A comparative analysis of dengue, chikungunya, and Zika manifestations in a pediatric cohort over 18 years

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ABSTRACT

Background. Dengue, chikungunya, and Zika are mosquito-borne diseases of major human concern. Differential diagnosis is complicated in children and adolescents by their overlapping clinical features (signs, symptoms, and complete blood count results). Few studies have directly compared the three diseases. We assessed clinical features of cases aged 2-17 years experiencing these diseases.

Methods. We characterized 1,405 dengue, 517 chikungunya, and 522 Zika pediatric cases occurring from January 2006 through December 2023 in a Nicaraguan cohort study. Clinical records and laboratory results across the first 10 days of illness were examined from a primary care health center. All cases were laboratory-confirmed. Data were analyzed with generalized additive models, generalized mixed models, and machine learning models.

Findings. The prevalence of many clinical features exhibited by dengue, chikungunya, and Zika cases differed substantially overall, by age, and by day of illness. Dengue cases were differentiated most by abdominal pain, leukopenia, nausea/vomiting, and basophilia; chikungunya cases were differentiated most by arthralgia and the absence of leukopenia and papular rash; and Zika cases were differentiated most by rash and lack of fever and lymphocytopenia. Dengue and chikungunya cases exhibited similar temperature dynamics during acute illness, and their temperatures were higher than Zika cases. Sixty-two laboratory-confirmed afebrile dengue cases, which would not be captured by any widely used international case definition, presented very similarly to afebrile Zika cases, though some exhibited warning signs of disease severity. The presence of arthralgia, the presence of basophilia, and the absence of fever were the most important model-based predictors of chikungunya, dengue, and Zika, respectively.

Interpretations. These findings substantially update our understanding of dengue, chikungunya, and Zika in children while identifying various clinical features that could improve differential diagnoses. The occurrence of afebrile dengue warrants reconsideration of current case definitions.

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Research in context

Evidence before this study: Dengue, chikungunya, and Zika are mosquito-borne diseases that co-occur in tropical and subtropical settings and give rise to fever, rash, and a variety of other clinical features. We reviewed the most widely used international case definitions for the three diseases – established by either the World Health Organization (WHO) or the Pan American Health Organization (PAHO); PAHO's 2022 report on differential diagnosis and treatment options for dengue, chikungunya, and Zika; and the 80 studies underlying PAHO's diagnostic recommendations. The available evidence suggests that dengue, chikungunya, and Zika have a broad and overlapping set of clinical manifestations that hamper differential diagnosis, and hence case management, in the absence of definitive laboratory testing. This similarity in disease presentation is believed to be especially characteristic of the first few days of illness. Most of the studies on the diseases' clinical manifestations are in adult populations, and these studies constitute the main evidence base for the existing case definitions. For example, only one of the 80 studies summarized in PAHO's recent report provided direct comparisons of the clinical features across cases experiencing dengue, chikungunya, and Zika, and that study mainly focused on adults. Disease presentation is more non-specific in children and adolescents than in adults, further impeding differential diagnoses in pediatric populations. Current guidelines suggest that the presence of thrombocytopenia, progressive increases in hematocrit, and leukopenia tend to distinguish dengue from chikungunya and Zika; that arthralgia is more common in chikungunya; and that pruritis is more common in Zika. Fever is considered a defining feature of dengue, such that all case definitions in widespread use for the disease require that patients exhibit fever. However, existing but limited evidence suggests that dengue cases can very rarely present without fever.

Added value of this study: This study follows a cohort of Nicaraguan children through multiple dengue epidemics, two large chikungunya epidemics, and one explosive Zika epidemic. By synthesizing 18 years' worth of primary care medical records, we find clinically meaningful differences in the prevalence of many clinical features, including by day of illness and across age, but not by sex. In addition to verifying the clinical features PAHO identified as key distinguishing features, we also identified others, including papular rash, nausea, hemorrhagic manifestations, abdominal pain, and basophilia, that could aid differential diagnoses. As the PAHO report is based on a multitude of studies with varied age ranges, health care accessibility, overall research quality, and patient populations, this study is a complementary and important counterpart that draws from a single, well-characterized source population.

Further, we identified 62 laboratory-confirmed cases of afebrile dengue (7·2% of all dengue cases since we started testing any suspected case exhibiting afebrile rash). The disease manifestations of afebrile dengue cases were generally clinically indistinguishable from afebrile Zika, although several displayed warning signs of severity. We found that cases of the three diseases exhibited different temperature dynamics across the acute period of illness. Machine learning models were best able to distinguish chikungunya (an alphaviral disease) from dengue and Zika (flaviviral diseases) based on clinical features alone. Our dengue model performed well, especially in classifying febrile dengue cases. However, our Zika model struggled to properly distinguish afebrile dengue cases from afebrile Zika cases, likely due to their very similar and minimal disease presentation.

Implications of all the available evidence: Despite clinical overlap in the pediatric manifestations of dengue, chikungunya, and Zika, we identify meaningful differences in their presentation and in laboratory markers that can be leveraged to improve diagnoses in the absence of definitive laboratory-based diagnostic testing. Afebrile dengue should be studied more and incorporated into future case definitions, as not accounting for its existence can impede surveillance, laboratory testing strategies, clinical management, and research efforts.

INTRODUCTION

Dengue virus (DENV), chikungunya virus (CHIKV), and Zika virus (ZIKV) are primarily transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes. Over four billion people live at risk of infection (1), and climate change is exacerbating the risk for billions more (2). Where viruses overlap geographically, explosive co-epidemics with high morbidity sometimes occur, as seen across Central and South America.

Four DENV serotypes (DENV-1-4) cause dengue, the world's most common mosquito-borne disease, which ranges from undifferentiated fever to life-threatening conditions (3–5). Fever and arthralgia are especially prominent during acute-phase chikungunya; some patients experience persistent arthralgia months or years afterward (6). Zika often presents non-specifically during childhood and as a mild, dengue-like disease in adolescence and adulthood (7). However, ZIKV infection during pregnancy can cause serious developmental complications in infants, including microcephaly.

