start in January, 2015, up to Nov 12, 2016. We did the first comprehensive report, to our knowledge, of the extent and the temporal and spatial contours of the Zika virus outbreaks and related microcephaly cases in Brazil. As of Nov 12, 2016, despite new major Zika virus outbreaks that disseminated throughout most of the country, only a minimal second rise in the risk of confirmed infection-related microcephaly was noted in the northeast, location of the initial reports of excess microcephaly. This rise was accompanied by quite small increases in the occurrence of microcephaly in three of the four remaining Brazilian regions.

Implications of all the available evidence

Reasons for these differences in microcephaly occurrence over time and place are not clear, but possibly include the intensity of the Zika virus outbreak, the success of public health responses, and the differential presence of yet to be described secondary factors. Our results, coupled with those now being reported in other countries, suggest that the striking increase in microcephaly initially reported in the northeast of Brazil does not correspond with the usual course of a Zika virus outbreak. Additional studies are needed, some are already underway in Brazil, to elucidate the reasons for this variability and, more importantly, to capture the full spectrum and burden of the congenital abnormalities due to Zika virus infection. Given the importance of the possible long-term complications of Zika virus infection during pregnancy, continued surveillance is mandatory. Moreover, the widespread and rapid emergence of chikungunya and now Zika virus signal the need to strengthen surveillance systems and their capacity to promptly respond to new outbreaks of emerging infectious agents of potential global consequence.

or CNS malformations, or both. Of these cases, 2106 (95%) were reported in Brazil.[15]

In view of the scale and extent of the Zika virus and microcephaly epidemics, we aim to describe their infectious courses in Brazil up to the first anniversary of the declaration of the Public Health Emergency of National Concern.

Methods

Study design

In this descriptive study, we used data collected from Jan 1, 2015, to Nov 12, 2016, by the Brazilian National Notifiable Diseases Information System (Sistema de Informação de Agravos de Notificação[16] [SINAN]; appendix) to obtain the number of suspected cases of dengue, chikungunya, and Zika virus (definition of a suspected case for each disease type are in the appendix). In Brazil, disease notification has been mandatory since 1961 for dengue fever and since 2011 for chikungunya fever. Sentinel surveillance of Zika virus disease began in June, 2015, in selected services and states, and was extended to universal surveillance in February, 2016.

To maximise inclusion of possible cases of Zika virus infection in view of the sparse availability of confirmatory testing and the initial inability to test and report the virus, we created an expanded case definition. Our expanded definition includes not only cases initially reported as suspected Zika virus infection, but also cases initially reported as suspected dengue or chikungunya fever that were later discarded because of the absence of positive RT-qPCR or ELISA test results, or, of the patients who were not tested, the absence or clinical findings meeting the epidemiological case criteria for these diseases.[17] Analogous to the previous use of the term dengue-like illness or syndrome in situations of uncertain cause,[18] we joined these cases with those of suspected Zika virus infection to compose the definition of possible Zika virus infection. For our analyses, we restricted possible Zika virus cases to those reported during pregnancy. We included data from women aged 10–49 years and who were pregnant. No primary data were collected, and informed consent was not required.

Microcephaly data

We identified cases of microcephaly from the Public Health Event Registry (Registro de Evento de Saúde Pública[16] [RESP]; appendix), which was created and implemented in November, 2015, during the emergency response for notification of cases of microcephaly or other congenital anomalies, and fetal loss, based on monitoring of pregnant women and their newborn babies.[16] Additional data for these cases from Jan 1, 2015, to Oct 31, 2016, was obtained through linkage to the Brazilian Live Birth Information System (Sistema de Informações sobre Nascidos Vivos[19] [Sinasc]). We redefined all suspected cases reported through RESP, in accordance with WHO's

Rapid Advice Guideline[20] of August, 2016, which considers microcephaly as a head circumference of at least 2 SDs below the mean for the baby's age and sex. These suspected cases of infection-related microcephaly were later confirmed, discarded, or remained under investigation. Confirmation, based on diagnostic approaches available at the time and location of their detection, was done by either neuroimaging techniques or laboratory tests (samples from the mother or newborn baby, or both). Additionally, in a few cases only clinical and epidemiological criteria were used. We classified confirmed microcephaly cases as all those stated in official notifications plus any non-officially confirmed cases with mention of brain calcifications in the reported diagnostic imaging summaries.[21] We excluded cases of congenital anomalies or death without microcephaly.

