Zika Virus Infection and Microcephaly in Infants: Is the Association Casual or Causal?

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Summary

In this case-control investigation to assess the association of microcephaly and Zika virus**,** cases reported to the national database for microcephaly (on the basis of their birth head circumference and total body length), born between Aug 1, 2015, and Feb 1, 2016, were enrolled. Controls were identified from the national birth registry and matched them to cases by location, aiming to enrol a minimum of two controls per case. Blood samples from mothers and infants were tested for Zika virus IgM and neutralizing antibodies as evidence of recent infection. Prevalence of microcephaly and its association with Zika virus infection was determined using a conditional logistic regression model.

Of the total 164 infants enrolled at birth, 91 (55%) had microcephaly on the basis of their birth measurements, 36 (22%) were classified as small head, 21 (13%) as disproportionate head, and 16 (10%) were classified as not having microcephaly. Forty-three (26%) of the 164 infants had microcephaly at follow-up for an estimated prevalence of 5·9 per 1000 live births. Investigators enrolled 114 control infants matched to the 43 infants classified as having microcephaly at follow-up. Infants with microcephaly at follow-up were more likely than control infants to be younger (OR 0·5, 95% CI 0·4, 0·7), have recent Zika virus infection (OR 21·9, 95% CI 7·0, 109·3), or a mother with Zika-like symptoms in the first trimester (OR 6·2, 95% CI 2·8, 15·4). Based on the presence of Zika virus antibodies in infants, authors concluded that 35-87% of microcephaly occurring during the time of the investigation in Northeast Brazil, was attributable to Zika virus, and an estimated 2-5 infants per 1000 live births had microcephaly attributable to Zika virus.

Commentaries

Evidence-based Medicine Viewpoint

Relevance: Zika virus (ZV) is a flavivirus that recently created a public health crisis in South America (and globally) [1]. Its transmission was detected during mid-2015 with a spurt in birth of infants with microcephaly in the North-East region of Brazil. The hallmark finding of microcephaly occurs in an estimated 2.3% (95% CI 1.0, 5.3%) infants of ZV-infected mothers [2]; although, this varies by timing (*i.e*., trimester) of infection. Affected infants have severe development disabilities and serious consequences, including seizures, motor disability, vision deficits and hearing defects [3,4]. There is a high mortality rate ranging from 7% to 10% [5]. Adult infections were associated with significantly increased risk of Guillain-Barré syndrome [6]. The recent Brazilian epidemic is the third such outbreak following those reported from Micronesia in 2007 and French Polynesia in 2013 [7], suggesting spread of the infection across the Pacific Ocean into South America.

This case control study in Paraiba (Brazil) reported that infants with microcephaly at the age of 1-7 months were more likely to have laboratory-confirmed recent ZV infection (OR 21·9, 95% CI 7·0, 109·3), and maternal history of ZV infection symptoms in the first trimester (OR 6·2, CI 2·8, 15·4) [8]. Microcephaly was not associated with the presence of ZV infection symptoms prior to pregnancy or during the other two trimesters. The overall population prevalence of microcephaly was calculated as 5.9 per 1000 live births, of which 35% to 87% could be attributed to ZV infection. There was no association between infant microcephaly and maternal age, education, household income, and a wide range of environmental factors, including mosquito exposure prior to pregnancy, type of water supply, consumption of fish, toxin exposure, and smoking or alcohol consumption during pregnancy

Critical appraisal: The investigators pursued three separate lines of inquiry in this case-control study [8] *viz*: (*i*) exploration of association between infant

microcephaly and ZV infection, (*ii*) prevalence of microcephaly following ZV infection outbreak, and (*iii*) factors other than ZV infection that could be responsible for the spurt in microcephaly. It can be argued that the case-control design is not the best suited design for these outcomes, especially for calculating prevalence. However, in the limited time span during (and after) the epidemic, it is perhaps the most feasible approach. ***Table* I** summarizes a critical appraisal of the study methodology. Several methodological refinements were applied during the design and conduct of this study. Highly specific definitions were used for almost all parameters. Although the focus was on microcephaly, the investigators categorized this further into true microcephaly, small head and disproportionate head. Microcephaly identified from the database was re-examined and re-categorized during a follow-up visit at 1 to 7 months of age. Extensive efforts were made to match controls to cases by area of residence. Efforts were made to ensure that the cases and controls had spent at least 80% of intrauterine life in the area of interest.

