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Amylee Anyoha _____ April 27th, 2020

Rapid Diagnostic Testing Identified Possible Zika Virus Cases in 6 Nigerian States Following Yellow Fever Outbreak Responses between 2015 and 2019

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Abstract

Rapid Diagnostic Testing Identified Possible Zika Virus Cases in 6 Nigerian States Following Yellow Fever Outbreak Responses between 2015 and 2019

In October of 2016, Nigeria Centre for Disease Control (NCDC) released a public health risk assessment of Zika virus in Nigeria and interim recommendations. The recommendations stress the need for surveillance to understand and monitor the epidemiology of Zika virus in the Nigerian population with the goal of developing appropriate interventions. This study estimated the prevalence of Zika virus in North Central Nigeria using Rapid Diagnostic Tests (RDTs) on stored serum samples that had tested negative for Yellow fever between 2015 and 2019. A convenience sample of 385 serum samples were tested for IgG and IgM antibodies to Zika virus. In the final sample of 280, 27 tested positive for IgG, IgM or both type antibodies (9.64%), which were specific to Zika. Higher mean annual temperature, population density and mean annual rainfall were significantly related to a higher number of observed cases. It is possible that areas experiencing Yellow fever outbreaks are likely to see cases of Zika virus due to the shared *Aedes aegypti* mosquito vector. In our study, all samples were tested because they showed typical symptoms of flavivirus type diseases, of which both Zika and Yellow fever are members of. In accordance with 80% of cases expected to be asymptomatic, as stated by WHO, it is probable that the prevalence of Zika virus in Nigeria is underestimated in our data. Persons with asymptomatic infections are less likely to observe precautions of passing the virus to sexual partners, increasing the number of babies unexpectedly born with microcephaly. In the future, outbreak response for flavivirus type diseases in Nigeria should include testing for Zika virus.

Keywords: Zika virus, flavivirus, rapid diagnostic testing

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List of Abbreviations

CDC	U.S. Centers for Disease Control and Prevention
CITI	Collaborative Institutional Training Initiative
DENV	Dengue virus
ELISA	Enzyme-linked Immunosorbent Assay
KAP	Knowledge, Attitudes and Practices
NCDC	Nigeria Centre for Disease Control
NHREC	National Health Research Ethics Committee
PCR	Polymerase Chain Reaction
RDTs	Rapid Diagnostic Tests
RNA	Ribonucleic Acid
SOP	Standing Operating Practice
VHF	Viral Hemorrhagic Fever
WHO	World Health Organization
ZIKV	Zika virus
YF	Yellow fever

Rapid Diagnostic Testing Identified Possible Zika Virus Cases in 6 Nigerian States Following Yellow Fever Outbreak Responses between 2015 and 2019

Zika virus (ZIKV) is a mosquito-borne Flavivirus first identified in a rhesus monkey in Uganda in 1947[1]. The first human case of ZIKV in Nigeria was confirmed in 1954[2]. By 2016, 72 countries and territories reported evidence of mosquito-borne ZIKV since 2007[2]. Zika virus is transmitted by various species of Aedes mosquitoes, including the *Steogmyia albopictus* and *Steogmyia aegypti* types. Mosquito vectors typically breed in domestic water-holding containers and are primarily daytime biters, feeding both indoors and outdoors[3]. Besides vector-based transmission, human activity has also been found to increase the spread of ZIKV beyond the normal bounds of vector transmission. Zika virus can be sexually transmitted from an infected person even if they are asymptomatic. There is also possible perinatal, in utero and blood transfusion transmission of ZIKV[4].

Based on a household survey conducted in 2007 in Micronesia and a systematic review produced in 2018, WHO estimates that approximately 80% of the population infected with ZIKV is asymptomatic[4,5]. The systematic review conducted by WHO, including all ages from any country, revealed a range of 25% to 82% in the prevalence of asymptomatic Zika virus infection[5]. The final review included 23 studies and 28 articles- 3 of which were of cross-sectional design in a general population and thus, more appropriate for estimating the prevalence of Zika virus. The majority of the studies included were case series from population health surveillance programs and systematic or hospital-based screenings of an at-risk population. The cross-sectional studies were conducted in Micronesia in 2007, French Polynesia from 2014-2015, and Puerto Rico in 2016. Across the 23 studies, 11,305 participants were found to be

positive for Zika virus, 6,921 of whom were asymptomatic. Using the 23 studies, the prevalence of asymptomatic ZIKV found in their meta-analysis was estimated at 61.8% (95% CI: 33.0–87.1%), however the authors mentioned significant heterogeneity ($Q = 3291$, $P < 0.001$, >I2 = 99%).