Statistical analysis

To estimate the monthly incidence of possible Zika virus infection per 10 000 pregnant women, we estimated the number of pregnant women for each month as the number of reported livebirths obtained from Sinasc, times nine, plus an additional 20% of the reported livebirths times 1·5. This calculation assumes livebirths result from pregnancies lasting for a mean of 9 months, and that for every 100 livebirths 20 pregnancies result in abortions which terminate at a mean of 1·5 months after initial prenatal health care.[22]

Given that an important fraction of suspected cases of infection-related microcephaly were under investigation at the closing of these analyses, we estimated the number of future confirmations and added them to confirmed cases for graphical presentation of the outbreaks. To estimate the future confirmations we multiplied the number of cases still under investigation by the fraction of closed cases that had been confirmed as infection-related microcephaly in each region. Given that the characteristics of the Zika outbreaks varied over time, we determined this fraction for three different periods (January, 2015–August, 2015; September, 2015–April, 2016; and May, 2015–September, 2016). We calculated the monthly risk of infection-related microcephaly as all confirmed cases, and separately as all confirmed plus estimated future cases per 10 000 newborn babies.[22] To improve understanding of the observed risk of microcephaly, we compared these risks with the mean historical risk of microcephaly in Brazil (two cases per 10 000 livebirths)[23] as estimated using data from the Latin American Collaborative Study of Congenital Malformations for 1965–2015.[24] This group defined microcephaly as a head circumference of at least 3 SDs below the mean for age and sex or at least 2 SDs below the mean for age and sex accompanied by neurological abnormalities.[20] We also present the monthly incidence of Zika virus infection in pregnancy and risk of confirmed infection-related microcephaly.

We statistically analysed the spatial point distributions of possible Zika virus infections in pregnant women and of confirmed infection-related microcephaly cases to obtain the kernel density estimation, an interpolating and smoothing technique to generalise point locations to detect areas with high occurrences of infections.[25]

We analysed data using Data Tabulator for Windows (Tabulador de Dados do Windows; version 4.1), Epi Info (version 7.2), and QGIS open source geographic information system (version 2.16). We completed all analyses using anonymised data from the Brazilian national surveillance systems.

Role of the funding source

There was no funding source for this study, and the corresponding author had full access to all of the data and the final responsibility to submit for publication.

Results

From Jan 1, 2015, to Nov 12, 2016, 8 429 735 individual-level mandatory notifiable diseases were reported in Brazil, of which 5 146 796 (61·1%) were suspected urban arbovirus cases of dengue fever (4 497 133 [87·4%]), chikungunya fever (339 880 [6·6%]), or Zika virus disease (309 783 [6·0%]; figure 1). Although suspected cases of Zika virus infection or chikungunya fever increased during 2016, dengue fever still accounted for most of the total suspected urban arbovirus cases.

After removal of epidemiologically confirmed cases of dengue and chikungunya fever, the remaining 1 673 272 (32·5%) notifications of 5 146 796 suspected urban arbovirus cases were considered possible cases of Zika virus infection during this period, most of which (1 318 593 [78·8%]) were cases initially suspected of dengue fever that had been discarded after investigation. For only pregnant women, the fraction of these possible cases initially reported as Zika virus infection during pregnancy (25 771 [62·1%] of 41 473) was much higher than that of all possible cases of Zika virus among notifications (309 783 [18·5%] of 1 673 272). From Jan 1, 2015, to Nov 12, 2016, the number of suspected cases of Zika virus infection increased notably in pregnant women from 2576 cases in 2015 to 23 195 cases in 2016.

Most cases of possible Zika virus infection during pregnancy were from the more populous northeast and southeast regions of Brazil, but important dissemination was seen in the north and centre-west. The monthly frequency of cases, according to the case definitions in the appendix, are provided in the appendix (pp 1–2).