The clinical spectrum of dengue, chikungunya, and Zika encompasses fever, rash, and other clinical features (*i.e.*, signs, symptoms, and complete blood count results) (5). The diseases tend to present distinctly in adults but more non-specifically in children and adolescents. Differential diagnosis and disease surveillance are thus especially difficult in pediatric populations absent definitive laboratory testing. In response, a recent Pan American Health Organization (PAHO) report identified clinical features useful for differential diagnosis by summarizing 80 studies with varied patient populations, age ranges, research quality, and health care settings (8). However, only one of 80 studies directly compared the prevalence of clinical features across the diseases, and that study (9) mainly focused on adults, leaving a wide gap in pediatric medicine.

To address this gap, we used 18 years of primary care observations to characterize dengue, chikungunya, and Zika manifestations in a Nicaraguan pediatric cohort. We identify multiple clinical features that vary in prevalence overall, by age, and by day of illness; highlight disease-specific differences in the temporal dynamics of fever; and ascertain the most important clinical features for disease classification via machine learning algorithms. Finally, we provide compelling evidence for the existence of afebrile dengue, despite the requirement for fever in all widely used dengue case definitions (3–5).

METHODS

Ethics statement

Institutional Review Boards of the University of California, Berkeley, the University of Michigan, and the Nicaraguan Ministry of Health approved the Pediatric Dengue Cohort Study (PDCS) protocol. Participants' parents or legal guardians provided written informed consent. Participants ≥6 years old provided verbal assent.

Study design

Briefly, in 2004, the PDCS began studying DENV infections among 2-9-year-old children in Managua, Nicaragua (10). The PDCS expanded its eligibility criteria to include CHIKV and ZIKV before they entered the study area in August 2014 and January 2016, respectively (11,12). After several age-based expansions (2-11 in 2008, 2-14 in 2013), the study has included 2-17-year-olds since 2018, after the chikungunya and Zika epidemics. PDCS participants receive 24/7 free medical care at the Health Center Sócrates Flores Vivas (HCSFV). Participants are encouraged to visit the HCSFV at the first indication of any illness and to return if new signs/symptoms occur; most (~95%) participants comply (10). Acute blood samples are collected at the first medical consult (97% within 1-3 days of illness). Convalescent samples are collected 14-21 days post-illness onset (94% of cases).

We assessed clinical features during the first 10 days of illness. Analysis was restricted to clinical findings either 1) present in the World Health Organization (WHO) or PAHO case definitions for dengue, chikungunya, or Zika (3–5,13–15) or 2) occurring in at least 30 cases of any of the three diseases. Fever was defined as a recent history of fever/feverishness by the patient/guardian or a measured temperature of ≥38·0°C (100·4°F) during the medical consult. *Rash*, unless otherwise specified, denotes any type of rash.

We included laboratory-confirmed cases evaluated at the HCSFV between January 2006 and December 2023. Patient and laboratory data undergo extensive validation to ensure accuracy (10). For this study, we reassessed the data for consistency and completeness.

Testing criteria and laboratory confirmation

Initially, PDCS cases suspected of dengue, chikungunya, or Zika were eligible for laboratory testing if they exhibited: 1) fever and ≥2 of the following: headache, retro-orbital pain, myalgia,

arthralgia, rash, hemorrhagic manifestations, and leukopenia (1997 WHO dengue case definition (3)) or 2) undifferentiated fever without evident cause, with or without other clinical findings. After ZIKV was introduced into Managua in 2016, PDCS testing criteria expanded to encompass a third clinical profile: afebrile rash, with or without other clinical findings (7).

Suspected dengue and chikungunya cases were laboratory-confirmed by 1) RT-PCR of acute blood samples, with some undergoing viral isolation, 2) seroconversion by IgM capture ELISA, and/or 3) seroconversion or $\geq$4-fold increase (DENV only) in antibody titers by Inhibition ELISA in paired acute/convalescent samples. DENV serotyping was achieved by RT-PCR. After the introduction of ZIKV, a flavivirus antigenically related to DENV, acute-phase serum (and/or urine samples for Zika patients) of suspected cases was tested by 1) a DENV-CHIKV-ZIKV multiplex real-time RT-PCR (rRT-PCR) (16,17) or 2) a ZIKV singleplex rRT-PCR (18) alongside a pan-DENV and CHIKV rRT-PCR (19). Paired acute/convalescent serum samples from suspected Zika cases were also tested by a validated algorithm consisting of five serological assays (7,20).

Exclusion criteria

Incomplete clinical records were excluded. Medical personnel reviewed moderate-to-severe discrepancies in patient data; medically irreconcilable data were excluded.

Statistical analyses

The prevalence of clinical findings across age and days of illness was estimated using binomial generalized additive models and day-specific means, respectively. Linear mixed-effects models estimated the temporal dynamics of fever. Continuous outcome data were compared first with Kruskal-Wallis rank sum tests and then Dunn tests with Hochberg corrections for multiple comparisons. We excluded temperature data for individuals on antipyretics at the time of medical consult. We developed machine learning models using boosted regression trees to classify diseases based on clinical findings, extracted the five most informative clinical findings, and generated predictions across the full dataset, using misclassifications as measures of disease similarity. The Appendix contains additional information on all aspects of study methodology.

Role of the funding source

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

RESULTS

Participant characteristics

From January 19, 2006, through December 31, 2023, 1,321 dengue, 517 chikungunya, and 522 Zika cases were identified (Table 1). Substantial numbers of dengue cases occurred most years, whereas chikungunya and Zika cases were concentrated in 2014-2015 and 2016, respectively (Figure S1). We also detected 89 flavivirus cases (participants with DENV or ZIKV infections that could not be unambiguously distinguished by laboratory testing). Of these, 84 occurred when only DENV was circulating in Managua; we thus classified them as dengue cases. The five remaining flavivirus cases and five co-infected cases were excluded from all analyses. Among 1,405 (1,321+84) total dengue cases, 255 (18·1%) were caused by DENV-1, 425 (30·2%) by DENV-2, 306 (21·8%) by DENV-3, and 191 (13·6%) by DENV-4.