The national microcephaly database identified 836 microcephalic infants, whereas only 352 (42%) had microcephaly by definition. This discrepancy has two implications. First, the reporting system was probably highly sensitive (but poorly specific) as may be expected in an outbreak situation. Second, it confirms that the database could not be blindly believed. Further, even among 127 infants confirmed to be microcephalic at birth, only 50 (39%) were microcephalic at the follow-up visit, and a substantial 44% of the infants did not have microcephaly. This suggests that birth head circumference may have poor correlation with subsequent classifications of microcephaly.

This study [8] is not the only, nor even the first report of an association between Zika virus infection and microcephaly in infants. During 2015-16, 15 Brazilian states having confirmed ZV transmission documented the birth prevalence of microcephaly as 0.28 per 1000 live birth, which was over 4 times higher than the corresponding prevalence in 4 states without confirmed viral transmission [4]. Another case-control study conducted in several hospitals in Recife (within the epidemic region in Brazil) compared neonates having

TABLE I CRITICAL APPRAISAL OF THE STUDY METHODOLOGY

Criteria	*Appraisal*
Did the study address a clearly focused issue?	The investigators focused upon three issues in Paraiba region of Brazil viz (*i*) Potential association between infant microcephaly (at the age of 1-7 months) and recent ZV infection, (*ii*) Prevalence of infant microcephaly attributable to the ZV outbreak, and (*iii*) Association between various maternal risk factors and infant microcephaly.
Did the authors use an appropriate method (in to answer their question?	The case-control design is acceptable for identifying associations between exposure this case recent ZV infection as well as environmental factors) and outcome (occurring of microcephaly in infants). Methodologically superior prospective observational studies are likely to be time and resource intensive.
Were the cases recruited in an acceptable way?	Potential cases were initially identified from the Brazilian national database created to detect microcephaly, defined by the national criteria (head circumference <33 cm in term infants until December 2015; <32 cm thereafter, or less than 3rd centile of mean in pre-term infants). The investigators then re-classified the potential cases based on the WHO's growth curves into (*i*) microcephaly (head circumference ≤3rd centile, and ratio of head circumference : length ≤0·65), (*ii*) small head (head circumference ≤3rd centile, and ratio of head circumference : length >0·65), (*iii*) disproportionate (head circumference >3rd centile, and ratio of head circumference : length ≤0·65), and (*iv*) no microcephaly (head circumference >3rd centile, and ratio of head circumference : length >0·65). All infants underwent a follow-up assessment at 1-7 months of age for re-measurement and re-classification of head circumference. Those with microcephaly at the follow-up visit were counted as cases. *A priori* sample size calculation was done and the intended size achieved.
Were the controls recruited in an acceptable way?	Controls were infants born in the same region who did not have microcephaly, hence had to be identified from another database that recorded information on live births in the country. Two or three controls were planned for each case. Controls were matched for residence (as close to cases as possible) but not matched by age, gender etc.

Contd...