Up to Nov 12, 2016, 10 555 cases of microcephaly or other neurological disorders were reported, of which 10 056 (95·3%) were newborn babies and infants. From the 10 056 cases, 5968 (59·3%) cases of microcephaly were recorded, of which 1950 (32·7%) were confirmed to be infection-related through official notifications (1708 [87·6%]) and based on mention of brain calcifications in the reported diagnostic imaging summaries (242 [12·4%]).[21] 2207 (37·0%) of the 5968 cases had an infection-related cause ruled out and 1811 (30·3%)
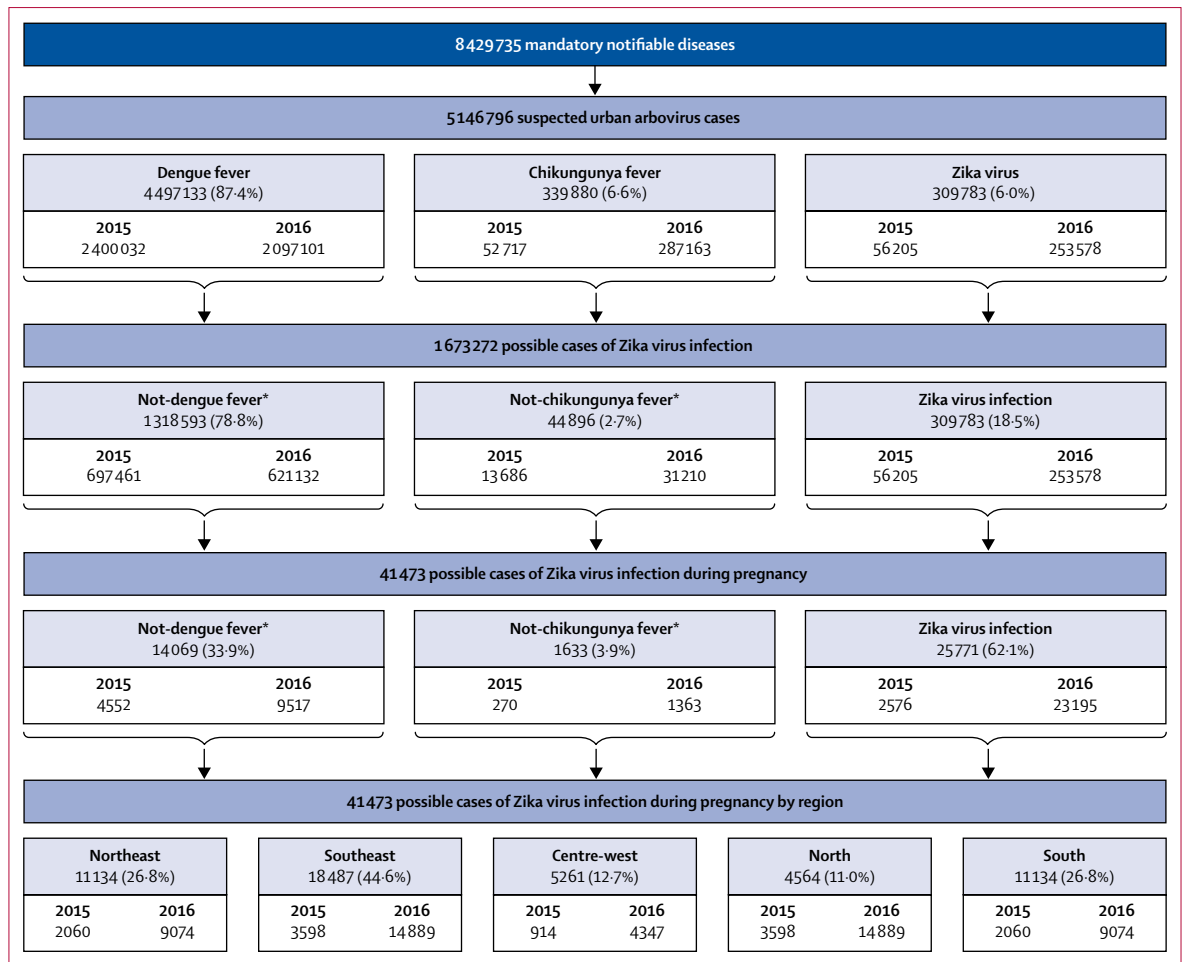
Figure 1: Possible cases of Zika virus infection during pregnancy based on mandatory notification of suspected cases of dengue, chikungunya, and Zika virus, Jan 1, 2015–Nov 12, 2016

Data are from the Brazilian National Disease Notification System.[16] *Cases originally suspected as dengue and chikungunya fever with negative laboratory findings or did not meet the protocol definition.

were still under investigation. Maternal age ranged from 13 years to 45 years (median 25 years), most (1200 [83·3%]) women were non-white. Rash was the most frequent symptom reported in pregnancy (891 [45·7%]), with 598 (67·1%) occurrences during the first trimester. Only 542 (27·8%) pregnant women reported fever. The table presents the diagnostic method for the 1950 confirmed cases of microcephaly. RT-PCR or ELISA techniques identified Zika virus in 304 (15·6%) women or their child's samples. Presence of a STORCH agent (infectious agents known to cause congenital infection, mainly syphilis, toxoplasmosis, cytomegalovirus and herpes simplex virus) was the basis for diagnostic confirmation in an additional 105 (5·4%) cases, most of whom also presented with imaging abnormalities. Notification did not always provide detail as to which STORCH agent was investigated or found. Neuroimaging was the diagnostic method of confirmation for infection-related microcephaly in 1674 (85·9%) cases, and the

only method used in 1378 (70·7%) cases. Most cases of microcephaly were diagnosed post partum, and CT was the most frequently used imaging technique (used in 704 [36·1%] of 1950 cases). 848 (43·5%) cases were registered as having brain calcifications.

Most infection-related microcephaly cases (1487 [76·3%]) were concentrated in the northeast region of Brazil. 1649 (84·6%) cases occurred during the first microcephaly epidemic wave (between September, 2015, and April, 2016), with 1373 (83·3%) of these recorded in the northeast.