The study population contained 1,980 unique participants who experienced 2,444 illness episodes and had 9,087 medical consults by HCSVF physicians (Table 1). Approximately 50% of all cases were female. Children 10-14 years-old constituted the largest age group. Dengue cases had more clinical findings, medical consults, and hospital referrals than chikungunya and Zika cases ($p<0·001$ all comparisons). Chikungunya cases reported significantly earlier to the HCSFV than dengue ($p<0·001$) and Zika cases ($p<0·001$).

Prevalence comparisons by disease

Dengue cases frequently exhibited fever (95·6%, 1,343/1,405), lymphocytopenia (83·4%, 1,158/1,405), headache (75·4%, 1,060/1,405), and leukopenia (71·8%, 997/1,405) (Table S1). Fever (100%, 517/517), arthralgia (86·3%, 446/517), lymphocytopenia (85·6%, 441/517), and headache (76·6%, 396/517) were the most common clinical features for chikungunya patients. Rash (79·5%, 415/522), specifically generalized rash (73·9%, 386/522), was the only clinical feature occurring in ≥70% of Zika cases. Fever was always present for chikungunya and the vast majority of dengue cases since we did not test participants with afebrile rash until the Zika epidemic started in 2016.

Large and significant differences in the prevalence of many clinical findings were observed across diseases (Figure 1, Tables S1-2). The largest differences were observed for arthralgia, which was 62·7 and 47·8 percentage points more prevalent among chikungunya than Zika and dengue cases, respectively. Myalgia, fever, headache, and lymphocytopenia were all ~40 percentage points more prevalent among chikungunya than Zika cases. Rash was 41·7 percentage points more prevalent among Zika than dengue cases. Conversely, headache, fever, myalgia, leukopenia, hemorrhagic manifestations, lymphocytopenia, and basophilia were 30-40 percentage points more prevalent among dengue than Zika cases. Based on absolute and relative differences in prevalence, dengue was most distinguished from chikungunya and Zika based on the presence of monocytopenia and abdominal pain; chikungunya was most distinguished by the presence of arthralgia and absence of papular rash and conjunctival injection; Zika was most distinguished by the presence of generalized, erythematous rash and absence of fever, headache, myalgia, and lymphocytopenia (Tables S2-3).

Several clinical findings occurred ~0% of the time, which can aid in excluding diseases during differential diagnosis. For example, papular rash was observed in 0·4% (2/517) of chikungunya cases, compared to 22·2% (116/522) of Zika cases and 7·5% (105/1,405) of dengue cases (Table S1); conjunctival injection displayed a similar pattern. Abdominal pain, thrombocytopenia, and monocytopenia were rare for chikungunya and Zika cases (≤3·3% prevalence) but were more common (11-24%) among dengue cases.

Prevalence trends by age

We first compared the age-varying presentation of clinical profiles we use for laboratory testing. As age increased, a growing percentage of dengue, chikungunya, and Zika cases met the 1997 WHO case definition for dengue (Figure S2). Conversely, the percentage exhibiting undifferentiated fever decreased with age across diseases. Zika cases were significantly more likely to exhibit afebrile rash than dengue cases at any age.

We then examined the age-prevalence trends of the underlying clinical features and found many significant differences (Figure 2). For example, leukopenia was >25% more prevalent in dengue than Zika cases at every age, confirming earlier findings (Figure 1). We also found disease-specific age patterns. Uniquely among dengue cases, the prevalence of all types of rash decreased linearly and nausea increased linearly by age. Additionally, the prevalence of basophilia was constant and high (50-63%), in contrast to rapid age-based waning for

chikungunya and Zika cases. Among chikungunya cases, the prevalence of generalized, erythematous rash decreased from ages 2-10 but then rebounded and continued increasing throughout adolescence. Among Zika cases, the prevalence of fever increased with age and the prevalence of lymphocytopenia decreased before rebounding in adolescence. Age-prevalence trends did not differ by sex (Figure S3).

Prevalence trends by day of illness

Significant differences were found after analyzing the data across the first 10 days of illness (Figure 3). For example, the prevalence of fever decreased below 50·0% for Zika and chikungunya cases on day four; dengue cases achieved this on day six. Rash was most commonly observed among Zika cases, particularly on days 1-4. In contrast, among chikungunya cases, the prevalence of erythematous, generalized rash increased from 11·3% on day five to ~46·0% on days 6-7 of illness before decreasing again. Arthralgia was most prevalent on days 1-4 among the chikungunya cases. Compared to dengue and chikungunya cases, headache was about half as prevalent among Zika cases during days 1-2 of illness.

Unlike dengue and chikungunya cases, which exhibited steady increases in the prevalence of leukopenia until peaking at ~73·0% on day five, the average prevalence of leukopenia was relatively stable at 32·4% for Zika cases throughout. The prevalence of basophilia was similarly low across diseases on days 1-3. Afterward, its prevalence increased slightly to plateau at ~25·0% during acute Zika illness. For dengue and chikungunya cases, conversely, the prevalence of basophilia substantially increased, exceeding 75·0% by day seven.

Fever dynamics

We then examined temperature readings taken at the HCSFV among cases not on anti-pyretic medication (n=1,833). Average temperatures were highest on day one across diseases (Figure S4). Whereas 74·0% of day one temperatures for dengue and chikungunya cases were $\geq$38·0°C (100·4°F), our fever threshold, only 31·6% of comparable Zika temperatures were $\geq$38·0°C. Indeed, Zika cases had mean temperatures <38·0°C across days 1-10. Only 0·1% (7/5,118) of temperature readings not influenced by antipyretics were $\geq$40·0°C (104·0°F), and all occurred among dengue cases.

Average fevers for dengue and chikungunya cases were significantly higher, by 0·5-0·9°C (0·9-1·6°F), than Zika cases during days 1-3 ($p<0·001$ all comparisons). By day three, average temperatures for Zika cases returned to the interquartile range of healthy temperatures for this

pediatric population (36·7-37·0°C, 98·0-98·6°F); for chikungunya and dengue cases, this occurred on days five and six, respectively. On days 4-5, fever tended to be more common among dengue than chikungunya (day four p=0·07, day five p=0·05) and Zika (p<0·001, p=0·01) cases.

