Age-dependent manifestations and case definitions of paediatric Zika: a prospective cohort study

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Summary

Background Paediatric Zika remains an understudied topic. WHO and the Pan American Health Organization (PAHO) Zika case definitions have not been assessed in children. We aimed to characterise clinical profiles and evaluate the diagnostic performance of the WHO and PAHO case definitions in a large cohort of paediatric Zika cases.

Methods From January, 2016 to February, 2017, encompassing the major 2016 Zika epidemic, participants in the Pediatric Dengue Cohort Study (PDCS) in Managua, Nicaragua, were encouraged to visit the study health centre at first indication of any illness. PDCS participants were aged 2–14 years, healthy at enrolment, and recruited before the initiation of the present study. Molecular and serological assays were used to test participants exhibiting any of four broad clinical profiles suspected of resulting from a symptomatic Zika virus infection. These clinical profiles were: fever and at least two of headache, retro-orbital pain, myalgia, arthralgia, rash, haemorrhagic manifestations, and leukopenia; fever and at least two of nausea or vomiting, rash, aches and pains, positive tourniquet test, leukopenia, and any dengue warning sign; undifferentiated fever without evident cause, with or without any other clinical finding; and afebrile rash with or without any other clinical finding. We characterised acute clinical findings (signs, symptoms, and complete blood counts) in both Zika cases and non-Zika cases.

Findings We prospectively followed a cohort of about 3700 children, of which 1110 were deemed eligible for inclusion. Four participants with laboratory-confirmed Zika (three co-infections with dengue virus, one missing complete blood count data) and two participants who were non-Zika cases (missing complete blood count data) were excluded from analysis. We analysed 556 laboratory-confirmed Zika and 548 non-Zika cases. The WHO case definition captured 176 confirmed Zika cases, and the PAHO definition 109 confirmed Zika cases, who presented with the most clinical findings and a dengue-like clinical profile. The remaining two thirds of Zika cases, principally characterised by undifferentiated fever or afebrile rash, were missed. Among Zika cases, rash (n=440)—particularly generalised erythematous rash (n=334)—fever (n=333), leukopenia (n=217), and headache (n=203) were most common and peaked within 3 days of illness onset. The most common Zika presentation over the first week of illness was rash only (n=80). The sensitivity of Zika case definitions increased across paediatric age (from 11·3% to 56·1% for the WHO case definition and from 6·0% to 36·6% for the PAHO case definition), as the prevalence of most clinical findings (particularly arthralgia) increased with age, irrespective of previous dengue virus infection. Consequently, Zika manifested differently across paediatric age; older Zika cases presented with a dengue-like clinical profile while younger Zika cases presented with undifferentiated fever or afebrile rash.

Interpretation We provide the most thorough description of paediatric Zika to date. Most paediatric Zika cases go undetected under the WHO and PAHO case definitions, suggesting that current standards for Zika case ascertainment require revision. Zika manifests with mild but differing clinical profiles across paediatric age, presenting major challenges to diagnosis, surveillance, and efforts to control future Zika epidemics.

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Introduction

Zika virus (ZIKV), a member of the Flavivirus genus usually transmitted by aedes mosquitoes, was believed to cause a self-limiting febrile illness without severe complications for decades after its discovery in 1947. However, ZIKV became a global public health concern when ZIKV infection caused severe congenital complications such as microcephaly and neurological disabilities during epidemics throughout the Pacific Islands and the Americas from 2013 to 2016. More than 828,000 Zika cases have been reported to the Pan American Health Organization (PAHO), the Regional Office for the Americas of WHO, since the Zika pandemic started in 2015. ZIKV infection is primarily concerning in pregnant women and their fetuses, as Zika in adulthood, except for rare neurological complications such as Guillain-Barré syndrome, presents with mild fever, maculopapular rash, conjunctivitis, and arthralgia, generally resolving within a week. The clinical profile of patients with Zika is thought
Added value of this study

This study used a wide case ascertainment strategy to identify potential Zika cases and employed a serology-based algorithm to supplement the traditional confirmation of cases by real-time RT-PCR. The study expands our understanding of Zika by showing that the disease primarily manifests as undifferentiated fever or afebrile rash throughout childhood, with paediatric Zika tending to manifest with a clinical profile similar to dengue in late childhood and early adolescence. Zika in children is often missed by current case definitions because of its mild presentation in childhood.