Criteria	*Appraisal*
	However, they were enrolled only if they were the same age or younger than the respective cases.
Was the exposure accurately measured to minimize bias?	Ascertainment of recent ZV infection in infants was done by identifying anti ZV IgM in blood and anti ZV neutralising antibodies against Zika virus by the plaque reduction neutralization assay. The effect of passively transferred maternal antibodies was taken into account by comparing the ratio of maternal : infant anti ZV antibodies to the corresponding ratio for dengue virus (since dengue is not transmitted vertically). Based on this, infants were categorised as confirmed ZV infection, presumed, possible and uninfected. All recent ZV infections in infants were assumed to be vertically transmitted. Ascertainment of exposure to other risk factors was carried out by asking about maternal demographic characteristics, illnesses during pregnancy, medications taken, exposure to toxins/pesticides, type of water supply, fish consumption, alcohol consumption, smoking, etc. Thus it is evident that robust ascertainment criteria for ZV infection were applied, whereas criteria for other exposures were not similarly stringent, thereby compromising specificity. Further, recall bias (seeking answers to potential exposures more than 6-12 months previously) compromises sensitivity also.
What confounding factors have the authors accounted for?	The investigators considered several potentially confounding factors including infant age and gender, maternal age, ethnicity, & education status, household income, exposure to mosquito bites (through surrogate questions), water source during pregnancy, fish consumption, maternal smoking, alcohol consumption, and toxin exposure (specifically pesticide, insecticide, rodenticide, fertiliser, and fumigator). However, there was no effort to confirm or rule out other intrauterine infections that could be associated with microcephaly.
What are the results of this study? How precise are the results?	Association with microcephaly (Cases, *n*=43 *vs* Controls, *n*=114) • ZV infection symptoms during the first trimester: OR 6·2 (95% CI 2·8, 15·4) • Confirmed ZV infection: OR 21·9 (95% CI 7·0, 109·3) • Confirmed or presumed ZV infection: OR 18·9 (95% CI 7·1, 70·3); • Confirmed, presumed, or possible ZV infection: OR 15·1 (95% CI 4·9, 75·3) • Data for only presumed and only possible infection not shown. • None of the other risk factors showed a statistically significant association. Prevalence of microcephaly following the ZV outbreak: 5·9 per 1000 livebirths. This was determined as follows: Numerator = proportion of infants in the microcephaly database who also had microcephaly at the follow-up visit x total number of infants in the microcephaly database during the study period. Denominator: Total number of live births during the same period.The mean attributable risk of microcephaly with Confirmed ZV infection was 35% (95% CI 26, 44%); Confirmed or presumed infection 58% (95% CI 46, 73%); and Confirmed, presumed, or possible infection 87% (95% CI 70, 100%).
Do you believe the results?	The results are valid and hence believable. Some issues compromising validity have been highlighted in the text. Evaluation of the Bradford Hill criteria is summarized in Table II.
Can the results be applied to the local population?	No. Please see details.
Do the results of this study fit with other available evidence?	See Table II for detailed analysis.

microcephaly with those born without microcephaly [9]. Serum polymerase chain reaction (PCR) testing for ZV in both groups and additional CSF testing of cases, was used to define infections. ZV infection was confirmed in about one-third of cases but none of the controls. A preliminary analysis, soon after the epidemic peaked, reported significant association of microcephaly with ZV infection (OR 55·5, 95% CI 8·6, ∞) [9]. A subsequent analysis by the same investigators again confirmed the same [10]. Population-based surveillance for detection of birth defects in the USA also identified an increase in birth defects reported to be associated with congenital

