

Viremia and Clinical Presentation in Nicaraguan Patients Infected With Zika Virus, Chikungunya Virus, and Dengue Virus

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Background. Zika virus (ZIKV), chikungunya virus (CHIKV), and dengue virus (DENV) cocirculate in Nicaragua. In this study, we sought to compare the quantified viremia and clinical presentation of patients infected with 1 or more of these viruses.

Methods. Acute-phase serum samples from 346 patients with a suspected arboviral illness were tested using a multiplex real-time reverse-transcription polymerase chain reaction for ZIKV, CHIKV, and DENV. Viremia was quantitated for each detected virus, and clinical information from request forms submitted with each sample was recorded.

Results. A total of 263 patients tested positive for 1 or more viruses: 192 patients tested positive for a single virus (mono-infections) and 71 patients tested positive for 2 or all 3 viruses (coinfections). Quantifiable viremia was lower in ZIKV infections compared with CHIKV or DENV (mean 4.70 vs 6.42 and 5.84 log₁₀ copies/mL serum, respectively; $P < .001$ for both comparisons), and for each virus, mean viremia was significantly lower in coinfections than in mono-infections. Compared with patients with CHIKV or DENV, ZIKV patients were more likely to have a rash ($P < .001$) and less likely to be febrile ($P < .05$) or require hospitalization ($P < .001$). Among all patients, hospitalized cases had higher viremia than those who did not require hospitalization (7.1 vs 4.1 log₁₀ copies/mL serum, respectively; $P < .001$).

Conclusions. ZIKV, CHIKV, and DENV result in similar clinical presentations, and coinfections may be relatively common. Our findings illustrate the need for accurate, multiplex diagnostics for patient care and epidemiologic surveillance.

Keywords. dengue virus; chikungunya virus; Zika virus; coinfection; viremia.

Dengue has been endemic in Nicaragua since 1985, with all 4 dengue virus serotypes (DENV1–4) circulating, generally with 1 serotype dominant in each epidemic [1–4]. Chikungunya virus (CHIKV) was first detected in Nicaragua in July 2014, and the first autochthonous cases were confirmed in September 2014 [5]. Limited CHIKV transmission occurred in 2014–2015, followed by a larger epidemic in 2015–2016. The first autochthonous cases of Zika virus (ZIKV) in Nicaragua were reported in January 2016, and ZIKV and CHIKV now cocirculate with dengue virus (DENV) throughout the country [6]. This complicates the diagnosis of patients with an acute febrile illness, as the

spectra of clinical manifestations that result from infection with these viruses overlap significantly [7–10]. Diagnosis is further complicated by cross-reactions observed in ZIKV-positive patients tested using immunoglobulin M or non-structural protein 1 assays for DENV and vice versa and by limited data on the duration of anti-CHIKV immunoglobulin M positivity following acute infection [7, 8].

Molecular diagnostics can be used to detect and differentiate ZIKV, CHIKV, and DENV in the acute phase, and real-time reverse-transcription polymerase chain reaction (rRT-PCR) can provide quantitative data in addition to qualitative detection [8, 11–14]. Quantitation of viremia has been widely reported in the DENV literature, where higher viremia at presentation has been associated with secondary DENV infections and disease severity [15, 16]. Fewer data are available regarding the level of viremia at presentation for patients with ZIKV and CHIKV [8, 9, 17–20]. As such, it is unknown how the level of viremia may correlate with clinical manifestations or clinical outcomes in these infections and whether the level of viremia varies in coinfections compared with mono-infections.

Our group previously described a single-reaction, multiplex rRT-PCR for the detection and differentiation of ZIKV, CHIKV, and DENV (referred to as the ZCD assay) [6]. The

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ZCD assay demonstrated similar sensitivity to a pan-DENV-CHIKV rRT-PCR and higher sensitivity than a published ZIKV rRT-PCR when evaluated using samples collected from Nicaraguan patients with suspected arboviral infections. The ZCD assay can also be performed as a quantitative test, though accurate quantitation of DENV viremia requires identification of the serotype [6, 21, 22].

Here, the ZCD assay and a companion serotype-specific DENV multiplex assay were used to study the level of viremia in patients infected with ZIKV, CHIKV, and/or DENV [21, 22]. This provides an extensive evaluation of the quantified viremia detected in patients presenting with ZIKV and CHIKV and how this compares to viremia in patients with DENV infections.