Implications of all the available evidence

Our study and evidence from the literature support the hypothesis that age is a key determinant of the clinical manifestations of Zika, with older cases presenting more signs and symptoms, and thus presenting more often with a dengue-like clinical profile, than younger cases. Current case definitions might require revision to capture the full clinical spectrum of Zika. Clinicians might need to use molecular and serological methods to confirm ZIKV infection, especially in children, as Zika’s mild and non-specific presentation complicates differential diagnosis.

Methods

Study design and participants

The Pediatric Dengue Cohort Study (PDCS) is an ongoing prospective cohort study of children aged 2–14 years based in Managua, Nicaragua, originally established in 2004 to study DENV infections and later expanded to include ZIKV. Articles describing Zika in adults, including pregnant women, and in children infected with ZIKV in utero were excluded. Only a few studies in the published literature describe paediatric Zika, and most are small case reports. The few modestly sized studies describing Zika in children have either selected participants predominantly using a febrile surveillance system or participants who travelled to areas with ZIKV transmission. These previous studies of children with Zika have not included a comparison group of non-Zika cases, evaluated the temporality of Zika signs and symptoms, investigated age trends with statistical methods appropriate for continuous data, or assessed the diagnostic accuracy of standard Zika case definitions.

Participants were encouraged to visit the study health centre at first indication of any illness and to revisit the study health centre upon the occurrence of new signs or symptoms. During the study period of January 2016, to February, 2017, PDCS cases that exhibited any of four broad clinical profiles suspected of Zika were eligible for inclusion; there were no exclusion criteria. These clinical profiles were fever and at least two of headache, rash, aches and pains, positive tourniquet test, leukopenia, and any dengue warning sign (2009 WHO dengue case definition); undifferentiated fever without evident cause, with or without any other clinical finding; and afebrile rash, with or without any other clinical finding. For analysis purposes, Zika cases meeting the 1997 or 2009 WHO dengue case definition, or both, were collapsed into a clinical profile termed WHO dengue case definition.

Institutional review boards of the University of California, Berkeley, the University of Michigan, and the Nicaraguan Ministry of Health approved the PDCS protocol. Participants’ parents or legal guardians provided written informed consent. Participants age 6 years or older provided verbal assent.

Procedures

Acute-phase serum samples or urine samples, or both, of eligible participants were tested for ZIKV infection by real-time RT-PCR (rRT-PCR). Paired acute-phase and convalescent-phase serum samples were tested using...
an algorithm based on five serological assays (appendix pp3–4). On the basis of such testing, cases were categorised as either Zika cases or non-Zika cases. The non-Zika case group contained all non-Zika illnesses that met one of the clinical profiles during the study period. We collected and analysed clinical findings (a collective term for signs, symptoms, and complete blood count findings) through the initial 7 days of illness (appendix pp 2–3). Conjunctival involvement collectively refers to conjunctivitis, conjunctival injection, or both. Rash without qualifications refers to any type of rash (appendix p 3).

Statistical analyses

The WHO case definition for Zika is rash or fever, or both, and at least one of arthralgia, arthritis, and conjunctivitis. The PAHO case definition for Zika is rash and at least two of fever, conjunctivitis, arthralgia, myalgia, and periarticular oedema. Using the Zika and non-Zika cases, we assessed the sensitivity, specificity, and predictive values of these case definitions (appendix p 4). Sensitivity was modelled as a function of age using generalised additive models, which capture non-linear trends (appendix p 4). We calculated the percentage of laboratory-confirmed Zika cases that would be missed by standard (WHO and PAHO) case definitions if cases had to meet both definitions to be captured, and, as a robustness check (sensitivity analysis), if cases had to meet only one definition to be captured.