Table II BRADFORD HILL CRITERIA FOR ASSESSMENT OF CAUSAL ROLE OF ZIKAVIRUS INFECTION ON MICROCEPHALY

Criteria	*Assessment*
Strength of association	This [8] and a few other studies [9,10] confirm strong association between infant ZV infection and microcephaly.
Temporality	The first case of Zika virus infection in Brazil was reported in May 2015, and the epidemic was well established by August. The Government declared a national emergency in November 2015. The epidemic was followed by a dramatic increase in neonatal microcephaly [10].An analysis of Brazilian data from January 2015 to November 2016 identified >70% of microcephaly cases to be associated with the ZV epidemic [14]. During the initial months of the epidemic, the prevalence was as high as 5.0 per 1000 live births. During the second wave of the epidemic, the monthly peak prevalence ranged from only 0.32 to 1.50 per 1000 live births. Initial studies among mothers of infants with confirmed congenital Zika syndrome, reported that over three quarters of affected mothers recalled ZV infection symptoms during pregnancy [15].This case control study [8] was able to demonstrate that maternal ZV infection symptoms occurring only during the first trimester of pregnancy was associated with microcephaly; whereas symptom occurrence during 30 days prior to pregnancy, as well as during the second or third trimesters, were not.
Consistency	There is data from other settings within Brazil [9,10,16,17] as well as other countries such as USA [11] that report similar observations. During the period 2015-16, fifteen Brazilian states having confirmed ZV transmission documented the birth prevalence of microcephaly to be >four-fold higher than the corresponding prevalence in 4 states without confirmed viral transmission [18]. Although most investigators confidently assert a causal association between ZV infection and microcephaly [19], occasional reports suggested that the prevalence of microcephaly in Brazil during 2015-16 was similar or even lower than the baseline prevalence rate [20]. Apparently, increases in the number of Zika virus infection corresponding to 11-18 weeks of gestation were not followed by statistically significant increases in the prevalence of neonatal microcephaly. Another group of authors reported that the affected (north-east) part of Brazil was recording microcephaly cases well before the ZV epidemic [21]. They also argued that active ZV infection and transmission occurred on more than 60 countries, but none showed spurt in microcephaly cases.
Theoretical plausibility	Vertical transmission is well documented for several viral infections (Hepatitis B, HIV, CMV etc) hence it is not surprising that ZV can also be transmitted in this way. Previous studies have demonstrated ZV footprints in the amniotic fluid of pregnant women, placenta and even fetal brain tissue. Other flaviviruses have been associated with neurotropic effects. Therefore, there is theoretical plausibility that ZV infections can cause microcephaly.
Coherence	A case-control study comparing neonates with, and without microcephaly born in Recife confirmed the association with ZV infection [10]. Additionally, the investigators did not find any association with suspected risk factors for microcephaly such as maternal immunization during pregnancy with TdaP, MMR or MR vaccines. Similarly, addition of the larvicidepyriproxyfen in drinking water was not associated with microcephaly. Another careful analysis of the microcephaly prevalence in municipalities of Recife using pyriproxyfen identified a comparable prevalence to municipalities using a biologic larvicide [22]. These data negate the hypothesis of pyriproxyfen as a cause of spurt in microcephaly.
Specificity in the causes	There are no obvious threats to specificity. However, this study [8] has not undertaken sufficiently specific methods to rule out the role of maternal exposures to toxins/teratogens/other infections as a potential cause of microcephaly.
Dose response relationship	It is difficult to confirm a dose-response relationship for ZV infection and microcephaly. However, one study showed that maternal rash during the third trimester (surrogate for ZV infection) could be associated with Zika virus related brain abnormalities even though the head circumference was normal [23]. The study reported that almost 20% infants with ZV infection had normal head circumference, suggesting an indirect dose-response relationship. Data from animal experiments have demonstrated a dose dependent effect of ZV infection in immune-competent mice [24].
Experimental evidence	Animal experiments in immune-competent mice have confirmed that ZV can be transmitted vertically, and infection resulted in brain development defects, eye abnormalities and spinal paralysis in affected offspring [24-26]. Experimental models also showed that ZV has a predilection for neuronal stem cells, dysregulating gene expression, and cell cycle progression, resulting in cellular death [27].
Analogy	Some other viral infections transmitted vertically have been associated with microcephaly. This most likely occurs by a depletion of the pool of neuronal progenitor cells in the developing brain, resulting in impaired development and microcephaly.

Zika virus infection, during the temporal period of the epidemic in Brazil [11].

Therefore, the present study [8] has to be viewed against the backdrop in which it was conducted. In early 2016, there was reasonable uncertainty whether ZV was actually associated with microcephaly [12], especially as other potentially responsible factors were also hypothesized, including maternal vaccination, pesticides, toxins *etc*. Putting all the available data together, ***Table* II** summarizes the Bradford-Hill criteria [13] for causality [8-10,14-27]. In order to establish a causal link between ZV infection and microcephaly, confirmation of baseline prevalence of microcephaly in the community (just prior to the epidemic) is important. This is somewhat hampered by multiple factors, including paucity of local data, variable reliability of data sources, different methods used to define microcephaly, variations in types of infants studied and inadequate investigations performed to identify cause.

During the period from 2005 to 2014, data from over 100 hospitals located in 10 South American countries reported a microcephaly prevalence of 0.44 per 1000 live births for hospital deliveries and 0.30 per 1000 live births in the community. However, there were significant inter-country, inter-region and even inter-hospital differences [28]. The traditional intrauterine infections were together responsible for less than 4% cases. Similarly, over a five-year timeframe prior to the ZV epidemic, the Texas Birth Defects Registry recorded the prevalence of microcephaly as 1.47 per 1000 live births. Severe microcephaly was recorded in 0.48 per 1000 live births. Another US-based birth defect surveillance system identified an overall microcephaly prevalence of 0.87 per 1000 live births [29]. The Quebec province in Canada reported an overall microcephaly prevalence ranging from 0.30 to 0.53 per 1000 live births during an observation period comprising nearly 2 million births over 23 years [30].