Afebrile dengue

We identified 62 dengue cases who neither reported recent histories of fever/feverishness nor met the 38·0°C fever threshold. Among the 36 females and 26 males, the average age was 9·5 years (SD=3·0). These cases represent 4·4% of all dengue cases (n=1,405) and 7·1% of dengue cases since we began testing patients exhibiting afebrile rash for DENV infection (n=873). Nine afebrile dengue cases were rRT-PCR-confirmed and caused by DENV-2. The maximum recorded temperature across the 62 cases was 37·5°C (99·5°F) (Figure S5). Fifty-three (85·5%) afebrile dengue cases first reported to HCSFV within days 1-2 of illness, when temperatures for febrile dengue cases are at their highest. If fever were instead defined as ≥1 temperature measurement >37·2°C (99·0°F), as is sometimes done for children, 44 dengue cases would still be considered afebrile.

Most afebrile dengue cases were very mild and required no hospitalization referrals (Table S4). However, five (8·1%) exhibited dengue warning signs, as defined by WHO in 2009 (4), with one warning sign each (two with mucosal bleeding, two with abdominal pain, one with persistent vomiting). Of the 62 cases, 12 (19·4%) had no clinical findings besides rash, and 17 (27·4%) exhibited only one clinical finding besides rash, predominantly (12/17, 70·6%) leukopenia. Rash was almost always erythematous and generalized. Afebrile dengue cases had significantly fewer per-case medical consults than febrile dengue cases (2·2 vs. 4·9, p<0·001), and they most closely resembled afebrile Zika cases in terms of per-case medical consults (2·2 vs. 1·8, p=0·19), day of first medical consult (1·7 vs. 1·7, p=1·00), and number of clinical findings exhibited over the first 3 (2·5 vs. 2·3, p=0·67) and 10 days of illness (2·7 vs. 2·6, p=0·81).

Model-based disease classification

Finally, we developed machine learning models that classified cases based on clinical findings. The full chikungunya model (covering days 1-10 of illness) had a sensitivity of 72·5% for chikungunya and ≥89% specificity for each of six non-chikungunya outcomes (*i.e.*, overall, febrile, and afebrile dengue and Zika cases) (Figure 4A). The presence of arthralgia and

absence of basophilia and leukopenia were the most important predictors of chikungunya vs. non-chikungunya disease status (Figure 4B). The full dengue model had a sensitivity of 86·1% for overall dengue and 89·6% for febrile dengue. However, it exhibited 15·0% sensitivity for afebrile dengue and misclassified 18·1% of febrile Zika cases as dengue. The dengue model's most important predictors were the presence of basophilia and leukopenia (Figure 4C). The full Zika model had sensitivities of 68·2% for overall Zika and 47·1% for febrile Zika. The model correctly classified 99·1% of afebrile Zika and misclassified 78·3% of afebrile dengue cases as Zika, confirming earlier findings that afebrile dengue and afebrile Zika cases presented very similarly. The absence of fever was most indicative of Zika vs. non-Zika status (Figure 4D).

All three full models had increasing sensitivity with increasing pediatric age (Figure 4E-G). The chikungunya model had modest (~50%) sensitivity in early childhood, likely because arthralgia, the model's most important variable, was absent or difficult to diagnose in ~25% of young children (Figure 2).

Reduced models focused on early illness (days 1-3) broadly resembled, but exacerbated the weaknesses of, their respective full models (Figure S6). The reduced chikungunya model correctly classified 28·8% (vs. 45·0% in the full model) of the youngest chikungunya cases. The reduced dengue and Zika models exhibited similar sensitivities, but lower specificities, than their full counterparts. The diminished performance of the reduced models is likely attributable to the loss of information from clinical findings that only differ significantly in prevalence after days 1-3 (Figure 3).

DISCUSSION

This study analyzed dengue, chikungunya, and Zika clinical manifestations within a single long-running pediatric cohort. We leveraged our prospective study design, broad laboratory testing criteria, and extensive clinical records to elucidate differences in disease presentation across days 1-10 of illness. We performed the most comprehensive analysis of afebrile dengue cases to date, and we used machine learning to classify cases based on clinical findings. Our conclusions are strengthened by direct comparisons of clinical features across dengue, chikungunya, and Zika, which only occurred in one (9) of the 80 studies summarized in PAHO's report on diagnostic guidelines (8). Further, this one report focused mostly on adults and was ~10 times smaller than our current study. Thus, our study meets an urgent need to characterize meaningful differences across these three diseases in pediatric populations.

The PAHO report suggested using the occurrence of thrombocytopenia, leukopenia, and progressive increases in hematocrit to distinguish dengue; arthralgia to distinguish chikungunya; and pruritus to distinguish Zika. Our findings agree with the use of thrombocytopenia, leukopenia, and arthralgia. We further identified several other key distinguishing clinical, especially at particular ages/days of illness (Table 2), that may aid diagnosis and case management. Both the PAHO report and our findings concur that complete blood counts are important sources of certain highly discriminating clinical features.

Our machine learning model reliably distinguished chikungunya from dengue and Zika cases, irrespective of fever status. Although the dengue model captured most febrile dengue cases, it classified 18% of febrile Zika and only 15% of afebrile dengue cases as dengue. The dengue model's poor ability to identify afebrile dengue cases likely stemmed from their non-specific and minimal clinical presentation. Interestingly, the most important predictor of dengue was basophilia. As it is not known to play a role in dengue pathogenesis, our study only establishes basophilia as a biomarker for dengue. The Zika model correctly classified 99% of afebrile Zika cases, but it also misclassified 78% of afebrile dengue cases as Zika, further showcasing their clinical similarity. Thus, afebrile dengue and afebrile Zika cases are only distinguishable by definitive laboratory testing. The lower performance of our reduced models using data only from days 1-3 of illness suggests that resource-limited settings experiencing concurrent epidemics can reduce diagnostic uncertainty by prioritizing cases in early illness for laboratory testing. Doing so also enables timelier management of severe complications, particularly for dengue.