METHODS

Clinical Samples

Serum samples, collected at Ministry of Health facilities as part of routine care, were sent to the Centro Nacional de Diagnóstico y Referencia (CNDR) in Managua, Nicaragua, for reference ZIKV, CHIKV, and/or DENV molecular testing. Samples were obtained at the discretion of care providers from patients with suspected ZIKV, CHIKV, and/or DENV infections. Deidentified, acute-phase (collected within 7 days of symptom onset) serum samples collected between 1 September 2015 and 3 April 2016 were tested for this study. Serum was separated from whole blood at the collection site and then stored at -20°C and shipped to CNDR on ice. Serum was stored at CNDR at -20°C until thawed for nucleic acid extraction.

Clinical information was obtained from the epidemiologic records that are submitted along with each sample. These documents contain the following information: age, gender, pregnancy status, dates of symptom onset and sample collection, temperature, symptoms, clinical diagnosis, and date of hospitalization. Submission of an epidemiologic form is required for specimen processing, but completion of all data fields is voluntary. The Nicaraguan Ministry of Health and the Stanford University Institutional Review Board reviewed and approved the research protocol for this study.

Nucleic Acid Extraction and rRT-PCR Performance

RNA was extracted from 140 μL of serum using the QIAamp Viral RNA Mini kit (Qiagen) with a 60- μL elution volume. RNA extracts were stored at -80°C . Samples were tested for ZIKV, CHIKV, and/or DENV using the ZCD assay as previously described [6]. Viremia for ZIKV- and CHIKV-positive samples was quantitated in the ZCD assay, and DENV-positive samples were serotyped and viremia was quantitated using a serotype-specific DENV multiplex assay [21, 22]. For quantitation, the ZCD and DENV multiplex assays were performed with 4-point standard curves (8.0, 6.0, 4.0, and 2.0 \log_{10} copies/ μL of eluate). Standard curves were prepared using quantitated ssDNA (Integrated DNA Technologies) containing the

target sequences for ZIKV, CHIKV, and the identified DENV serotypes. For quantitation, clinical samples and the standard curve were tested in duplicate on a single run. The mean cycle threshold (C_t) was used for all calculations. The concentration of RNA in the eluate (expressed as \log_{10} copies/ μL of eluate) was calculated from the linear regression equation for the standard curve. Viremia in \log_{10} copies/mL of serum was then calculated from this value, accounting for volumes used in extraction. Data regarding the precision of ZIKV and CHIKV detection, linear range of the ZCD assay, and performance of external controls are provided in the [Supplementary Data](#).

Mixing studies were performed to evaluate possible interference between channels in the ZCD assay. Quantitated ssDNA standards for ZIKV, CHIKV, and DENV-2 were diluted in nuclease-free water at concentrations equivalent to 8.0, 6.0, and/or 4.0 \log_{10} copies/mL serum; standards were mixed in different combinations to create simulated coinfections, including triple infections ([Supplementary Table 3](#)).

Definitions

The term *viremia* is used generally to describe the presence of detectable viral RNA in serum using the ZCD assay. *Quantifiable viremia* is used to denote viremia that is within the linear range of the ZCD assay. The linear range for each target in the ZCD assay extends from 8.0–1.0 \log_{10} copies/ μL of eluate ([Supplementary Figure 1](#)). For serum samples tested in this study, this linear range corresponds to viremias of 10.6–3.6 \log_{10} copies/mL. *Low-positive viremia* describes viremia that is detected but falls below the lower limit of quantitation for the ZCD assay (3.6 \log_{10} copies/mL of serum) [6].

Statistics

Basic statistics were calculated using Excel software (Microsoft). Categorical variables were compared using Fisher exact tests, and continuous clinical variables were compared using *t* tests. Kruskal–Wallis tests were performed to compare viremia distributions that included low-positive viremia (below the limit of quantitation in the ZCD assay), and Welch *t* tests were used to compare quantifiable viremia. Fisher exact tests, *t* tests, and Kruskal–Wallis tests were performed with GraphPad software (GraphPad, San Diego, California). Pearson and Spearman correlation coefficients were calculated at socscistatistics.com. For the multivariable analysis of factors associated with hospitalization, R software was used to evaluate generalized linear models that included the following variables: viral etiology (CHIKV and/or DENV infection vs ZIKV infection), age, gender, viremia, temperature, and day post-onset of symptoms.