Characteristic clinical presentation of ZIKV is similar to that of Lassa fever, Yellow fever, Dengue fever, and Chikungunya, and include acute onset fever, headache, maculopapular rash, arthralgia, and conjunctivitis. Illness is usually mild, lasting from several days to a week, therefore the larger concern is the reproductive implications of ZIKV infection. ZIKV infection, including asymptomatic infections during pregnancy, is a cause of microcephaly, a birth defect in which a baby's head is smaller than expected when compared to babies of the same age and sex. Congenital Zika syndrome is a combination of birth defects associated with 5 features: severe microcephaly, decreased brain tissue, damage to the back of the eye, joints with limited range of motion, and an excess of muscle tone resulting in restricted body movement. Not every woman infected with ZIKV will deliver a baby with birth defects, however, ZIKV infection does increase health risks both during delivery and development[6].

For the reasons described above, the clinical diagnosis of Zika virus is challenging. Laboratory testing can involve identifying the presence of specific IgM antibodies using ELISA and the detection of viral hemorrhagic fever antigens in tissues by immunochemistry and virus isolation using PCR. Research by the Centers for Disease Control and Prevention (CDC) that compares ZIKV to other similar infections, suggests that once a person has been infected with ZIKV he or she would be protected from future infection. Moreover, evidence supports that among non-pregnant women, once ZIKV has cleared from the blood, future pregnancies would not be jeopardized.

Nigeria Centre for Disease Control and the World Health Organization currently recommend two major actions against ZIKV infection: 1) preventing mosquito bites through the use of insect repellants, wearing of light-colored clothing that covers the body, using and sleeping under physical barriers such as mesh screens or insecticide-treated nets especially during the day, and 2) Practicing of safe sex or abstinence. WHO recommends that sexually active adults be counseled on all available contraceptive methods. Other guidelines for personal prevention include postponing non-critical travel to areas with ongoing ZIKV transmission as well as possibly delaying pregnancy[7].

The CDC suggests that men and women who have traveled to areas at risk for ZIKV wait 3 and 2 months respectively after symptom onset or after travel before attempting to conceive. The wait time is longer for men because Zika virus particles have been observed to remain in semen longer than any other bodily fluid, though an exact estimate of the persistent period is unknown[20,21]. Calvet et. al report that all cases of sexual transmission of ZIKA have implicated a symptomatic male index case. We speculate that some Nigerian adults are not engaging in reproductive planning to account for the risk of ZIKV; therefore, there is a possibility that future generations will be heavily affected by microcephaly. A 2018 study conducted in a tertiary hospital in Edo state, Nigeria found that among 191 females aged 15-49, only 69.0% (n=132) were using any form of family planning. The use of condoms was identified as the most frequently used contraceptive method (60%)[24].

To our knowledge, there are no studies conducted in Nigeria that estimate the prevalence of Zika virus explicitly within a population known to be exposed to possible Yellow fever infection. However, there have been several clinic-based studies of Zika infection prevalence in Nigeria among asymptomatic people. In 2016, a cross-sectional study conducted across 6 health

care facilities in Northern Nigeria estimated the rate of ZIKV diagnosed via IgM and IgG antibodies at 6% and 4% respectively (n=468)[13]. Participants with and without fever symptoms were included in this study, 24.3% being symptomatic with fever. Among pregnant women only, who made up about 60% of the sample, this rate was found to be 4% and 3% for IgM and IgG antibodies respectively. This is less than the prevalence reported by a 2019 study that found 10% were seropositive for ZIKV IgM, 12% for ZIKV IgG and 20% for ZIKV IgM, IgG or both among patients with fever presenting to 9 randomly selected secondary health care facilities in southeastern Nigeria (n= 100)[14]. Another study examined samples collected between 2004 and 2016 in a cohort of 188 HIV positive adults over the age of 15 presenting to a teaching hospital in Jos, Nigeria. They found the ZIKV seroprevalence to be (6.4%)[12].