The temporal distribution of possible Zika virus infection in pregnant women and of confirmed infection-related microcephaly are shown by region, in figure 2. Figure 2A shows that the increase in confirmed infection-related microcephaly in the northeast, which began in August, 2015, was preceded by a rise in the incidence of possible cases of Zika virus about 6 months earlier (March, 2015). In the northeast region, the maximal frequency of notified microcephaly reached 49·9 cases

	Region of Brazil					Brazil
	Northeast	Centre-west	Southeast	North	South	
Confirmed cases of infection-related microcephaly						
By laboratory determination (Zika virus*)	242 (16·3%)	21 (21·2%)	28 (10·4%)	11 (15·5%)	2 (8·7%)	304 (15·6%)
Laboratory only						
Zika virus	35 (2·4%)	6 (6·1%)	13 (4·8%)	6 (8·5%)	..	60 (3·1%)
STORCH† plus Zika	8 (0·5%)	1 (1·0%)	1 (0·4%)	1 (1·4%)	..	11 (0·6%)
Imaging and laboratory						
Zika virus	194 (13·0)	12 (12·1%)	11 (4·1%)	4 (5·6%)	1 (4·3%)	222 (11·4%)
STORCH plus Zika	5 (0·3%)	2 (2·0%)	3 (1·1%)	..	1 (4·3%)	11 (0·6%)
By laboratory determination (non-Zika virus)	33 (2·2%)	13 (13·1%)	37 (13·7%)	8 (11·3%)	14 (60·9%)	105 (5·4%)
STORCH						
Laboratory only	8 (0·5%)	4 (4·0%)	22 (8·1%)	1 (1·4%)	7 (30·4%)	42 (2·2%)
Imaging and laboratory	25 (1·7%)	9 (9·1%)	15 (5·6%)	7 (9·9%)	7 (30·4%)	63 (3·2%)
By means other than laboratory determination						
Imaging	1074 (72·2%)	63 (63·6%)	186 (68·9%)	48 (67·6%)	7 (30·4%)	1378 (70·7%)
Clinical or epidemiological only	138 (9·3%)	2 (2·0%)	19 (7·0%)	4 (5·6%)	..	163 (8·4%)
Confirmed cases of infection-related microcephaly after the first epidemic wave of Zika virus infection (September, 2015, to April, 2016)	1373 (92·3%)	53 (53·5%)	175 (64·8%)	38 (53·5%)	10 (43·5%)	1649 (84·6%)
Total cases of confirmed infection-related microcephaly	1487 (100·0%)	99 (100·0%)	270 (100·0%)	71 (100·0%)	23 (100·0%)	1950 (100·0%)

Data are n (%). Data are from the Brazilian Public Health Event Registry (Registro de Evento de Saúde)[16] on Nov 12, 2016. STORCH=infectious agents known to cause congenital infection: mainly syphilis, toxoplasmosis, cytomegalovirus, and *herpes simplex*; testing for these multiple agents was not always performed; rubella and measles are not endemic in Brazil. *Zika virus laboratory detection was not available to support notification in many cases.

Table: 1950 confirmed cases of infection-related microcephaly by diagnostic method and occurrence after the first epidemic wave of Zika virus infection in regions of Brazil; January, 2015–November, 2016
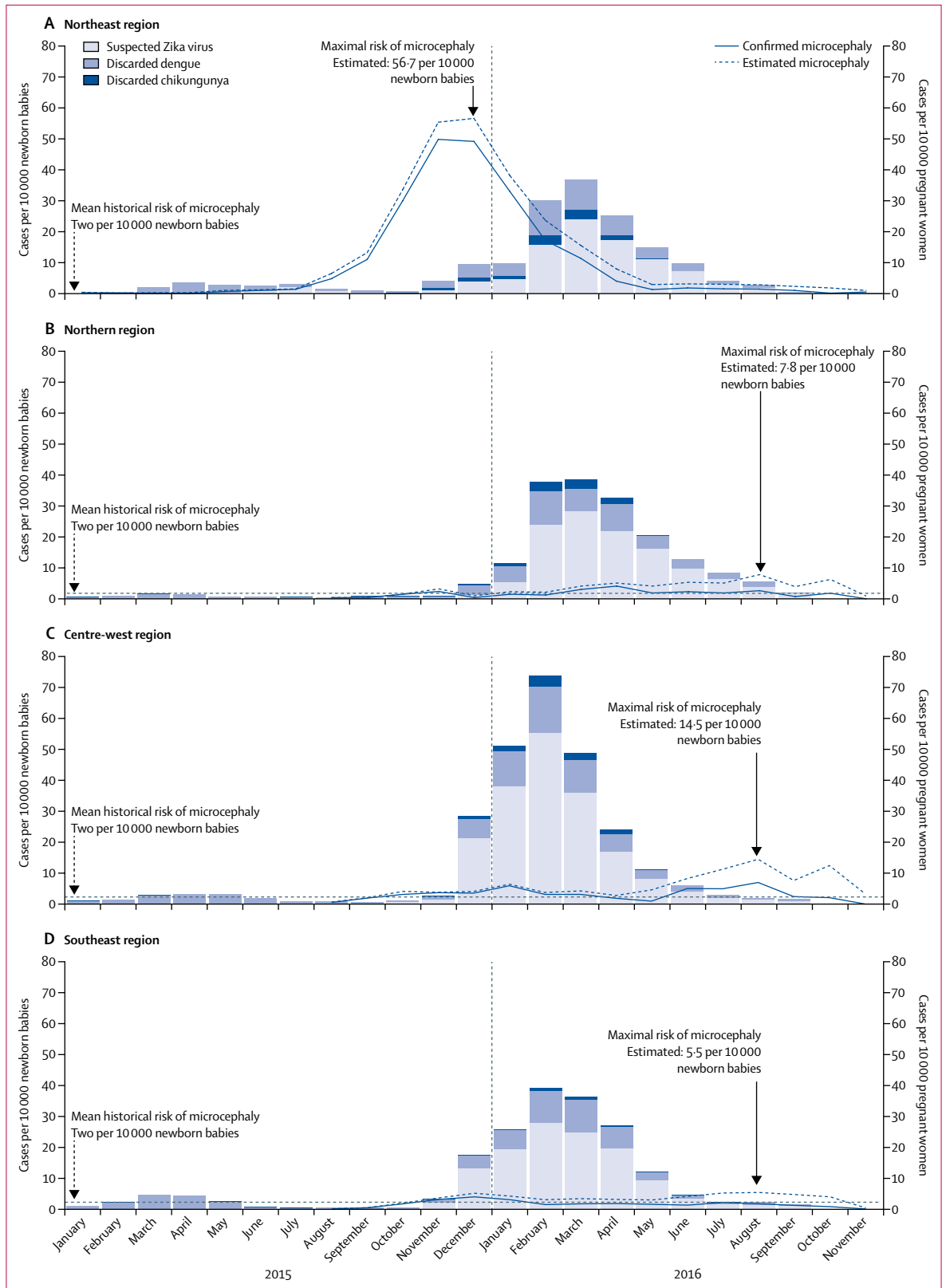
per 10 000 newborn babies in November, 2015 (figure 2A), a peak that is 24 times higher than the historical mean occurrence of microcephaly in Brazil (two cases per 10 000 newborn babies) during the studied period. This wave of cases continued until April, 2016. A new and much larger wave of Zika virus infection in pregnancy was recorded from November, 2015, to August, 2016. However, up to November, 2016, and even considering estimated cases yet to be confirmed, only a small increase in microcephaly was noted in the northeast region, with risks since May, 2016 (0·22 cases per 10 000 newborn babies), close to the historical mean for Brazil.