The age and sex distributions of the non-Zika cases, the Zika cases, and the Zika cases stratified by clinical profile were examined (appendix p 4). The prevalence of each clinical finding for confirmed Zika and non-Zika cases was calculated. Clustering of clinical findings among Zika cases was visualised through a co-occurrence dendrogram (appendix p 4). We used generalised additive models to characterise the prevalence of each clinical finding across age. We created a severity score for each Zika case by summing the number of clinical findings (appendix p 4) and modelled this severity score with zero-truncated generalised additive models, which capture non-linear trends (appendix p 4). We calculated the percentage of laboratory-confirmed Zika cases that would be missed by standard (WHO and PAHO) case definitions if cases had to meet both definitions to be captured, and, as a robustness check (sensitivity analysis), if cases had to meet only one definition to be captured.

Role of the funding source

The study funder had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Participant characteristics

Approximately 3700 PDCS participants were followed from January, 2016, to February, 2017, spanning the initiation and cessation of Managua’s first Zika epidemic. Of these, 1110 participants exhibited any of the four broad clinical profiles suspected of Zika, and were eligible for inclusion. Of the 1110 PDCS cases, 560 were laboratory confirmed by either rRT-PCR or serology as Zika cases and the remaining 550 as non-Zika cases. Four Zika cases (three co-infections with DENV, one missing complete blood count data) and two non-Zika cases (missing complete blood count data) were excluded from the analysis.

Data from 548 non-Zika cases (appendix p 23) and 556 Zika cases were assessed: of the Zika cases, 370 (67%) were rRT-PCR-confirmed and 186 (33%) were rRT-PCR-negative but confirmed by serology. 296 of 370 rRT-PCR-positive Zika cases had ZIKV in the acute-phase serum sample, and 140 of 370 rRT-PCR-positive Zika cases had ZIKV in the acute-phase urine sample. Zika was identified in both acute-phase serum and urine samples of 66 of 370 rRT-PCR-positive Zika cases. Zika cases were older and more frequently female than were participants confirmed as non-Zika cases (appendix pp 6, 23). Both groups had similar numbers of medical consultations, but 28 participants who were non-Zika cases were referred to hospital compared with five participants with confirmed Zika (1%). No participants with Zika developed Guillain-Barré syndrome. 107 Zika cases (19%) presented with undifferentiated fever and 223 (40%) with afebrile rash (table 1). Only 226 (41%) of the 556 Zika cases met the WHO dengue case definition.

Paediatric Zika is often missed by standard case definitions

Overall, 176 (32%) and 109 (20%) of 556 laboratory-confirmed Zika cases met the WHO and PAHO Zika case definitions, respectively (table 2, appendix p 24). Only 100 Zika cases were captured by both case definitions (sensitivity 18·3%, 95% CI 15·0–21·4), and 185 Zika cases were captured by either case definition (sensitivity 33·3%, 29·5–37·3; figure 1A).

To understand clinical differences between captured and missed cases, we compared the 100 Zika cases that were captured by both case definitions with