WHO Zika virus prevention guidelines to avoid mosquitos bites, and to practice safe sex are not expected to be successful in Nigeria, where mosquitos are common and use of repellants is not adequate. Notably, ZIKV shares the same vector as Dengue fever, Yellow fever, and Chikungunya, all *Aedes aegypti* mosquito-transmitted infections[8]. A 2013 study conducted by Idris et. al in northern Nigeria found that antibody prevalence of DENV- was 38%, while antibody prevalence of DENV-2 and DENV-3 was 45% and 10% respectively. There has also been documentation of DENV-3 infections in northern Nigeria. Nigeria Centre for Disease Control (NCDC) also sites an increase in the prevalence of antibodies against DENV with age, suggesting endemicity[9-11].

We find that the guidelines above lack region-specific advice that is pertinent to Nigeria given the heavy burden of mosquito-transmitted infections. We hypothesize that many people in the general public are not aware of the current risk or the guidelines that NCDC and WHO have issued. Moreover, it is plausible that the current coverage and use of insecticide-treated bed nets

are inadequate for the prevention of ZIKV because other mosquito-transmitted diseases have remained endemic in Nigeria.

The goal of this study is to estimate the prevalence of Zika virus in Nigeria using RDTs in a population with known exposure to *Steogmyia aegypti*. The serum samples used for this study were originally collected during Yellow fever outbreak responses to North Central Nigeria and tested for Yellow fever antibodies. In this study, these samples underwent further testing to determine the presence of IgG/IgM antibodies for Zika virus. Because of the shared vector between Yellow fever and Zika virus, it is hypothesized that there would be evidence of historic infection of Zika virus in the population and this prevalence would overestimate the prevalence of Zika virus in the general population.

Researchers also performed a supplement to this study in which a questionnaire was administered to assess knowledge, attitudes, and practices (KAP) towards Zika virus among adults presenting to a general hospital in Abuja, Nigeria, as well as the determining factors. Knowing the prevalence of ZIKV and current adherence to prevention guidelines will provide NCDC with an initial population risk assessment. Given the results of this study, population-specific measures for outbreak control and future interventions can be defined proactively.

Methods

Setting

The study took place in Abuja, Nigeria at the National Reference Laboratory and Maitama General Hospital. Approval from the Nigeria Health Ethics Research Committee extended to allow research to be conducted at both locations. The National Reference Laboratory encompasses the Central Public Health Laboratory in Lagos, a WHO-designated reference lab for the diagnosis of measles, rubella, and Yellow fever. The National Reference Laboratory

provides diagnostic services for diseases of public health importance in the country including viral hemorrhagic fevers meningitis, cholera, measles, Yellow fever, rubella, and polio. Nigeria Centre for Disease Control (NCDC) began Yellow fever (YF) serology testing in April 2008 and 4 labs were identified to test based on 6 geo-political zones. Maitama General Hospital is charged with the sample analyses for measles, rubella, and Yellow fever from NCDC outbreak response in the North Central Zone which consists of 7 states. This study uses serum samples collected in the North Central Zone and sent to Maitama General Hospital for testing. Samples from two States in the North East Zone- Borno and Adamawa- were also analyzed by Maitama General Hospital between 2015 and 2019.

Data collection

Standard operating protocols (SOP) were applied to the current study. The lab scientists testing serum samples were trained by an NCDC laboratory manager on the SOP for handling infectious serum samples. Involved lab scientists reviewed the NCDC provided recommendations for safety when working with Zika virus, which suggests Biosafety Level 2 precautions including the use of gloves, a laboratory gown or coat, and eye protection[16]. Each researcher involved in data collection completed the Biomedical Focus and Social/Behavioral Modules from the Collaborative Institutional Training Initiative (CITI) as well as the Good Clinical Practice Module to be in accordance with the Emory Institutional Review Board requirements. Researchers are also compliant with the additional Nigeria Health Research Ethics Committee (NHREC) training requirements for in-country research activities.