In the other regions of Brazil (figure 2B–E), we also observed two waves of Zika virus infection occurring from March, 2015, to July, 2015, and from September, 2015, to August, 2016. The centre-west region presented the highest incidence of notified possible cases of Zika virus infection (74 cases per 10 000 pregnant women) in February, 2016, during the second wave of Zika virus infection. For confirmed infection-related microcephaly, much smaller increases were notified outside of the northeast region after the first wave of the Zika virus outbreaks (figure 2). After the second wave of infections, and in view of estimated cases yet to be confirmed, we observed quite small monthly peaks, ranging from
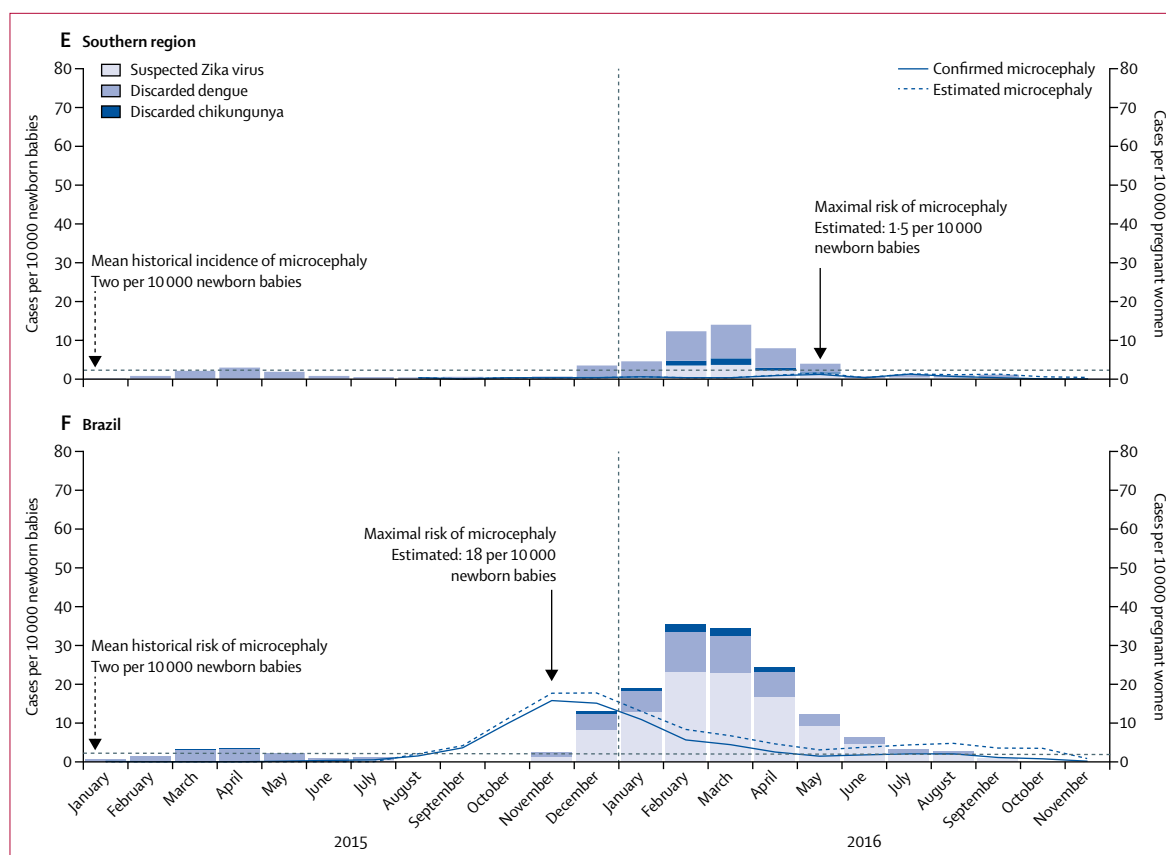
5·5 cases (southeast) to 14·5 cases (centre-west) per 10 000 newborn babies. No increase was observed in the south of Brazil at any time during 2015–16. Additional details on the timing and notified size of the epidemics for the whole country are provided in the appendix (p 3).