<table>
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<tr>
<th>Identification method</th>
<th>Clinical profile proportion (%)</th>
<th>Identification method proportion (%)</th>
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</thead>
<tbody>
<tr>
<td>rRT-PCR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHO dengue case definition</td>
<td>169/226 (75%)</td>
<td>169/370 (46%)</td>
</tr>
<tr>
<td>Undifferentiated fever</td>
<td>59/107 (55%)</td>
<td>59/370 (16%)</td>
</tr>
<tr>
<td>Afebrile rash</td>
<td>142/223 (64%)</td>
<td>142/370 (38%)</td>
</tr>
<tr>
<td>Serology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical profile proportion (%)</td>
<td>Identification method proportion (%)</td>
<td></td>
</tr>
<tr>
<td>WHO dengue case definition</td>
<td>57/226 (25%)</td>
<td>57/186 (31%)</td>
</tr>
<tr>
<td>Undifferentiated fever</td>
<td>48/107 (45%)</td>
<td>48/186 (26%)</td>
</tr>
<tr>
<td>Afebrile rash</td>
<td>81/223 (36%)</td>
<td>81/186 (44%)</td>
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Table 1: Summary of identification method and clinical profile for participants with laboratory-confirmed Zika
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the remaining 456 cases. 14 of the 22 clinical findings assessed were significantly more prevalent in the group that met both case definitions, and no clinical findings were significantly more prevalent in the missed group (appendix p 7). Therefore, participants with Zika that did not meet the definitions for both criteria displayed milder manifestations than did the captured cases. The overall distribution of the Zika severity score (determined by total number of clinical findings; appendix p 8) showed that Zika cases mostly had few clinical findings. The most common severity score (128 [23%] of 556 Zika cases) involved only two clinical findings, most commonly rash and leukopenia (47 [37%] of 128). Such children would not be captured by the WHO or PAHO case definition for Zika, as leukopenia (appendix p 9) does not appear in either. Additionally, 92 (17%) Zika cases had one clinical finding (usually rash, 80 [87%] of 92), too few to meet either case definition. Rash alone was the first and rash with leukopenia the second most common paediatric Zika manifestations when we considered combinations of the four most common clinical findings (rash, fever, headache, and leukopenia; appendix p 10). Among all 556 Zika cases, 12 (2%) only had fever as a symptom.

A bimodal distribution was apparent when the overall distribution of the severity score was stratified by whether cases were captured by both standard case definitions (figure 1B). Missed cases had a median of three (IQR 2–4) clinical findings, whereas captured cases had a median of six (IQR 5–7) clinical findings. A similar bimodal distribution was recapitulated when stratifying by clinical profile (figure 1C). Zika cases meeting the WHO dengue case definition had a median of five (IQR 4–7) clinical findings. Cases with undifferentiated fever and those with afebrile rash had a median of two clinical findings (IQR 2–3 for undifferentiated fever and 1–3 for afebrile rash). Therefore, the standard case definitions for Zika primarily captured cases meeting the WHO dengue case definition (appendix p 25). As a robustness check (sensitivity analysis), we repeated the above analyses by comparing the 185 cases that were captured by either standard case definition with the remaining 371 cases, with equivalent conclusions (appendix pp 11–12, 26).

Separately, we observed that stratifying the severity score by DENV infection history did not recapitulate a bimodal distribution (figure 1D).

### Paediatric Zika manifests with mild and non-specific acute clinical findings

Among Zika cases, rash (440 [79%] of 556), fever (333 [60%]), leukopenia (217 [39%]), and headache (203 [37%]) were the four most common clinical findings (appendix p 27). Although both case definitions include conjunctivitis, only 15 (3%) Zika cases had conjunctivitis, and only two (<1%) had periarticular oedema, a clinical finding appearing in the PAHO case definition. Respiratory and gastrointestinal conditions were rare among the paediatric Zika cases. Zika cases predominantly presented with generalised rash (404 [73%] of 556) and erythematous rash (359 [65%]; appendix p 13); hence, generalised erythematous rash was common (334 [60%]). Maculopapular rash (105 [19%] of 556) and other rash subtypes were less common.

Rash and leukopenia were significantly more prevalent among Zika cases than non-Zika cases (figure 2A). Paediatric Zika presents non-specifically, as most clinical findings were more prevalent among non-Zika cases. No evidence of a difference in prevalence between Zika and non-Zika cases regarding arthralgia (in both case definitions) and myalgia and periarticular oedema (in the PAHO case definition) was found.

Among the Zika cases, the average number of signs and symptoms per medical visit remained relatively constant over the first 3 days after illness onset and subsequently declined, as expected (appendix p 14). The prevalence of the more common clinical findings showed a similar pattern (appendix p 15). Leukopenia was the exception with a relatively stable prevalence over 7 days after illness onset.