RESULTS

A total of 346 samples were tested using the ZCD assay. Among patients with information regarding day of symptom onset, 303/306 (99.0%) presented on days 1–6, where day 1 was defined as the day on which symptoms began. A total of 263 patients

Table 1. ZCD Assay Results for 346 Patients With Suspected Zika Virus, Chikungunya Virus, and/or Dengue Virus Infections

ZCD Assay Result	Number, n (% of all Samples)
Positive	263 (76.0)
<i>Monoinfections</i>	192 (55.5)
ZIKV	47 (13.6)
CHIKV	91 (26.3)
DENV ^a	54 (15.6)
<i>Coinfections</i>	71 (20.5)
ZIKV-CHIKV	16 (4.6)
ZIKV-DENV ^a	6 (1.7)
CHIKV-DENV ^a	43 (12.4)
ZIKV-CHIKV-DENV ^a	6 (1.7)
Negative	83 (24.0)

Abbreviations: CHIKV, chikungunya virus; DENV, dengue virus; ZCD, multiplex real-time reverse-transcription polymerase chain reaction for the detection and differentiation of ZIKV, CHIKV, and DENV; ZIKV, Zika virus.

^a Serotypes of 109 DENV-positive samples: DENV-2, 107; DENV-1, 1; DENV-4, 1.

(76.0%) tested positive for 1 or more viruses (Table 1), and the mean day post-onset of symptoms at presentation was not significantly different for patients with positive samples and those with negative samples (Supplementary Table 4). RNA from a single virus (monoinfection) was detected in 192 patients (55.5% of all patients tested), and RNA from more than 1 virus (coinfections) was detected in 71 patients (20.5%; Table 1).

Clinical Presentation

Clinical data were available for 163 positive (62.0% of all positives) and 41 negative patients (49.4% of all negatives). Patients with positive ZCD assay results were clinically similar to patients with negative results based on the variables analyzed (Supplementary Table 4). A comparison of clinical data for patients with ZIKV, CHIKV, and DENV is shown in Table 2. Patients with ZIKV were significantly older than patients with either CHIKV or DENV (aged 36.0 vs 20.4 years; $P < .001$).

Table 2. Comparison of the Clinical Presentation for all Patients With Zika Virus, Chikungunya Virus, and Dengue Virus Infections

Patient Data	Zika Virus	Chikungunya Virus	Dengue Virus	P Value ^a
Number, n	37	103	66	...
Gender, % female, % male	72.2, 27.8	60.8, 39.2	63.6, 36.4	NS
Age in years, mean (SD)	36.0 (16.0)	20.4 (15.8)	20.4 (15.2)	<.001, <.001
Day post symptom onset, mean (SD)	3.4 (1.1)	3.2 (1.3)	3.5 (1.3)	NS
Pregnant, n	6	8	6	...
<i>Symptoms, positive/total (%)^b</i>				
Rash	32/35 (91.4)	49/87 (56.3)	25/50 (50.0)	<.001, <.001
Conjunctivitis	18/22 (81.8)	13/17 (76.5)	8/11 (72.7)	NS
History of fever	28/35 (80.0)	81/90 (90.0)	45/50 (90.0)	NS
Headache	27/34 (79.4)	54/85 (63.5)	34/49 (69.4)	NS
Arthralgia	21/30 (70.0)	59/85 (69.4)	32/48 (66.7)	NS
Myalgia	20/31 (64.5)	44/83 (53.0)	28/48 (58.3)	NS
Retro-orbital pain	13/27 (48.1)	25/66 (37.9)	18/42 (42.9)	NS
Nausea	7/26 (26.9)	24/58 (41.4)	17/39 (43.6)	NS
Abdominal pain ^c	2/17 (11.8)	10/57 (17.5)	16/41 (38.1)	NS
Hemorrhage	1/17 (5.9)	2/56 (3.6)	4/42 (9.5)	NS
Vomiting	0/17 (0.0)	6/58 (10.3)	6/42 (14.3)	NS
<i>Clinical Data</i>				
Temperature, mean (SD)	36.9 (0.6)	37.4 (0.9)	37.3 (0.8)	.005, .018
Febrile ^d , number/total (%) ^b	2/27 (7.4)	29/86 (33.7)	16/56 (28.6)	.007, .044
Clinical diagnosis, n	35	97	63	...
Chikungunya	1	29	7	...
Dengue	0	41	40	...
Zika	27	15	14	...
Multiple viruses Listed ^e	7	11	2	...
Hospitalized, positive/total (%) ^b	7/23 (30.4)	55/70 (78.6)	43/55 (78.2)	<.001, <.001