In 1952, following intentional DENV infection of human volunteers (21), Sabin first reported mild cases of afebrile dengue with rash and leukopenia, the most common presentation of our afebrile dengue cases. While our 62 afebrile dengue cases were generally mild, five exhibited warning signs. Moderate-to-severe afebrile dengue has been reported from surveillance (22) and hospital (23–26) data. In the PDCS, 7·2% of captured dengue cases were afebrile after we began testing cases exhibiting afebrile rash. This percentage is higher than other studies documenting afebrile dengue (22–29), probably because only our study systematically tested afebrile cases for many years. However, 7·2% likely represents a *lower bound* on the hidden magnitude of afebrile dengue because afebrile cases without rash have been reported (26,28,29). Importantly, afebrile dengue has been observed in DENV-2 cases and in DENV-1 and DENV-4 outbreaks (27), so most, if not all, serotypes can cause afebrile dengue.

Since the WHO 1997 and 2009 and PAHO 2023 dengue case definitions require fever (3–5), the existence of afebrile dengue complicates diagnosis, treatment, surveillance, risk assessments for severe manifestations upon a secondary DENV infection, and transmission/epidemiological studies. For example, key outbreak parameters, like R_0, are likely systematically underestimated by not considering afebrile cases. Consequently, afebrile dengue should be considered in future diagnostic guidelines for dengue, and further studies on its epidemiological, immunological, and clinical aspects are warranted. As we have previously shown for Zika (7), the spectrum of some flaviviral diseases is wider, but less febrile, than traditionally understood.

Systematic collection of medical data in a single population, large sample sizes, and modern analytic methods strengthened our study. We were limited by our testing criteria, which prevented cases of afebrile dengue and potentially afebrile chikungunya from being captured before 2016. However, our criteria reflected contemporaneous knowledge about disease presentation. Moreover, analyzing the full complexities of dengue (*e.g.*, multiple serotypes, primary vs. secondary infections) were beyond the scope of this study, but they are explored in our companion paper using primary and tertiary care data (30).

In summary, we showed that pediatric dengue, chikungunya, Zika exhibit distinguishing features overall, by age, and by day of illness. Exploiting these differences can enable more reliable diagnoses and better case management in the absence of confirmatory laboratory methods. Our afebrile dengue cases, particularly those with warning signs, emphasize that this undercharacterized presentation of dengue should be incorporated into future guidance. With climate change accelerating the threat posed by mosquito-borne diseases (2), updated knowledge will allow for improved diagnostic, surveillance, and research endeavors.

AUTHOR CONTRIBUTIONS

FABC 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 data. AB oversaw the laboratory testing that was performed by DC, TM, and SS. BLM, JCM, SA, LC, and ZC organized and verified the data. FBC analyzed the data and performed the statistical analyses, with CJC consulting on the machine learning models. FABC 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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TABLES

Table 1. Characteristics of the dengue, chikungunya, and Zika cases in the PDCS in Managua, Nicaragua, by disease (January 2006 – December 2023).

	Dengue N (%)	Chikungunya N (%)	Zika N (%)
Overall	1,405 (100·0)	517 (100·0)	522 (100·0)
Epidemic years	Most years	2014 and 2015	2016
Female	739 (52·6)	252 (48·7)	288 (55·2)
Age			
2-4 years	105 (7·5)	70 (13·5)	104 (19·9)
5-9 years	531 (37·8)	182 (35·2)	220 (42·1)
10-14 years	640 (45·6)	265 (51·3)	198 (37·9)
15-17 years[1]	129 (9·2)	0 (0·0)	0 (0·0)
RT-PCR confirmed[2]	1,180 (84·0)	510 (98·6)	350 (67·0)
Number of medical consults	6,722 (100·0)	1,215 (100·0)	1,150 (100·0)
Mean day of first presentation to the health center per case (SD)[3]	2·0 (1·1)	1·7 (0·8)	1·9 (0·8)
Mean number of medical consults per case (SD)[4]	4·8 (3·6)	2·4 (1·3)	2·2 (1·1)
Mean number of clinical findings over days 1-10 of illness per case (SD)[5]	8·2 (3·3)	6·5 (2·1)	4·1 (2·3)
Mean number of clinical findings over days 1-3 of illness per case (SD)[6]	6·7 (2·9)	6·2 (2·0)	3·6 (2·0)
Referred to pediatric hospital[7,8]	446 (31·7)	42 (8·1)	1 (0·2)

[1] The PDCS expanded its age range from 2-14 years to 2-17 years in August 2018, after the chikungunya and Zika epidemics subsided.

[2] DENV serotype information was not available for three of the dengue cases that were RT-PCR-positive.

[3] Chikungunya cases presented to the health center significantly earlier than dengue ($p<0{\cdot}001$) and Zika cases ($p<0{\cdot}001$). There was no evidence of a difference between dengue and Zika cases.

[4] Dengue cases had a significantly higher number of visits to the health center than chikungunya ($p<0{\cdot}001$) and Zika cases ($p<0{\cdot}001$). There was no evidence of a difference between chikungunya and Zika cases.

[5] Over the first ten days of illness, dengue cases had a significantly higher number of clinical findings than chikungunya ($p<0{\cdot}001$) and Zika cases ($p<0{\cdot}001$). Chikungunya cases had a significantly higher number of clinical findings than Zika cases ($p<0{\cdot}001$). Rash was considered collectively for this analysis; for example, if a child exhibited a rash that was both localized and macular, it was only counted once.

[6] Over the first three days of illness, Zika cases had a significantly fewer number of clinical findings than dengue ($p<0{\cdot}001$) and chikungunya cases ($p<0{\cdot}001$). There was no evidence of a difference between dengue and chikungunya cases. Rash was considered collectively for this analysis.