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**Table 2: Sensitivity, specificity, and predictive values for the WHO case definition and the PAHO case definition for the 556 Zika and 548 non-Zika PDCS cases reported between January, 2016, and February, 2017**

<table>
<thead>
<tr>
<th>Elements of case definition</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>Positive predictive value (95% CI)</th>
<th>Negative predictive value (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO</td>
<td>Rash or fever, or both, and at least one of arthralgia, arthritis, l or conjunctivitis</td>
<td>31.7% (27.9–35.6)</td>
<td>74.3% (70.4–77.8)</td>
<td>55.5% (50.0–60.9)</td>
</tr>
<tr>
<td>PAHO</td>
<td>Rash and at least two of fever, conjunctivitis, arthritis, myalgia, periarticular oedema</td>
<td>19.6% (16.5–22.1)</td>
<td>98.2% (96.6–99.1)</td>
<td>91.6% (88.1–95.5)</td>
</tr>
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</table>

PDCS=Paediatric Dengue Cohort Study. PAHO=Pan American Health Organization. *An expanded version of this table, with the raw data and additional diagnostic indices, is provided (appendix p 24).†During the Zika outbreak that occurred during the study period from January, 2016, to February, 2017, uncharacteristically few cases of dengue and chikungunya were observed in the study population. Therefore, the specificity and predictive values are higher than they would be if the non-Zika cases included typical levels of dengue and chikungunya. ‡Arthritis is not a recorded variable for medical assessments of our paediatric population. Instead, doctors record proximal and distal arthralgias. The estimated indices are lower for the WHO case definition than they would be if arthritis were a recorded variable. The four indices of interest are calculated with conjunctival involvement (conjunctivitis or conjunctival injection, or both, as conjunctivitis and conjunctival injection were uncommon (appendix p 3 and p 27). The estimated indices are slightly higher than if they were estimated using conjunctivitis alone.
Standard case definitions do not reflect observed patterns of paediatric Zika clinical findings

In the co-occurrence dendrogram, rash formed its own co-occurrence cluster among Zika cases (figure 2B). This clustering of rash by itself is consequent of the many (n=80) Zika cases that had rash and no other clinical finding. Fever, leukopenia, headache, and arthralgia, in the second cluster, were the second, third, fourth, and fifth most common clinical finding, respectively. All other clinical findings are in the third cluster. These co-occurrence patterns of clinical findings were discordant with the clinical findings in the WHO and PAHO case definitions. Neither standard case definition captured Zika cases with rash only. Myalgia, conjunctival involvement, and periarticular oedema clustered together, but did not cluster with the other clinical findings in the PAHO case definition (ie, rash, fever, and arthralgia).

Age drives the prevalence of clinical findings, the clinical profile, the severity score, and the sensitivity of Zika case definitions

We examined the clinical profile and severity score of the laboratory-confirmed Zika cases for an explanation underlying all previous observations. Among Zika cases, there was no evidence that clinical profile varied by sex; however, Zika cases with a dengue-like clinical profile were significantly older than were Zika cases with other clinical profiles (appendix 16). Similarly, there was no evidence that the severity score was associated with sex (appendix p 17). However, the adjusted risk ratio per
Figure 2: Analyses of clinical findings

(A) Forest plot showing prevalence differences between the 556 Zika cases and 548 non-Zika cases for each clinical finding. Prevalence differences along with associated 95% CIs and p values are presented. The dashed line at 0 represents the null value for prevalence differences; 95% CIs including the dashed line correspond to non-significant prevalence differences. The prevalence differences are ordered by the prevalence of each clinical finding in the Zika group, as shown in the appendix (p 27).