Sample Selection

A convenience sample of 385 serum samples stored at the Maitama General Hospital was tested for ZIKV antibodies using RDTs. Samples were originally tested for Yellow fever after

subjects reported characteristic symptoms of the disease. For this study, researchers used the Artron One Step Zika IgG/IgM Antibody Test from Maternova and the associated protocol.

Laboratory

CDC recommends that only those with reported clinical symptoms for Zika virus (acute onset fever, headache, maculopapular rash, arthralgia, and conjunctivitis) undergo testing. However, this may only be applicable to US-based laboratories or areas where mosquito-borne Flavivirus infections are rare. Due to the temporal nature of biological analytes in the infected person, multiple assays and sample types are necessary to achieve an accurate clinical diagnosis of Zika virus[18]. Viral RNA from Zika virus can be detected using Nucleic acid testing within the first 6 weeks after the onset of symptoms while IgM antibodies persist longer and maybe detected 12 weeks past the onset of symptoms. The standard practice of diagnosis of ZIKV includes molecular plasma testing, molecular urine testing, as well as serology. If possible, paired serum samples taken 2 weeks apart may be analyzed with ELISA to monitor titer changes, however during the study period, duplicate samples were not available[19].

IgM for Zika virus is typically detectable around three to five days after infection, but cross-reactivity between Zika and closely related flaviviruses such as Yellow fever, Japanese encephalitis, and West Nile virus is possible. Notably, cross-reactive results have been found to be more common in patients that presented with prior flavivirus infections than patients with primary Zika virus infection.

Rapid Diagnostic Testing

One Step Zika IgG/IgM Antibody Test is an antibody-capture immunochromatographic assay for the simultaneous detection and differentiation of IgG & IgM antibodies to Zika virus in human serum, plasma, and/or whole blood samples. For this study, serum samples were tested.

Zika virus-specific antigens are conjugated to a colloidal gold tagged antibody and deposited on the conjugate pad. A unique combination of anti-human IgG & IgM antibodies is immobilized on the test zone of the nitrocellulose membrane as two individual test lines in the test window of the test device (Figure 1). When the sample is added, the gold-antigen conjugate is rehydrated and the ZIKV IgG and/or IgM antibodies, if any in the sample, will interact with the gold conjugated antigen. The antigen-antibody-gold complex will migrate towards the test window until the test zone, where it will be captured by the relevant anti-human IgG (T1) and/or anti-human IgM (T2). This process will form a visible pink line that indicates a positive result. If Zika virus antibodies are absent in the sample, no pink line will appear in the test zone, indicating a negative result. The absence of a pink control line in the control zone (C) is an indication of an invalid result.

This test was internally validated by Maternova during a reproducibility and repeatability test in which five ZIKV positive specimens and ten negative samples were tested with 5 replicate tests repeated on three different days. For each trial, all positive samples tested positive and all negative samples tested negative. An evaluation study was carried out with 100 recently infected specimens diagnosed as positive for Zika virus IgM antibodies by the EUROIMMUN Anti-Zika Virus ELISA (IgM), 40 specimens with other infections such as Zika virus and Chikungunya, and 10 samples negative for all the tests. The diagnostic sensitivity and specificity of the One Step Zika IgG/IgM Antibody test were reported to be 96.15% and 100% respectively.

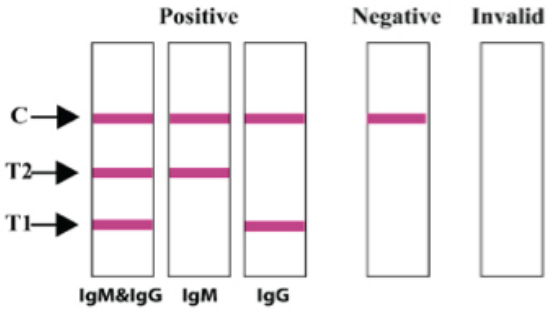

Figure 1. One Step Zika IgG/IgM Antibody Test

Data Management and Analysis

The results of 303 Zika tests provide the lab identification number (lab ID), the result of the test, and any additional notes on the sample. The lab ID was used to match samples with demographic data including age, sex, local government area, and state of residence.