Abbreviations: NS, not significant; SD, standard deviation.

^a P values are shown for comparisons of Zika virus (ZIKV) and chikungunya virus (CHIKV; first value) and ZIKV and dengue virus (DENV; second value). If results of both comparisons were not significant, only NS is shown.

^b Reported as the number of positives over the total number with recorded information for each variable.

^c $P = .022$ for comparison of CHIKV and DENV.

^d Defined as a temperature $\geq 38^{\circ}\text{C}$.

^e One CHIKV-positive patient had suspected leptospirosis.

Rash, conjunctivitis, fever, and headache were the most common symptoms in patients with ZIKV, but only rash was significantly more common among ZIKV-positive patients (32/35, 91.4%) than among CHIKV-positive (49/87, 56.3) or DENV-positive patients (25/50, 50.0%; $P < .001$ for both comparisons). Although a similar proportion of patients with each virus had a history of fever, patients with ZIKV were significantly less likely to be febrile ($\geq 38^{\circ}\text{C}$) at the time of presentation.

A suspected clinical diagnosis was recorded for 153/163 (93.9%) positive samples (Table 2), and sensitivity of the clinical diagnosis varied by the virus identified. ZIKV infection was correctly diagnosed in a higher percentage of cases (32/35, 91.4%) than DENV infection (42/63, 66.7%; $P = .007$), and both ZIKV and DENV infections were correctly diagnosed in a higher percentage of cases than CHIKV (40/97, 41.2%; $P < .001$ and $P = .002$, respectively).

Quantitation of Viremia

The distribution of viremia detected in ZIKV, CHIKV, and DENV mono-infections and coinfections is shown in Figure 1, and a comparison of quantifiable viremia in mono-infections and coinfections is shown in Table 3. Mean ZIKV viremia was significantly lower than mean viremia for either CHIKV or DENV ($P < .001$ for both comparisons). Six pregnant women tested positive for ZIKV, and mean ZIKV viremia in

those women (5.05 \log_{10} copies/mL serum; standard deviation [SD], 0.91) was higher than viremia detected in patients who were not pregnant (3.73 \log_{10} copies/mL serum; SD, 1.02; $P = .006$). The highest viremias identified were in CHIKV-positive samples. In particular, neonates diagnosed with CHIKV in the first month of life had the highest levels of CHIKV detected (6/6 patients with $>11.0 \log_{10}$ copies/mL serum). Despite these findings, the mean quantifiable viremia for CHIKV and DENV were not significantly different (Table 3, $P = .1$).

The distribution of DENV viremia differed significantly from distributions for ZIKV and CHIKV ($P \leq .001$ for both comparisons, Kruskal–Wallis). Of all DENV-positive samples, only 15 (13.8%) were low positives. This was significantly lower than the proportion of ZIKV low-positive (36/75, 48.0%) or CHIKV low-positive samples (89/156, 57.1%; $P < .001$ for both comparisons).

The numbers of CHIKV samples with either quantifiable viremia or low-positive viremia were sufficient to investigate these categories further. The percentage of samples with quantifiable and low-positive CHIKV viremia was similar for mono-infections and coinfections (Table 3). Detection of quantifiable CHIKV viremia decreased from a high of 58.3% (7/12) of samples collected on the first day of symptoms to 19.0% (4/21) of samples collected on days 5 and 6 post-symptom onset (Supplementary Figure 2; Spearman, $R = -0.943$; $P = .005$). The