(B) Hierarchical clustering dendrogram showing co-occurrence of clinical findings among the 556 Zika cases. Clinical findings closer together along branches of the tree structure (vertical axis) are more likely to either co-occur or jointly not occur in the same person. The high cophenetic distance correlation coefficient indicates that the dendrogram is reproducing an underlying hierarchical structure found in the original Manhattan distances used to construct the dendrogram. Clusters of clinical findings are colour-coded. Rash, in the first cluster (blue), was the most common clinical finding among the Zika cases (appendix p 27).
Figure 3: Model predictions from logistic generalised additive models

For all models, exact age is based on the date of birth and the date of the first medical consultation for a Zika-associated illness. All models were run with the sample of Zika cases (n=556). (A) The predicted sensitivity of the WHO case definition and the PAHO case definition across paediatric age is shown, with pointwise 95% confidence bands. The dashed line indicates a sensitivity of 50%, the expected sensitivity if true Zika cases were randomly categorised as Zika-positive or Zika-negative. Additionally, the predicted prevalence of arthralgia (from B) is shown. The near parallelism exhibited by the three curves implies that the main determining factor for whether the case definitions classify a true case as Zika-positive is the presence of arthralgia. (B) The predicted prevalence for each clinical finding in the PAHO case definition across paediatric age is shown, with pointwise 95% confidence bands. PAHO=Pan American Health Organization.
additional year of age was 1.04 (95% CI 1.02–1.05), indicating that the number of Zika manifestations significantly increased with age. Although the expected change in severity score per year of age is small, the model implied that Zika cases that age out of the PDCS cohort on their 15th birthday had, on average, 1.6 times (95% CI 1.4–2.0) the number of clinical findings than did the Zika cases aged 2 years (the youngest in the cohort), after accounting for sex, the number of medical consultations, and the number of days from illness onset to the last medical consultation. We further observed that the predicted sensitivity of the WHO case definition ranged from 11.3% (95% CI 7.1–17.5) at age 2 years to 56.1% (46.3–65.4) at age 15 years (figure 3A). Similarly, the predicted sensitivity of the PAHO case definition ranged from 6.0% (95% CI 3.0–11.6) at age 2 years to 36.6% (26.5–48.0) at age 15 years.

Among Zika cases, the predicted prevalence of all measured clinical findings in the standard case definitions also varied by age, except for periarticular oedema, which was rare (figure 3B). Rash and fever were common across age since children with either sign were tested for ZIKV infection. However, the prevalence of both signs increased by about 10–15 percentage points across paediatric age. The positive age-prevalence trend for rash reflected similar trends for generalised erythematous rashes (appendix p 18); the prevalence of maculopapular rashes decreased across age. The predicted prevalence of arthralgia increased from 8.4% (95% CI 4.7–14.6) at age 2 years to 44.0% (33.7–55.0) at age 15 years, an average increase of 2.7 percentage points per year of age. The age-prevalence curve for arthralgia displayed near parallelism to the age-sensitivity curves for the WHO and PAHO case definitions (figure 3A), implying that the presence of arthralgia principally determined whether standard case definitions correctly classified laboratory-confirmed Zika cases as Zika positive. The prevalence of several other clinical findings also varied across age, including leukopenia, which increased about 40 percentage points in prevalence across paediatric age (appendix p 19).

Zika manifests differently across paediatric age, independent of previous DENV infection

The proportion of Zika cases meeting the WHO dengue case definition increased by about 35 percentage points across paediatric age (appendix p 20); 26 of the 35 percentage points were offset by commensurate decreases in the proportion of undifferentiated fever, with the remaining nine explained by the decreasing proportion of afebrile rash. Thus, the clinical spectrum of Zika changed across paediatric age. The severity score (sum of clinical findings) did not differ by DENV infection history (figure 1D). Similarly, the predicted prevalence of clinical findings by DENV infection history (appendix p 21) and the predicted prevalence of clinical profiles by DENV infection history (appendix p 20) did not differ from the overall results (figure 3B; appendix p 20).

Therefore, our major finding that the clinical presentation of Zika changes substantially across paediatric age was not explained by the positive association between existing humoral immunity to DENV and age (appendix p 22).