The outcome of interest in this analysis is positive or negative IgG and or IgM results. Researchers followed the protocol developed by Maternova for the Artron One Step Zika IgG/IgM Antibody Test to analyze serum samples. We used descriptive analyses to estimate means and proportions, measures of variability, and confidence intervals around these variables in SAS. Chi-squared test or Fisher's exact test was used for 2x2 data and we used logistic regression for multivariate analysis of the outcome of interest. We compared positive results for the antibody test between month and year of sample collection, state and local government area (LGA) of residence, age and sex, and environmental factors such as mean annual temperature, mean annual rainfall, and population density. Environmental data was included at the State level (n=9). All variables were tested as possible significant predictors of RDT confirmed Zika virus infection in a logistic regression model. Continuous variables age, year, month, number of days between first reported symptoms, and the day the specimen was tested for YF, temperature, population density, and annual rainfall were also tested for significance when dichotomized

using the median value. Nigerian meteorology data also informed the division of months into the rainy season (March-July) and dry season (November- February) for further analysis.

Results

The final sample for this analysis included 303 serum samples tested for Zika virus among 1673 samples that had been collected and tested for Yellow fever between 2015 and 2019 prior to the study (Figure 2.). Only samples that had tested negative for Yellow fever antibodies were eligible for convenience sampling. Figure 2. is an administrative map of Nigeria showing the number of samples collected by NCDC during Yellow fever outbreak responses to 9 Sates between 2015 and 2019.

Table 1. shows the median and interquartile range for continuous variables compared by the result of the RDT. 283 of 303 samples (93%) were successfully matched to available demographic data via lab identification numbers. Of these, 27 samples tested positive for Zika virus (9.54%). 3 samples that were not matched with demographic data also tested positive. Spearman correlation analyses revealed significant correlations between some variables, notably between population density and mean annual rainfall ($R=0.73$, $p<0.0001$), population density and the number of days between first reported symptoms and the day the specimen was tested for YF ($R=0.28$, $p<0.0001$) and population density and mean annual temperature ($R=0.21$, $p<0.0004$). Pearson correlation also showed that the number of suspected Yellow fever cases in an LGA was correlated with a positive RDT result for ZIKV by 55% ($p<0.0001$). Figure 3. shows the location of samples tested for Zika virus in relation to the number of suspected Yellow fever cases in that LGA. The median age of the study sample was 13.0 years (IQR= 22.8). Among the samples that tested positive for ZIKV, the median age was 6.9 years, compared to 13.4 years among the samples that tested negative (IQRs= 19.2 and 22.3, respectively).