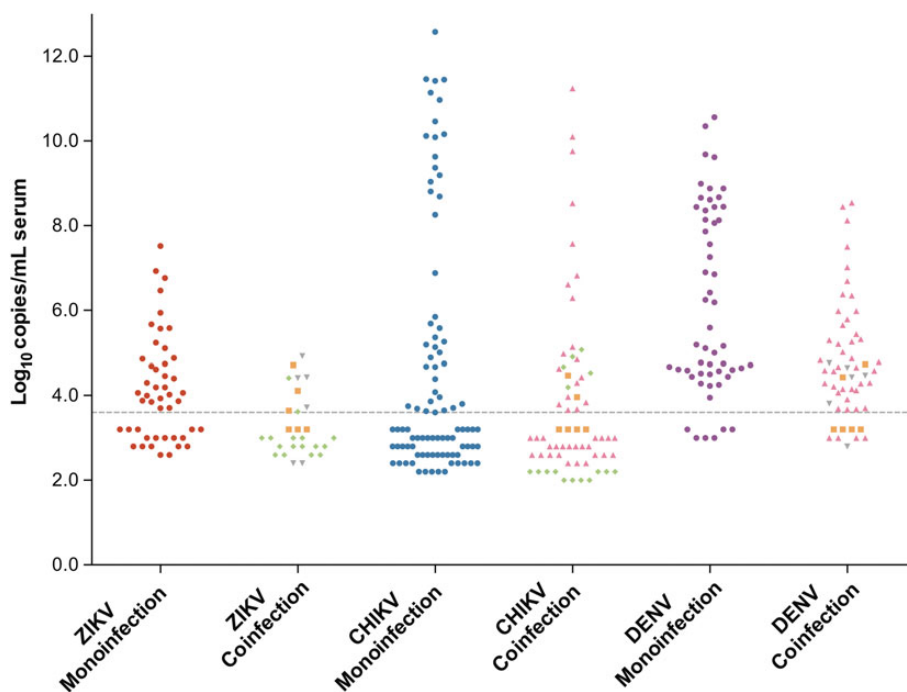


Figure 1. Levels of viremia in Zika virus (ZIKV), chikungunya virus (CHIKV), and dengue virus (DENV) mono-infections and coinfections. Mono-infections are represented by filled circles (●): ZIKV, red; CHIKV, blue; and DENV, purple. Coinfections are represented by the following: ZIKV-CHIKV (◆), ZIKV-DENV (▼), CHIKV-DENV (▲), and ZIKV-CHIKV-DENV (■). Viremia for each virus detected in a coinfection is displayed. The limit of quantitation for the multiplex real-time reverse-transcription polymerase chain reaction for the detection and differentiation of ZIKV, CHIKV, and DENV assay is displayed as a dashed gray line (3.6 \log_{10} copies/mL serum). Samples with viral RNA that was detectable but below the limit of quantitation (low positives) are shown below this line; marker positions for these samples do not represent estimated viremia.

Table 3. Quantifiable Viremia for Mono-infections and Coinfections With Zika Virus, Chikungunya Virus, and Dengue Virus

ZCD Assay Result	Number of Samples, n (%) ^a	Quantifiable Viremia, n (%) ^b	Viremia, mean (standard deviation)	P Value
Zika virus	75	39 (52.0)	4.70 (0.97)	...
Mono-infections	47 (62.7)	30 (63.8)	4.84 (1.04)	.018
Coinfections	28 (37.3)	9 (32.1)	4.22 (0.48)	
Chikungunya virus	156	67 (42.9)	6.42 (2.72)	...
Mono-infections	91 (58.3)	41 (45.1)	6.92 (2.94)	.040
Coinfections	65 (41.7)	26 (40.0)	5.62 (2.15)	
Dengue virus	109	94 (86.2)	5.84 (1.82)	...
Mono-infections	54 (49.5)	48 (88.9)	6.53 (2.01)	<.001
Co-infections	55 (50.5)	46 (83.6)	5.11 (1.25)	

Abbreviation: ZCD, multiplex real-time reverse-transcription polymerase chain reaction for the detection and differentiation of ZIKV, CHIKV, and DENV.

^a % of samples positive for a given virus with mono- or coinfections.