Kernel density estimation analysis based on the distribution of possible Zika virus cases in pregnant women at the municipal level shows a large cluster of cases in the northeast region (figure 3), mainly in Bahia state, which was the first state to use universal mandatory notification in 2015. In 2016, the epidemic extended to other areas of the northeast and southeast of Brazil (figure 3B). In both years, Zika virus presented most extensive clustering in the northeast, with accompanying frequent hotspots in the southeast extending to the centre-west and to the border state of the south of Brazil.

With respect to confirmed infection-related microcephaly (figure 4), cases following the first Zika virus wave were noted predominantly in the northeast, although some hotspots were present in the centre-west and southeast regions. No major clusters were observed after the second Zika virus wave (ie, during the period starting in May, 2016), although some hotspots were recorded in the northeast and southeast regions.

A Northeast region

Suspected Zika virus
Discarded dengue
Discarded chikungunya

Maximal risk of microcephaly
Estimated: 56·7 per 10 000 newborn babies

Confirmed microcephaly
Estimated microcephaly

Mean historical risk of microcephaly
Two per 10 000 newborn babies

B Northern region

Maximal risk of microcephaly
Estimated: 7·8 per 10 000 newborn babies

Mean historical risk of microcephaly
Two per 10 000 newborn babies

C Centre-west region

Maximal risk of microcephaly
Estimated: 14·5 per 10 000 newborn babies

Mean historical risk of microcephaly
Two per 10 000 newborn babies

D Southeast region

Maximal risk of microcephaly
Estimated: 5·5 per 10 000 newborn babies

Mean historical risk of microcephaly
Two per 10 000 newborn babies

(Figure 2 continues on next page)

Figure 2: Monthly incidence of possible Zika virus infection and monthly frequency of confirmed infection-related microcephaly in Brazil, 2015–16, compared with the historical mean risk of microcephaly in Brazil

States included per region: Distrito Federal, Goiás, Mato Grosso, and Mato Grosso do Sul (centre-west); Alagoas, Bahia, Ceará, Maranhão, Paraíba, Pernambuco, Piauí, Rio Grande do Norte, and Sergipe (northeast); Acre, Amapá, Amazonas, Pará, Rondônia, Roraima, and Tocantins (north); Espírito Santo, Minas Gerais, Rio de Janeiro, and São Paulo (southeast); and Paraná, Rio Grande do Sul, and Santa Catarina (south). The vertical dotted line shows the start of 2016.

Discussion

Coinciding with the first anniversary of the National Emergency Declaration of Nov 11, 2015, we present, to our knowledge, the first comprehensive description of the dual Zika virus infection and microcephaly epidemics observed in Brazil from Jan 1, 2015, up to Nov 12, 2016. In the northeast, a marked increase in the occurrence of infection-related microcephaly was observed following the first wave of possible Zika virus infection while in the north, centre-west, and southeast only minor increases in the occurrence of microcephaly were observed (figures 2B–D). Although a second wave of Zika virus infection in pregnancy has been well documented in all regions of Brazil, no increase in confirmed infection-related microcephaly has yet been observed, except for a small rise in the centre-west. However, in view of estimated additional confirmations of suspected cases still under investigation, we expect monthly peaks of infection-related microcephaly ranging from 5·5 cases to 14·5 cases per 10 000 newborn babies in the southeast, north, and centre-west regions, but no peak in the northeast and south regions.