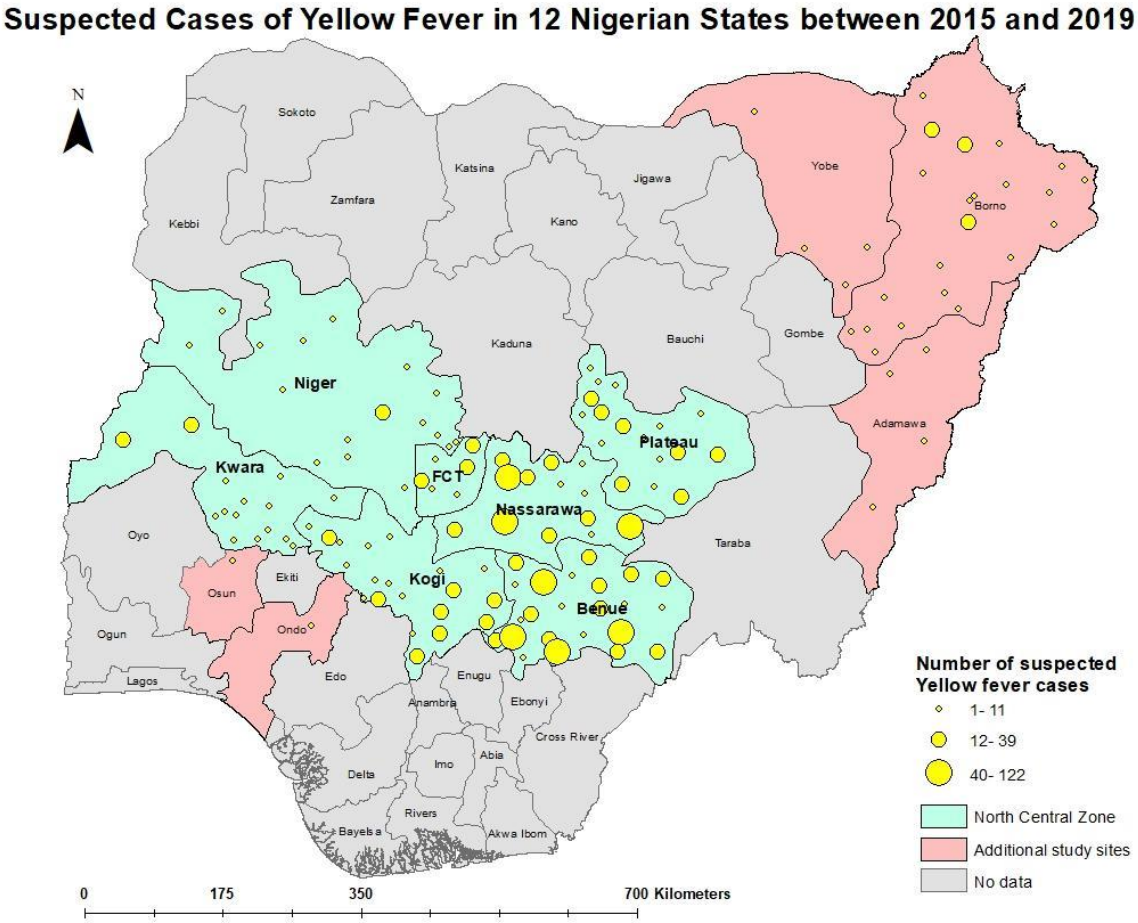

Figure 2. The NCDC Measles Laboratory (NML) is responsible for the analysis of samples collected in the North Central Zone following outbreak response to Yellow fever. Several samples from outside the North Central Zone were obtained and analyzed by NML. FCT is Federal Capital Territory. *Projected in Minna UTM Zone 31 Source: GADM database (www.gadm.org), version 2.5, July 2015*

Table 1. Median and Interquartile Range for Continuous Variables Compared by Positive or Negative IgG/ IgM Result from RDT

	Total (n=283)	**Positive (n=27)**				**Negative (n=253)**
Variable*		**All (n=27)**	**IgG (n=13)**	**IgM (n=13)**	**IgG & IgM (n=1)**	
Age	13.0 (22.8)	6.9 (19.2)	9.3 (17.8)	5.3 (19.3)	9.0	13.4 (22.3)
Number of days between first reported symptoms and the day the specimen was tested for YF	17.0 (13.0)	22.0 (31.0)	23.0 (34.0)	20.0 (12.0)	28.0	17.0 (13.0)
Mean Annual Rainfall (mm)	1231.0 (743.0)	1248.0 (17.0)	1248.0 (17.0)	1248.0 (0)	456.0	1231.0 (743.0)
Mean Temperature (°C)	27.2 (0.4)	27.2 (0.08)	27.2 (0.2)	27.2 (0)	26.8	26.9 (0.4)
Population Density (persons/km2)	150.0 (75.5)	168.6 (18.6)	168.6 (18.6)	168.6 (0)	93.1	135.9 (75.5)
Month**	5 (4.0)	5 (4.0)	5 (4.0)	4 (4.0)	12	5 (4.0)
Year**	2018 (2.0)	2018 (2.0)	2018 (1.0)	2018 (2.0)	2017	2018 (2.0)