Discussion

We describe the clinical presentation of 556 Zika cases aged 2–14 years in a prospective cohort in Managua, Nicaragua, using an extensive molecular and serological laboratory workup on a wide range of clinical profiles. Zika cases had mild and non-specific clinical findings, but age was positively associated with additional clinical findings (including signs of fever, generalised erythematous rash, cervical lymphadenopathy, and leukopenia) and hence the spectrum of severity, regardless of any previous DENV infection. Paediatric Zika cases infrequently had maculopapular rash. Our analyses indicate that the WHO and PAHO Zika case definitions anticipate a more severe Zika presentation than was typically found in paediatric cases. Accordingly, these case definitions missed most laboratory-confirmed cases (68% for WHO definitions; 80% for PAHO definitions) in an age-dependent manner and largely captured older children who met the WHO dengue case definition. Most Zika cases that presented with undifferentiated fever or afebrile rash were not captured. To our knowledge, this study is the most extensive analysis of paediatric Zika to date.

A mild and self-limiting illness is conventionally of little medical concern. However, the mild and non-specific nature of paediatric Zika presents numerous clinical and public health challenges. In our study, 40% of Zika cases presented with at most two clinical findings and were captured only because of our expansive testing scheme. Because such cases do not meet standard case definitions, physicians could miss many cases, particularly those presenting in the younger age range. Consequently, people living with children who have even a potentially quite mild Zika illness—especially women of child-bearing age—might be exposed to ZIKV without their knowledge.

Future Zika case definitions should prioritise sensitivity over specificity; because a high degree of false negatives can hinder time-sensitive outbreak control. A score-based case definition in a general population with DENV and chikungunya virus transmission has been reported to have high levels of sensitivity and specificity.19 However, the study’s sole reliance on RT-PCR to confirm ZIKV infection suggests that its published sensitivity is overestimated because RT-PCR-negative, serology-positive cases were misclassified as non-Zika cases.

The description of rash in Zika case definitions should be re-examined. We find little evidence that paediatric Zika presents with maculopapular rash, the typical type of rash reported in adults with Zika. In our paediatric study, Zika-associated rash was predominantly characterised as generalised and erythematous without macules or papules. Only a few paediatric Zika cases exhibited maculopapular rash. Future Zika case definitions should capture cases
presenting with rash only and rash and leukopenia, given that 23% of our large sample exhibited only these clinical findings.

Children with Zika tended to present with mild and non-specific clinical findings, which complicates the physician's task of differential diagnosis, especially in settings with co-circulating dengue and chikungunya viruses. The use of broad or data-driven\(^2\) case definitions, combined with extensive and expensive laboratory testing to overcome immunological cross-reactivity, will be necessary to capture and monitor paediatric Zika cases, especially in dengue-endemic areas. These issues will only become more urgent over time, as climate change over the next century is projected to expand the niche of aedes mosquitoes poleward. As a result, nearly a billion people, primarily in Europe and high-elevation tropical and subtropical areas, will be newly exposed to aedes-associated arboviruses, including ZIKV.\(^2\)

The low sensitivity of standard case definitions in children suggests that the size of Zika epidemics might have been systematically underestimated in an age-dependent manner, wherever these and similar case definitions have been used. Consequently, transmission models for Zika, mostly estimated from adult Zika data,\(^2\) might also be systematically biased. The large paediatric population of Latin American countries indicates the potential for a sizable degree of bias in the epidemiological literature.\(^2\) The proportion of asymptomatic ZIKV infections varies considerably across populations (range 0–100%),\(^2\) our results suggest that different case definitions and the different age structures of studied populations contribute to this heterogeneity. To resolve this issue, future epidemiological studies should employ an extensive ZIKV testing scheme and use consistent case definitions that capture both paediatric and adult Zika cases. Moreover, future meta-analyses of the proportion of asymptomatic ZIKV infection should examine individual-level data for differences in this proportion by age.