The two Zika virus waves were noted during the season of high rainfall and humidity, which offers conditions favourable for mosquito breeding and consequently a greater mass of vectors for Zika virus, dengue, and other arboviruses.[26] It is still too early to predict how the outbreaks of Zika virus will unfold in the future in Brazil. Large areas of Brazil have yet to experience an outbreak of Zika virus. Data from countries that previously had major outbreaks of the virus suggest that it rapidly spreads in communities once introduced, but disappears after the major outbreaks.[27] The epidemic rise in infection-related microcephaly observed 5–8 months after the Zika virus epidemic corroborates the causal link between Zika virus and microcephaly. However, the marked variation in the frequency of infection-related microcephaly across regions and time is puzzling. The northeast was the only region reaching a monthly peak (48 cases per 10 000 newborn babies) higher than what WHO considers to be the endemic range of microcephaly (0·5–20 cases per 10 000 livebirths).[20]

The remaining regions reached monthly peaks within WHO's range, but in 2016, the north, centre-west, and
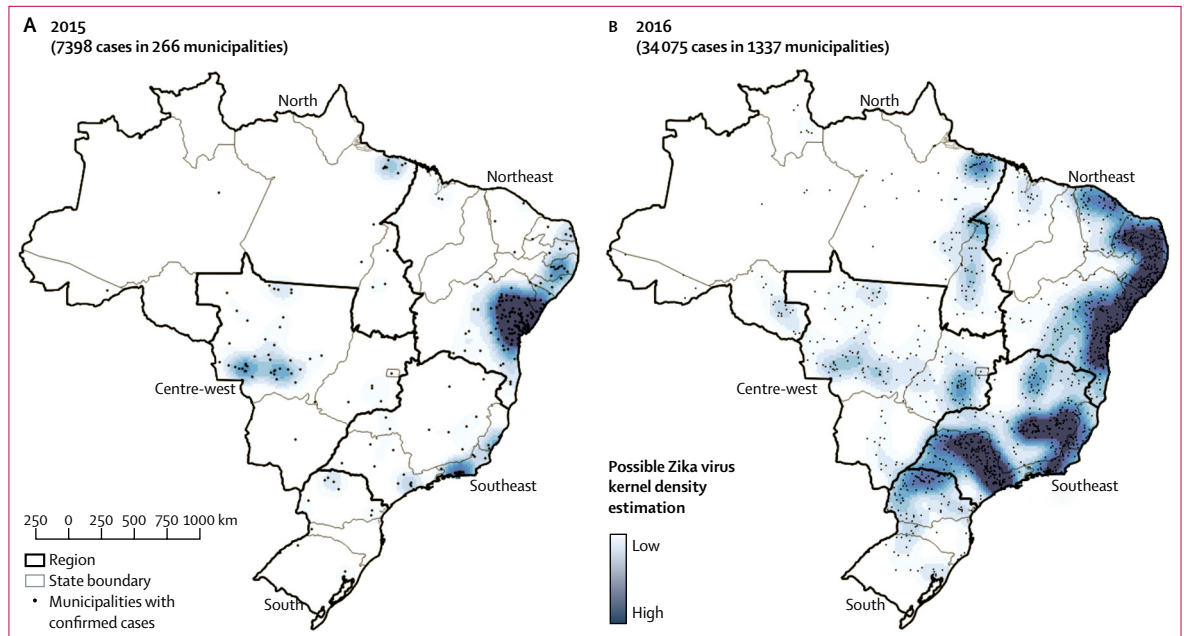
Figure 3: Kernel density estimates of the distribution of possible Zika virus infections in pregnant women in Brazil in 2015 (A) and 2016* (B)
*Cases are up to Nov, 12, 2016.
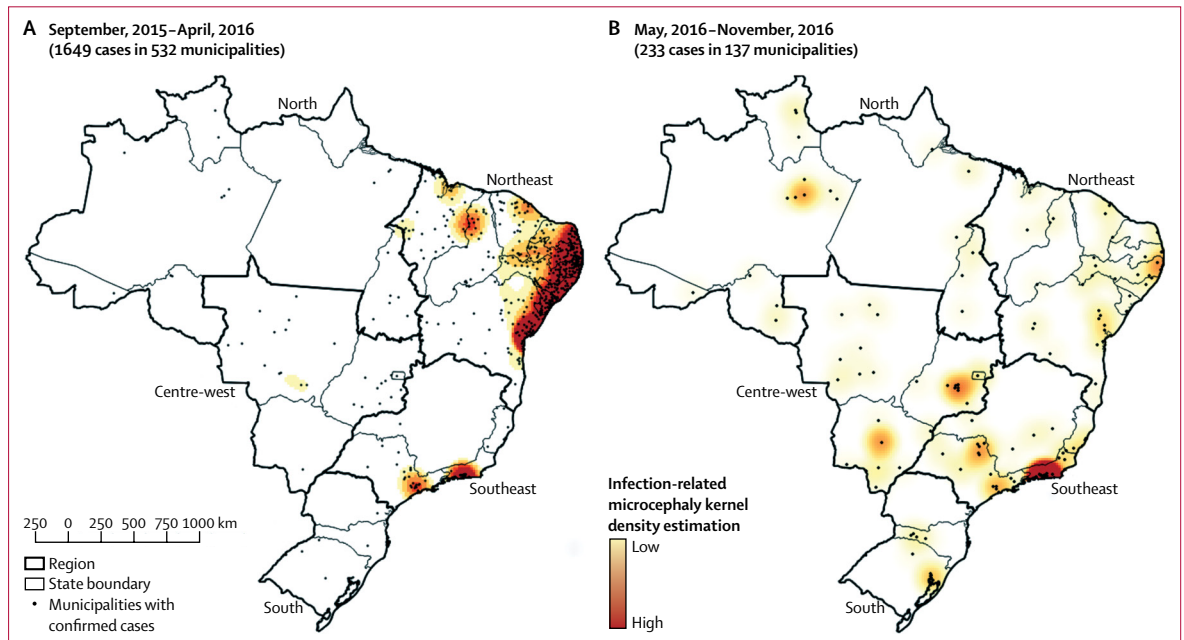
Figure 4: Kernel density estimates of the distribution of confirmed infection-related microcephaly in Brazil
Cases in Brazil after the first wave of Zika virus outbreaks (September, 2015–April, 2016; A), and after the second wave up to data closure
(May, 2016–Nov 11, 2016; B).