*Table lists median value and IQR in parenthesis.
**n=281

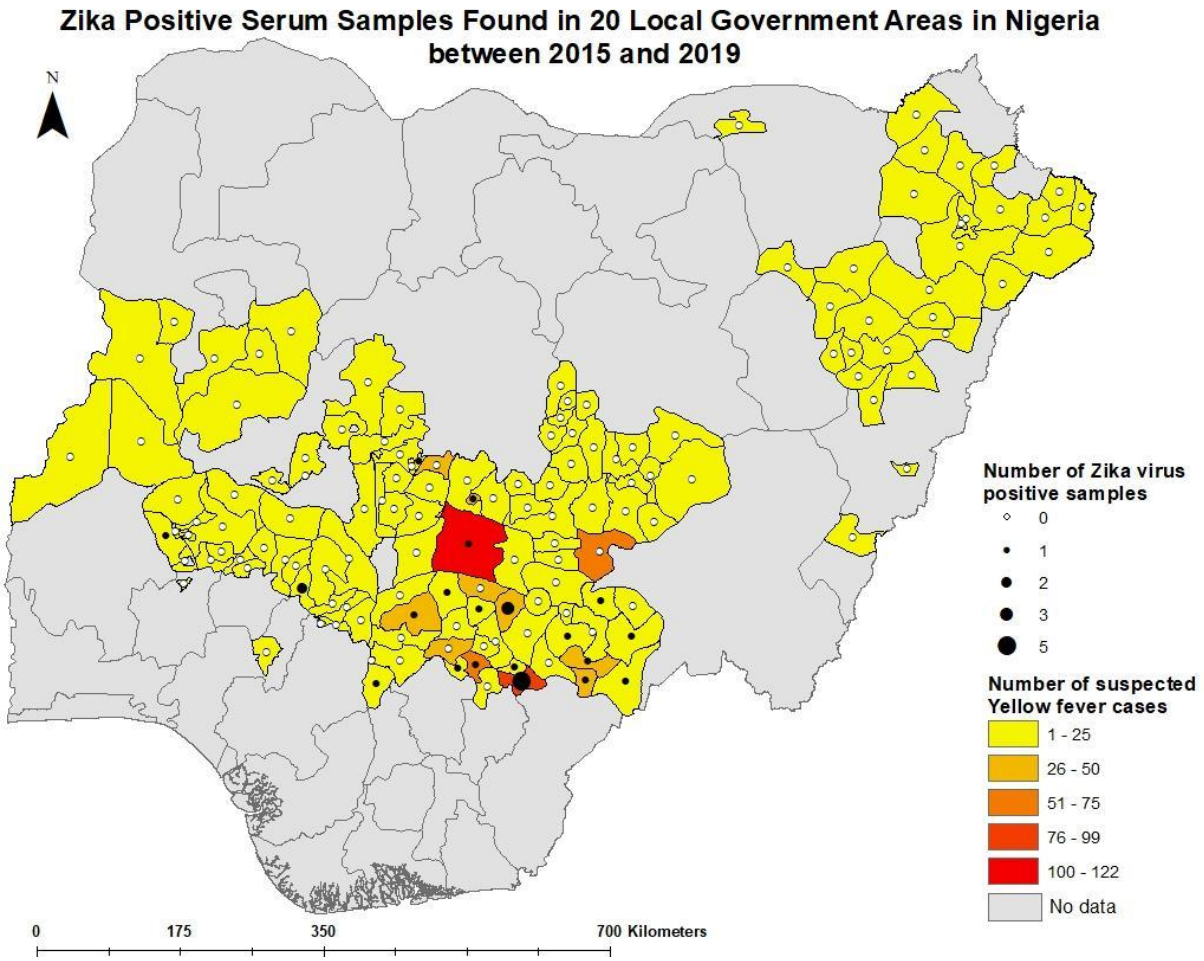

Figure 3. A convenience sample of serum specimens that already tested negative for Yellow fever was tested again for Zika virus using RDTs (n=303). *Projected in Minna UTM Zone 31 Source: GADM database (www.gadm.org), version 2.5, July 2015*