Arthralgia was the key (although not the only) age-varying factor determining whether the standard case definitions correctly classified a laboratory-confirmed Zika case, despite arthralgia not being considered a major aspect of Zika. The observed prevalence of arthralgia reflects both its true occurrence and the ability of a child to articulate his or her pain. If the prevalence of arthralgia were fully dependent on a child’s ability to articulate pain, the age-prevalence curve would have plateaued during late childhood, in contrast to the increasing age-prevalence trend observed at the upper end of paediatric age. Regardless of the degree to which the observed prevalence of arthralgia reflects occurrence or reporting, many Zika cases did not meet standard case definitions because study physicians, quite experienced with paediatric arthralgic diseases (ie, dengue, chikungunya, and Zika), concluded that young children did not experience much Zika-associated arthralgia. The increasing prevalence of arthralgia, rash, and fever across paediatric age has been previously noted.\(^9\)

The WHO and PAHO case definitions are likely based on adult data, as mostly adult data from Yap Island\(^6\) and French Polynesia\(^7\) were available when the case definitions were created and updated.\(^2\)\(^1\)\(^1\) Because Zika manifests differentially across childhood, and standard case definitions include clinical findings rarely observed in our large paediatric population, symptomatic ZIKV infection could manifest differently across the lifespan. This hypothesis is supported by the age-varying prevalence of various Zika clinical findings, observed in comparisons of younger children with older children (in our study and others)\(^9\) and in comparisons of children with adults.\(^7\) A comparison of Zika clinical findings in paediatric and general population studies provides further evidence in support of this hypothesis (appendix pp 28–30). Specifically, general population Zika studies report a higher prevalence of arthralgia, myalgia, conjunctival involvement, headache, periarticular oedema, and gastrointestinal and respiratory clinical findings than do studies of paediatric Zika. Our hypothesis that Zika manifests differently by age should be tested empirically with a broad case ascertainment strategy, rigorous laboratory testing, and appropriate methods for continuous data. Importantly, there was no evidence that Zika manifested differently with previous DENV infection. Thus, there is no epidemiological evidence that a mild form of antibody-dependent enhancement explains the age trends we documented.

We used rRT-PCR and a serological algorithm based on five assays to confirm ZIKV infection. If rRT-PCR alone were used (as reported in previous studies)\(^6\)\(^7\)\(^2\)\(^8\) and only cases with a dengue-like clinical profile were included,\(^6\)\(^7\)\(^2\)\(^9\) the sample size would have been reduced by 70%, artifically making the sample more concordant with Zika’s typical presentation in adults. Thus, commonly used approaches to ascertain and confirm paediatric Zika cases miss many true cases and substantially mischaracterise paediatric Zika. Better diagnostic and surveillance tools are needed to understand the Zika pandemic in 2015–16 and prepare for future Zika epidemics.\(^2\)

Our study has several limitations. First, the number and schedule of medical consultations varied by participant, potentially leading to an incomplete record of clinical findings. However, the severity model adjusted for these factors and PDCS participants were encouraged to visit the health centre at the first indication of any illness, and to revisit the health clinic upon the occurrence of new signs or symptoms. Second, there were few dengue and chikungunya cases (compared with historical data)\(^6\)\(^1\) in the non-Zika group; standard Zika case definitions would have lower specificity with contemporaneous arboviral transmission. Third, symptoms are subjective and therefore prone to age-dependent misreporting. Despite study physicians’ extensive attempts to diagnose symptoms such as arthralgia...
(appendix p 3), potential symptom misclassification might have occurred.

Overall, our results showed that paediatric Zika manifestations are mild, non-specific, and vary by age, explaining why paediatric Zika cases are often missed by the WHO and PAHO case definitions. As our observations concern how paediatric Zika manifests, our results are likely to be generalisable to other settings, increasing the need for more accurate Zika case definitions.

Contributors
FBC, RB-C, LG, and EH conceived the study. AB, AG, GK, and EH developed the study design. SO, NS, MP, AB, and GK implemented the study design and collected field data. AN and AB oversaw the laboratory testing. BLM, JCM, DE, and SA organised and verified the data. FBC, RB-C, and LG analysed the data and did the statistical analyses. FBC, RB-C, LG, IK, and EH drafted and revised the manuscript, and all authors reviewed the manuscript.

Declaration of interests
All authors declare no competing interests.

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