In Brazil itself, a report of microcephaly prevalence during 2011-15 among >8200 infants in neonatal intensive care units located in areas not associated with the ZV epidemic, reported an overall prevalence of 5.6% (95% CI 5.1%, 6.1%) with severe microcephaly in 1.5% (95% CI 1.2% to 1.7%) [31]. Data from two urban Brazilian birth cohorts comprising >7300 and 4200 live births reported a pre-Zika microcephaly prevalence of 3.5% and 2.5% [32]. The corresponding prevalence of severe microcephaly were 0.7% and 0.5%. These represent unusually high prevalence rates. These variations highlight the importance of recognizing the pre-epidemic microcephaly prevalence in the area of interest.

Since neonatal microcephaly is associated with several non-infectious maternal risk factors (age >35-40 y as well as <20 y, ethnicity, low education levels, smoking, diabetes, exposure to teratogens, *etc*) [28-30,33] that vary with country/society, data from other settings cannot be extrapolated to the local setting. Therefore, besides the baseline prevalence of microcephaly from birth records, analysis of the possible causes is also important to determine the role of infections such as ZV. This is also missing in this study [8].

In general, ZV infections are associated with severe microcephaly [15]. A study of 87 infants with confirmed congenital ZV infection had mean (SD) head circumference of only 28.1 (1.8) cm, despite mean (SD) birth weight being 2577 (260) g and >80% being term deliveries [15]. A comparative analysis of infants without ZV infection *versus* those with probable or confirmed infection reported a difference of 1·45-1·72 (mean 1.58) Z-scores in head circumference [23]. Unfortunately, this study [8] did not provide such data.

The global Zika virus crisis also highlights the variability in microcephaly definitions used around the world. The commonly used 'International Fetal and Newborn Growth Consortium' definition is head circumference <–2 standard deviations of the mean for age and gender. This has been used in most studies [10,17,32]. In contrast, the European Surveillance of Congenital Anomalies (EUROCAT) program defines microcephaly as head circumference <–3 Z-scores below the mean for sex, gender, and ethnicity, with reduced brain size [34]. This definition corresponds to 'severe' microcephaly in the other system. However, a study of 16 European registries comprising >5.7 lakh births across 15 countries, showed that only approximately half these registries applied the EUROCAT definition of microcephaly, whereas some used a cut-off of <–2 Z-score, and over a third of the registries defined microcephaly on the basis of criteria used by individual clinicians [34]. One registry changed the definition during the review period. Not surprisingly, there was a ten-fold variability in the prevalence across the registries. Interestingly, those using the more stringent EUROCAT definition reported a higher prevalence of 0.17 compared to 0.12 per 1000 live births with the –2 Z-score cut-off.

Even during the recent epidemic in Brazil, the definitions of microcephaly for active surveillance of ZV infection underwent modifications. The initial cut-off provided by the Ministry of Health was head circumference ≤33 cm in term infants, whereas this was

changed to <32 cm after a few weeks [35]. It appears that this was appropriate since it best corresponded to the definition of microcephaly using the gold standard criteria of head circumference below the 3rd percentile. In the present study [8] also, the authors reported that the Brazilian Ministry definition switched from <3rd centile to an 'updated, more specific' definition of <–2 Z-scores. However, both these are not very different; hence, it is difficult to accept that the latter can be more specific.

Extendibility: Data from this study [8] confirming a causal role of recent ZV infection with infant microcephaly, can be extended (in principle) across the world, as the bulk of evidence points in this direction. However, a meaningful epidemiological interpretation necessitates estimates of local baseline microcephaly prevalence, a robust surveillance system, and an action-oriented public health response system. These are currently not well developed in our setting. Further, the other causes of microcephaly and their relative distribution in various infant cohorts are unknown. For these reasons, the data from the study [8] cannot be meaningfully extended to our setting.