[7] Dengue cases were more often referred to the study hospital, Hospital Infantil Manuel de Jesús Rivera, a pediatric tertiary referral hospital, than chikungunya ($p<0{\cdot}001$) and Zika cases ($p<0{\cdot}001$). Chikungunya cases were more often referred to the study hospital than Zika cases ($p<0{\cdot}001$).

[8] A high percentage of dengue cases were referred for hospitalization because guidelines from the Nicaraguan Ministry of Health require a dengue case with any warning sign, per the 2009 WHO guidelines for dengue (5), to be referred for hospitalization.

Abbreviations: CHIKV, chikungunya virus; DENV, dengue virus; PDCS, Pediatric Dengue Cohort Study; RT-PCR, reverse transcription polymerase chain reaction; SD, Standard deviation; ZIKV, Zika virus

578 **Table 2.** Most distinguishing clinical characteristics of dengue, chikungunya, and Zika cases in the PDCS
579 in Managua, Nicaragua, by disease (January 2006 – December 2023).[1,2]

	Dengue	Chikungunya	Zika
The most distinguishing clinical features based on presence	Monocytopenia Abdominal pain Thrombocytopenia (days 5-7) Nausea Hemorrhagic manifestations (days 2-6) Vomiting Basophilia (>age 5) Leukopenia Sore throat (age 2-5) Retro-orbital pain	Arthralgia (days 1-4) Facial flushing Localized rash	Generalized rash (days 1-4) Erythematous rash (days 1-4) Maculopapular rash (days 1-4)
The most distinguishing clinical features based on absence	Rash (days 1-3 and >age 13)	Papular rash Maculopapular rash Conjunctival injection Basophilia Leukopenia	Fever (days 1-3) Lymphocytopenia (>age 4 and days 1-6) Headache (days 1-2) Myalgia (days 1-3) Appetite loss Pharyngeal erythema (age 2-10)

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581 [1] Data in the table summarize our results examining the prevalence of clinical features overall, by age,
582 and by days of illness, as well as the results of the classification models. Clinical features had to
583 consistently differ across multiple analyses to be placed in this summary table. The information in

parentheses indicates ages (in years) and days of illness where the presence or absence of the corresponding clinical feature is especially pronounced.

[2] While differences in the temporal dynamics of fever are not presented here, we note that average temperatures among Zika cases not on antipyretics were sub-febrile across days 1-10 of illness; average temperature for dengue, chikungunya, and Zika cases returned to normal on days six, five, and three, respectively; and fever tended to be more prevalent among dengue cases than chikungunya and Zika cases on days 4-5 of illness.

FIGURE LEGENDS

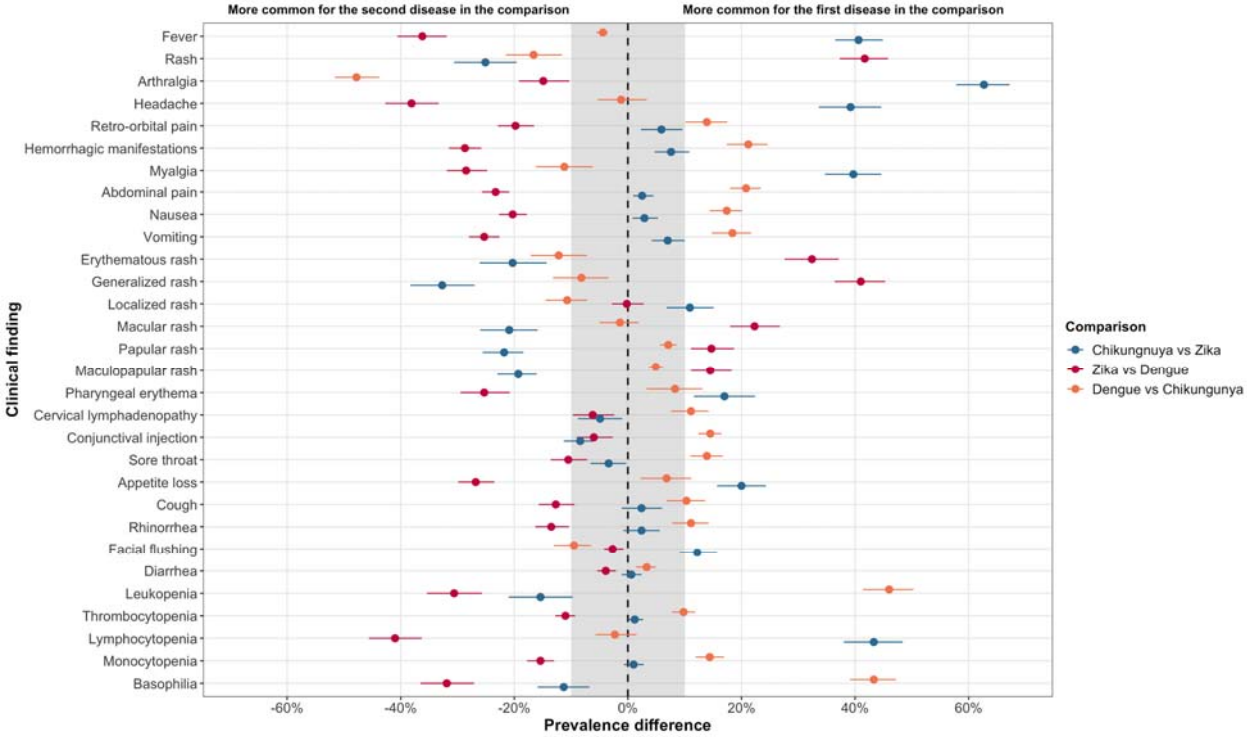
Figure 1. Prevalence differences for each clinical finding among all pairwise comparisons of dengue, chikungunya, and Zika PDCS cases in Managua, Nicaragua (2006-2023). Positive differences (those to the right of the dashed line) indicate that the clinical finding is more prevalent among the first case group in the comparison, and vice versa. For example, fever was observed among 100% of the chikungunya cases and 60% of the Zika cases. Therefore, the prevalence difference is +40%, as shown on the right side of the figure in blue. Dots indicate the point estimate of the average and the length of the bar indicates its 95% confidence interval. Intervals that include the null value of 0 are not statistically significant at the alpha=0·05 level; intervals that exclude the null correspond to statistically significant differences. The arbitrary range of -10% to +10% is shaded light gray to indicate small prevalence differences, regardless of statistical significance, that may not be clinically important. A clinical finding was considered present if a case reported experiencing it during the first 10 days of illness.