^b % of samples in each category with quantifiable viremia.

documented clinical presentations of patients with quantifiable or low-positive CHIKV viremia were similar. However, among patients with low-positive viremia, chikungunya was the clinical diagnosis significantly less often and Zika was the clinical diagnosis significantly more often than among patients with quantifiable viremia (Supplementary Table 5).

Coinfections in the ZCD Assay

For all 3 viruses, mean quantifiable viremia in mono-infections was significantly higher than viremia for the same virus detected in coinfections (Table 3). There was no evidence of interference in mixing studies using samples with viremias similar to those observed in these clinical samples. Specifically, there was no interference observed among simulated triple infections with viruses mixed at equal concentrations of 8.0, 6.0, or 4.0 log₁₀ copies/mL of serum (Supplementary Table 3). The clinical presentations of patients with coinfections and mono-infections were similar (data not shown), though there was a trend toward more frequent hospitalization in patients with coinfections (25/30, 83.3%) compared with those with mono-infections (55/86, 64.0%; *P* = .066).

Hospitalization

A total of 116 patients with positive ZCD test results had hospitalization status available: 80 hospitalized and 36 not hospitalized. Factors associated with hospitalization in univariate analysis are shown in Table 4. The best-fit multivariable model included temperature, viremia, and ZIKV mono-infection. Viremia and temperature were higher among hospitalized cases. Although there was a weak, positive correlation between viremia and recorded temperature (Pearson *R* = 0.207, *P* = .026), both variables remained significant in the final model. Patients with ZIKV mono-infections were significantly less likely to be hospitalized than patients with CHIKV and/or DENV infections, regardless of whether the latter group of patients had mono- or coinfections. Results from the multivariable analysis were similar when all patients without a documented hospitalization status were considered to be “not hospitalized.”

DISCUSSION

Here, we present a comparative analysis of the level of viremia and clinical manifestations that resulted from infections with ZIKV, CHIKV, and/or DENV among patients in a single endemic country. The clinical presentations caused by these viruses were similar, and only the presence of a rash and a documented fever differed significantly between patients with ZIKV and both patients with CHIKV and patients with DENV. As a result, clinical suspicion was only correct in 41.2% of CHIKV infections and 66.7% of DENV infections. The apparent sensitivity of a clinical diagnosis of Zika (91.4%) may have resulted from heightened awareness of and concern for Zika during the study period, as 38/70 patients (54.3%) with suspected Zika tested negative for ZIKV RNA. However, a portion of these cases may have been detected by serology, which was unavailable for this study. Taken together, our findings highlight the difficulty of providing an accurate clinical diagnosis in a region where patients may be infected with any one of these pathogens and support the use of a testing protocol for ZIKV, CHIKV, and DENV in all suspected cases. This would be expected to improve case detection, inform

Table 4. Univariate and Multivariable Analysis of Factors Associated With Hospitalization Among Patients With Zika Virus, Chikungunya Virus, and/or Dengue Virus Infections

Patient Variables	Hospitalized	Not Hospitalized	Univariate, <i>P</i> Value	Multivariable Analysis ^a		
				Log Odds (Ln)	Standard Error	<i>P</i> Value
Number, n	80	36				
Female gender, n (%)	42 (52.5)	26 (74.3)	.039
Age in years, mean (SD)	18.2 (15.5)	28.2 (14.4)	.002
Day post symptom onset, mean (SD)	3.1 (1.5)	3.6 (1.2)	.079
Viremia, log ₁₀ copies/mL serum, mean (SD)	7.1 (2.7)	4.1 (1.5)	<.001	0.339	0.151	.025
Temperature, mean (SD)	37.5 (0.9)	36.8 (0.6)	<.001	0.978	0.391	.012
Zika virus mono-infection, n (%)	4 (0.5)	12 (33.3)	<.001	-1.536	0.714	.032

Abbreviation: SD, standard deviation.

^a Variables indicated by an ellipse (. . .) were not included in the best-fit multivariable model.

management decisions, and allow for appropriate patient referral and follow-up.