Conclusion: This case-control study provided strong evidence of an association between infant ZV infection and microcephaly in early life. However, there are limitations in the validity of the data ruling out other possible causes for the spurt in microcephaly in the local setting of the study.

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Pediatric Neurologist's Viewpoint

In this case control study, Krow-Lucal *et al*. [1], provide confirmative evidence of association of maternal antenatal Zika virus infection with microcephaly in infants. Based on the presence of Zika virus antibodies in infants, the authors concluded that 35–87% of microcephaly occurring during the time of their investigation in northeast Brazil was attributable to Zika virus. They estimated that 2–5 infants per 1000 live births in Paraíba, Brazil had microcephaly attributable to Zika virus.

Even though, so far Zika virus is not an etiological consideration in the evaluation of microcephaly in infants in India (unless there is a history of maternal travel to Zika affected regions during pregnancy), there are a number of interesting learning points from this study for pediatricians and pediatric neurologists.

Microcephaly has been traditionally defined as significant reduction in the occipito-frontal head circumference (HC) compared with age- and gender-matched controls. Controversies persist whether a cut-off of less than –2SD or less than –3SD should be considered to define microcephaly. Some authors have advocated for defining severe microcephaly as an HC more than 3 SDs below the mean [1]. However, in the current study, and in most other studies, microcephaly was defined as HC more than 2 SDs (*i.e*., <3rd centile) below the mean for age and gender.

The importance of head circumference measurement at birth and serial follow- up measurements during clinic visits are essential but often missed. Head circumference is measured in infants who present with developmental delay or neurological problems but is frequently missed during well baby visits and visits for other childhood illnesses. For term babies, a head circumference at birth less than 32 cm was considered as microcephaly in this study. For preterm babies, pediatricians need to use the INTERGROWTH-21st Charts which provide standards for postnatal preterm growth [2].

The other issue in the assessment of microcephaly is the relationship of head circumference with other growth parameters such as length and weight. Traditionally, if the length and weight are also less than –2 SD for age, the infant is said to have proportionate microcephaly. If the head circumference is <–2 SD for age, and the weight and length are normal, the infant is said to have disproportionate microcephaly. In this study, however, the authors classified infants into 4 groups: microcephaly (head circumference ≤3rd percentile, head circumference: total body length ratio ≤0.65), small (head circumference ≤3rd centile) disproportionate (head circumference >3rd percentile, head circumference: body length ≤0.65), and no microcephaly (head circumference >3rd percentile, head circumference: body length >0.65). The authors selected a head circumference: body length cutoff of 0.65 on the basis of consultation with infant dysmorphology experts and data on newborn infants from British Columbia indicating that fewer than 10% of newborns have a ratio of 0.65 or lower. This classification is interesting and needs to be validated in future studies.

The importance of follow-up measurements is excellently demonstrated in this study. Out of 91 infants detected to have microcephaly at birth, only 34 (37%) had microcephaly at follow-up. Interestingly, out of the 21 infants who were classified as disproportionate at birth (normal head circumference but disproportionately small head as compared to the length), 3 infants (14%) were detected to have microcephaly at follow-up. This fact underscores the importance of interpreting the head circumference in relation to the length of the infant. Also, as the authors suggest, birth measurements have insufficient precision and several measurements might be needed to classify an infant as having microcephaly.

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Public Health Viewpoint

This was a retrospective case control study to assess the association of microcephaly and Zika Virus (ZV) conducted in North-East Brazil and included cases – infants reported to national database for microcephaly – and age-matched controls from the same geographic area in the same period [1]. The national case definition for microcephaly was an infant with head circumference (HC) of 33 cm or less, which was later changed to HC of 32 cm or less for term infants and HC lower than 3rd centile for gestational age for preterm infants. To control the effect of sex and intrauterine growth retardation (IUGR), the researchers classified the children in 4 categories depending upon HC and ratio of HC to body length – microcephaly, small, disproportionate and no microcephaly. Mothers and infants were tested for Zika virus and dengue virus IgM antibodies and neutralizing antibodies. All infants had repeat measurements of HC and length at follow-up.