Figure 2. Age-prevalence trends for clinical features among the dengue, chikungunya, and Zika PDCS cases in Managua, Nicaragua (2006-2023). The solid lines indicate the point estimate of the average, and the shaded area indicates its 95% confidence band. Trends were estimated with logistic generalized additive models. A clinical finding was considered present if a case reported experiencing it during the first 10 days of illness. For this figure, we changed five chikungunya cases to not having fever to prevent a model convergence error, as a meaningful 95% confidence band could not be estimated in the presence of all chikungunya cases' exhibiting fever (as was reported).

Figure 3. The prevalence of clinical features by disease and day of illness in the PDCS in Managua, Nicaragua (2006-2023). Dots show the day-specific average and the vertical bars correspond to its exact 95% confidence interval. A clinical finding was considered present if a case reported experiencing it during a given day within the first ten days of illness. Exceedingly few chikungunya and Zika cases had blood samples taken on days 8-10 of illness. We therefore removed the unreliable point and interval estimates for leukopenia, thrombocytopenia, lymphocytopenia, monocytopenia, and basophilia for these days.

Figure 4. Machine learning model results for PDCS cases in Managua, Nicaragua (2006-2023). (A) Three separate models were constructed to classify, based on clinical findings from days 1-10 of illness, dengue from chikungunya and Zika (the dengue model), chikungunya from dengue and Zika (the chikungunya model), and Zika from dengue and chikungunya (the Zika model). The values shown indicate the percentage of true cases (y-axis) that were classified as a given disease by the disease-specific model (x-axis). For example, 72·5% of chikungunya cases were classified as chikungunya cases by the chikungunya model, and 2·8% of all dengue cases were classified as chikungunya cases by the chikungunya model. Percentages in panel A that are bold and underlined denote sensitivity (which an ideal classifier maximizes), and those in regular font represent 1-specificity (which an ideal classifier minimizes). Thus, the 72·5% represents the chikungunya model's sensitivity for chikungunya cases, and the 2·8% represents the chikungunya model's 1-specificity (equal to 97·2% specificity) for all dengue cases. Model results are reported for all three diseases as well as by fever status for dengue and Zika cases. **(B-D)** The five most important clinical findings that helped correctly classify cases are shown for the chikungunya (B), dengue (C), and Zika (D) models. Each dot represents the variable importance value of a given clinical finding across 100 model iterations. Variables further to the right are more important for classification purposes. If the presence of a clinical finding was important to the classification, it is shown in dark blue; if its absence was important, it is shown in light blue. **(E-G)** Age-specificity trends are shown for the chikungunya (E), dengue (F), and Zika (G) models. Jittered points indicate whether cases of each disease were classified corrected (assigned a value of 1) or incorrectly (assigned a value of 0) by their respective model. Trend lines and 95% confidence bands were estimated with logistic generalized additive models.