southeast presented monthly peaks which surpassed the Brazilian mean historical risk of two cases per 10 000 livebirths.[23] Perhaps of even greater surprise, was the minimal second epidemic rise of microcephaly after the second wave of Zika virus infection in the northeast region. This new scenario in the northeast and that of

much lower peaks in the three other regions (north, centre-west, and southeast) is consistent with the ongoing experience of microcephaly now reported in many other countries.[27,28]

Thus, although our findings, together with the scientific literature, support the hypothesis that Zika

virus infection during pregnancy causes microcephaly, we do not know the reasons for the wide range of monthly peaks of microcephaly over time and regions after the Zika virus outbreaks.[29] Possible explanations include the intensity of the Zika virus outbreak, the presence of co-factors, intentionally increased pregnancy termination, and public health actions implemented to decrease exposure to the mosquito by pregnant women. Although it is too early to expect a decrease in the number of livebirths as a result of concern about risk of microcephaly, we observed no such decrease up to September, 2016, the last month for which reasonably complete data are available.

In this respect, limitations to our surveillance-based analyses need to be taken into account. First, to be inclusive, we assessed the incidence of Zika virus in-fection in pregnancy as possible cases of Zika virus infection, in view of the limitations of current diagnostic methods available for use during outbreaks. Yet, we believe rates are still greatly underestimated, especially during the first epidemic wave, as our surveillance data required patient contact with health professionals for notification, which is likely to have occurred in only a fraction of total cases. Additionally, rates of Zika virus cases are likely to be biased, with increased reporting following the declaration of the epidemic. This bias is the likely explanation for the greater increase in reported suspected cases of Zika virus infection in pregnant women than in the general population, given the high concern about the consequences of Zika virus infection in pregnancy both by pregnant women and by those responsible for the notification. Thus, our possible Zika virus infection data serve more to indicate the presence of the yearly epidemics and their shape over time than their magnitude. Second, we used infection-related microcephaly as a proxy to Zika virus microcephaly, since the cause in most cases could not be established. To be inclusive again, we also considered cases not confirmed during the surveillance period, but whose diagnostic image summaries contained a mention of brain calcification. Although 409 (20·1%) of 1950 cases had a laboratory determination of an infectious agent, 848 (43·5%) cases had brain calcifications, thus strengthening the probability that the microcephaly was infection related. We also recognise that our definition of infection-related microcephaly might include some cases that are not of infectious origin. Finally, a large proportion of cases suspected of congenital syndrome or microcephaly are still being investigated, which reduces the reliability of estimates .

Despite these limitations, our study is, to our knowledge, the most comprehensive analysis so far of the 2015–16 dual epidemics. The epidemics were expressed in a vast country with territories spreading from the Tropics to the Southern Temperate Zone, thus enabling important reflections in terms of nature and scope of this emergent public health problem. However, the question of what

caused the apparent large difference in risk of microcephaly after the initial Zika virus outbreak in northeast Brazil versus the subsequent outbreaks in Brazil and elsewhere must await results of future research, including the many ongoing, or soon to start, cohort and case-control studies, and additional laboratory research. Moreover, although the results of our microcephaly analyses suggest that the burden of the Zika virus epidemic might be lower than originally feared, one must recognise that not all cases of Zika virus congenital syndrome present microcephaly, and a major disease burden will likely result from additional but less evident congenital anomalies due to Zika virus infection during pregnancy.[30–32]

In conclusion, based on cases of possible Zika virus infection in pregnant women and of confirmed infection-related microcephaly notified during two distinct waves of the vast Zika virus epidemic that spread across Brazil in 2015–16, we found a striking increase in the ensuing risk of microcephaly in only the northeast region and only after the first wave. Smaller peaks were observed in three of the four other regions following both waves. Reasons for these major differences in the apparent risk of complications of Zika virus infection over time and space remain to be elucidated.

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