The study strengthens the evidence of causal association of ZV infection in pregnancy with microcephaly. Since no other factor was significantly associated with microcephaly, the article lays to rest the speculation of role of alternative risk factors such as environmental toxins. The role of effect modifier such as past or concurrent dengue infection remains uncertain as the study did not have sufficient power. A case-control study from same region similarly attributed the microcephaly epidemic in the area to ZV infection in pregnancy [2]. A recent meta -analysis of sample size of 2941 pregnancies of which 2648 were live births, provided a pooled prevalence of ZV-associated microcephaly of 2.3% of pregnancies and 2.7% of the live births, which is rather lower than expected [3]. A more reliable estimate of risk of Zika-associated birth defects depending upon the time of infection in pregnancy was provided by a prospective study from US territories that studied completed pregnancies with confirmed ZV infection. The percentages of fetuses or infants with possible Zika-associated birth defects with maternal infection with Zika virus infection in 1st , 2nd, and 3rd trimester was 8%, 5%, and 4%, respectively [4]. It is recognized that the risk of sequelae with ZV is not limited to first trimester alone, and infants born to mothers with ZV infection need close follow-up and monitoring.

The study documents importance of repeat measurements after birth as at follow-up only about one-fourth of the reported cases actually had microcephaly. The use of a single cut-off value of 33 cm at birth, which was used in the study, lacks specificity. The head circumference grows at rapid pace in the last trimester;

therefore, using sex- and gestational age- specific head circumference charts such as Intergrowth-21 is recommended for preterm infants and term infants in whom exact gestational age is known [5]. In LMIC countries in which Low birth weight rates are high, use of a single cut-off, as used in Brazil will overestimate the burden of microcephaly.

A major challenge with ZV is difficulty in diagnosis since the PCR assay detects viral RNA and is therefore positive only during the brief period of viremia. The serological tests show significant cross-reactivity with other flaviviruses such as dengue which are often circulating in the same areas . The fetal abnormalities are detected late in pregnancy when it is often too late for termination of pregnancy. Besides it is not yet known whether asymptomatic infection poses a risk to the fetus.

Currently in absence of a vaccine against ZV infection, prevention remains the only method to reduce the burden of complications of ZV infection. In populations with established ZV transmission, congenital Zika Virus syndrome can be prevented by good vector control measures, preventing sexual transmission, and reducing the number of unplanned pregnancies. In countries with low or no transmission of ZV, checking importation of ZV and surveillance of congenital Zika virus syndrome and Guillian-Barre Syndrome should be in place. Clustering of microcephaly or suspected Congenital Zika virus syndrome can give a clue to the outbreak.

The public health implications of outbreak of Zika virus in India can be enormous. The weak surveillance system coupled with difficulty in clinically differentiating Zika virus infection from dengue and chikungunya can hamper the control measures. WHO has classified countries according to Zika virus circulation and transmission. As per this classification India falls in category 2 since there is historical evidence of virus circulation before 2015. The report of two patients with febrile illness testing positive for Zika Virus at Ahmedabad in 2017 raised alarm bells in our country. Till date 4 cases with acute febrile illness have tested positive for Zika Virus (3 from Gujarat and one from Tamil Nadu) suggesting foci of local transmission. However, no virus has been detected from mosquito population tested [6]. Needless to say that given the conducive environment in India, over a variable period, the mutations in the virus might render mosquitoes more susceptible, which in turn may increase transmission and result in outbreaks [7]. A similar situation has been seen with Chikungunya virus in recent past.

To estimate the extent of Zika virus infection in India, a long-term robust surveillance network is needed. Vector surveillance needs to be scaled up. Existing acute febrile illness and birth defect surveillance at sentinel sites needs to be strengthened. It may be added that phenotype of congenital Zika virus infection is expanding and besides microcephaly other congenital abnormalities such as club foot, arthrgryoposis and ocular abnormalities should be added for surveillance. Surveillance for Guillian Barre Syndrome (GBS) is another strategy that can be used in addition. Cases of Acute Flaccid Paralysis are reported routinely to the National Polio Surveillance Program. Detection of an increase in the number of cases of GBS in the AFP surveillance system may provide early warning of a Zika virus outbreak.

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