Patients infected with ZIKV in the current study had lower quantifiable viremia, on average, than patients with CHIKV and/or DENV. Additionally, the maximum viremia detected among ZIKV-positive patients was 1000 to 100 000-fold lower than the maximum viremia detected for DENV and CHIKV, respectively. Studies to date have focused on the qualitative detection of ZIKV in serum or plasma, and fewer data have been published regarding the level of viremia in ZIKV-positive cases [8–10, 23]. In a study by Lanciotti et al, the mean estimated ZIKV viremia detected in 17 patients on Yap Island (4.4 log₁₀ copies/mL of serum, SD 0.94) was very similar to the mean quantifiable viremia observed in our patients (4.7 log₁₀ copies/mL of serum, SD 0.97) [8]. Such low-level viremia at presentation provides a likely explanation for the short window of ZIKV RNA detection in serum or plasma that has been reported [8, 9, 23, 24]. However, it remains possible, though unlikely, that peak viremia in ZIKV infections is similar to peaks observed in CHIKV and/or DENV infections but occurs earlier relative to symptom onset.

Patients with low-positive CHIKV viremia have been identified in other series, but the clinical presentation of and outcomes for such cases have not been evaluated [17–20, 25]. In our study, 57.1% of CHIKV-positive patients had viremia that was below the quantifiable range in the ZCD assay. The documented clinical presentation of patients with quantifiable and low-positive viremia was similar (Supplementary Table 5). Patients with low-positive viremia were more likely to present later in the course of illness, which is consistent with declining viremia in acute CHIKV infections over the first week of illness [17–19]. It is important to note that the lower limit of quantitation in the ZCD assay is dependent on characteristics of the test and the volumes of serum and elution buffer used during RNA extraction. This value bears no a priori biological significance.

Zika was suspected in a higher proportion of patients with low-positive CHIKV viremia (and chikungunya was suspected less often) compared with patients with quantifiable viremia. This may indicate that patients with low-positive CHIKV viremia had a milder clinical presentation despite similar reported symptoms. In addition, this finding is consistent with the associations between higher viremia (among all patients), fever at presentation, and hospitalization. These data, when considered along with a documented association between viremia and disease severity in the dengue literature [15, 16, 26, 27], suggest a more general correlation between viremia at the onset of clinical symptoms and illness severity in acute arboviral infections.

Despite transmission of ZIKV, CHIKV, and DENV in many regions, reports of coinfections between these viruses have been rare, particularly in comparison to the 20.5% of patients who had detectable RNA from 2 or all 3 viruses in our study. This figure is consistent with our earlier findings, which included a subset of patients from this analysis, as well as those from

Guayaquil, Ecuador, where the ZCD assay has also been implemented [6, 28]. The apparent difference in rates of coinfection may then result from a number of factors, including a reliance on serological testing in many endemic areas; the performance of individual tests for each virus, which increases test cost and may decrease utilization; and a lack of signs or symptoms that clinically distinguish co-infections from mono-infections. This latter point was illustrated in findings from our patients and has also been observed in the few cases described in the literature [29, 30].

Given the nature of national surveillance sample collection in Nicaragua, only acute-phase specimens were available for testing. Therefore, serological testing on paired acute and convalescent serum samples could not be performed. An additional limitation to this study is the reliance on voluntarily completed epidemiologic forms for clinical information. Given that many forms were not fully completed, we focused the statistical analysis on clinical variables for which data were available for ≥50% of patients in at least 1 category (positive or negative ZCD result, viral etiology, low-positive or quantifiable CHIKV viremia). Finally, in simulated triple infections, high concentrations of CHIKV and DENV (8.0 log₁₀ copies/mL of 1 virus plus 8.0 or 6.0 log₁₀ copies/mL of the second) interfered with the detection of ZIKV at 4.0 log₁₀ copies/mL (Supplementary Table 3). Although viremia in detected triple infections was far lower than these simulated infections, we cannot rule out that low-level ZIKV viremia was missed in CHIKV-DENV coinfections with high viremia.

In conclusion, the co-circulation of ZIKV, CHIKV, and DENV presents a number of challenges for clinical care and laboratory diagnosis in endemic areas. Patients infected with 1 or more of these viruses can present with similar clinical manifestations over a wide range of viremia. In addition, coinfections may be quite common, requiring that all patients be tested for each virus. This demonstrates the need for sensitive, accurate, multiplex diagnostics for clinical care, disease research, and epidemiologic surveillance of these arboviral diseases.

Supplementary Data

Supplementary materials are available at <http://cid.oxfordjournals.org>. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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