649 **Figure 1**



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654 **Figure 2**

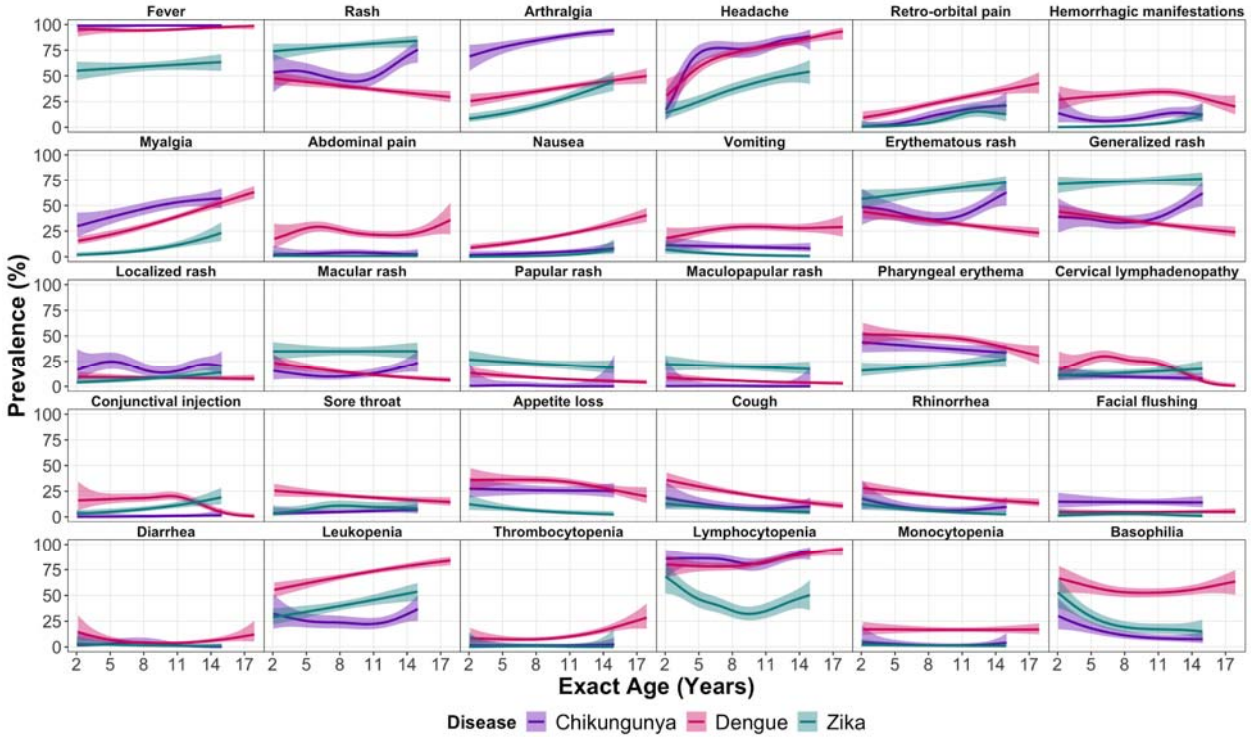

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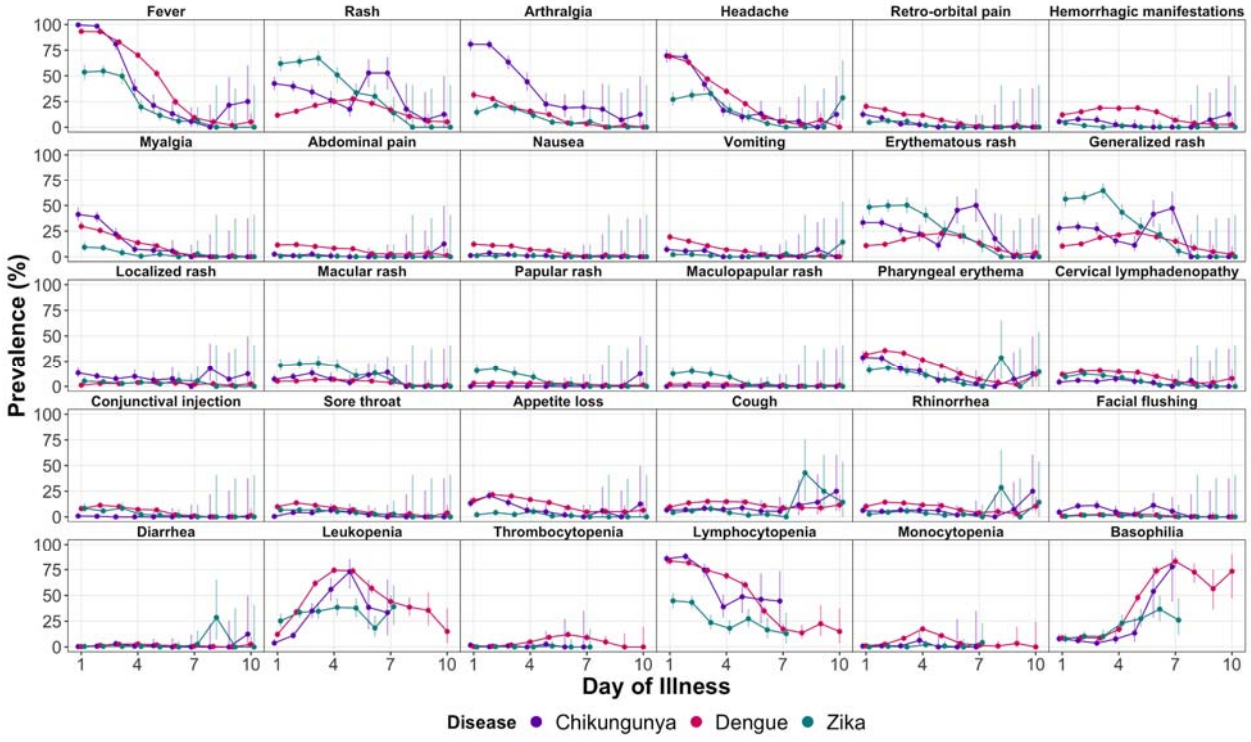
Figure 3



659 **Figure 4**
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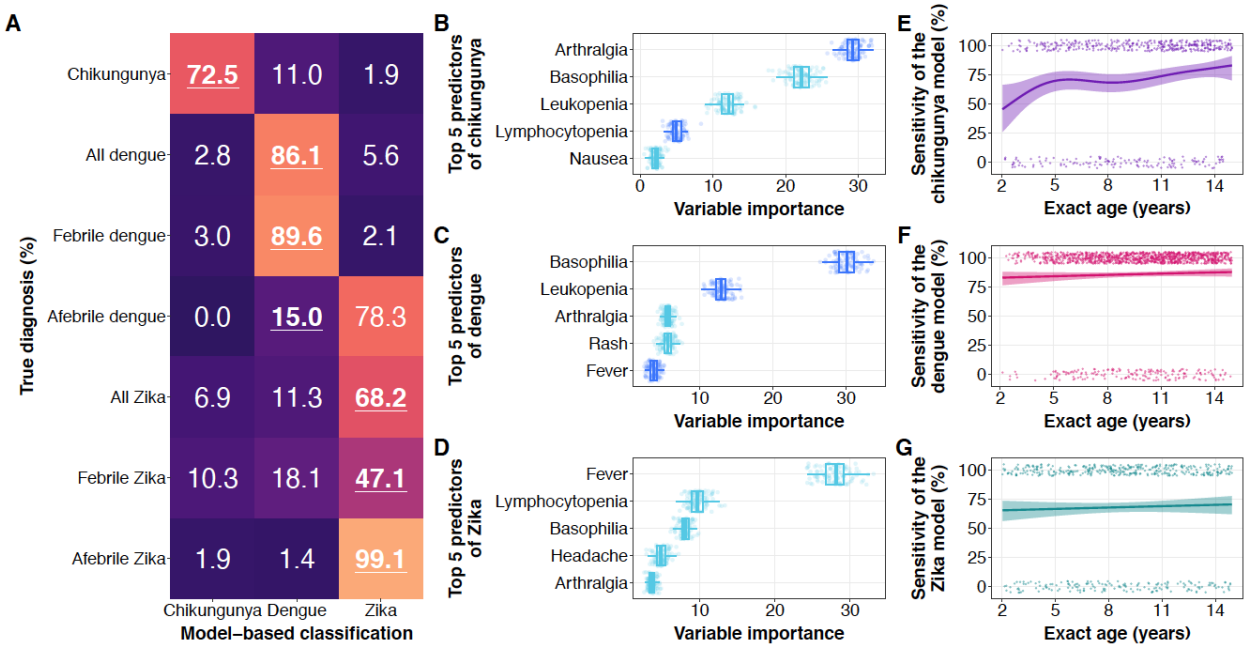

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