The mean annual temperature among negative samples was 0.72°C lower than the corresponding temperature among positive samples (Std Err of difference=0.32) and this difference was statistically significantly different (Satterthwaite $p< 0.0001$). The mean annual rainfall for negative samples was 1000.1mm (SD= 371.3mm) which was significantly lower than the corresponding rainfall for positive samples (Satterthwaite $p=0.027$), which was 1131.6mm (SD= 271.5mm). Continuous variables age, temperature, population density, and rainfall and the number of days between first reported symptoms and the day the specimen was tested for YF were modeled as dichotomous variables and tested for differences in Zika virus positivity in a

chi-square test. For population density, mean temperature, and mean annual rainfall, values above the median were associated with an increased number of expected positive ZIKV samples (Table 2.). Age, sex, year and month of sample collection, and the mean number of days between first reported symptoms and the day the specimen was tested for Yellow fever were not significantly different when comparing positive and negative Zika virus samples. Similarly, there was no significant difference between positive and negative samples when months in the rainy season (March- July) were compared to other months or when months in the dry season (November- February) were compared to the other months.

Table 2. Chisq Comparing Samples Positive or Negative for ZIKV at Median Value of Mean Annual Rainfall, Mean Annual Temperature and Population Density (n=280)

		Level*	
Variable		0	1
Average Annual Rainfall (mm)			
	Positive	4	23** (p=0.0327)
	Negative	89	164
Average Temperature (°C)			
	Positive	3	24** (p=0.0011)
	Negative	110	143
Population Density (persons/km2)			
	Positive	9	18** (p<0.0006)
	Negative	169	84

*Environmental data above was not continuous. It is included at the State level (n=9). Level 0 represents values below the median rainfall, temperature, and population density
**significant at .05 level

Table 3. shows the observed number of ZIKV positive samples by State and the percent positive. Benue had more cases than any other state and this difference was statistically

significant. However, when making a comparison between states in the east of the study area (Plateau, Nassarawa, and Benue) to the other localities, there was no observed association.

Table 3. Observed Number of ZIKV Positive Samples by State (n=9)

	Adamawa	**Benue**	**Borno**	**FCT, Abuja***	**Kogi**	**Kwara**	**Nassarawa**	**Niger**	**Plateau**
Positive	0	18	1	0	4	1	2	1	0
Negative	5	70	17	14	41	6	57	10	33
% Positive	0%	20.5%	5.6%	0%	8.9%	14.3%	3.4%	9.1%	0%
Total	5	88	18	14	45	7	59	11	33

*Federal capital territory, Abuja

Researchers developed 2 different models to predict Zika virus positivity using continuous, categorical, and dichotomous representations of the data. Both models tested mean annual rainfall (mm), temperature (°C), mean population density (person/km2), age, year, month, sex and interaction between temperature and rainfall, rainfall and population density and temperature and population density in a logistic model with 280 observations and explored using forward selection. In Model 1, mean annual rainfall and temperature, population density, and age were considered as continuous variables. Forward selection to build Model 1 included continuous variables mean annual temperature and mean annual rainfall as significant predictors of a positive RDT at the 0.05 level. None of the three interaction terms we tested were significant. A 0.69 °C increase in temperature was associated with about 2 times increased odds of a positive result (95% CI: 1.15, 3.46). However, when controlling for temperature, rainfall was insignificant (Table 4.). An increase of 19cm of rainfall annually was associated with a 20% increase in the odds of a positive result. Model 1. yielded a Likelihood test statistic of 14.78 (p=0.0006).

$$Model\ 1: log\ (odds\ of\ positive\ RDT) = -22.9483 + 0.6906 * MeanTemp + 0.00194 * MeanRain$$

Table 4. Odds ratio estimates for Model 1. at significance level a=.05 (n=280)

	Point Estimate	95% Confidence Interval
Mean annual temperature (°C)	2.0*	1.15 – 3.46
Mean annual rainfall (mm)**	1.23*	1.04 – 1.42

*significant at .05 level
**Estimate and 95% Confidence interval are provided per 100 mm of rain

All the variables included in Model 1 were also tested in Model 2, however, Model 2 uses dichotomized dummy variables for mean annual rainfall and temperature, population density, age, and the number of days between first reported symptoms and the day the specimen was tested for YF using the median values. Only temperature and population density were significant predictors in this model. Samples collected in states with annual temperatures greater than or equal to 26.9°C were associated with over 4 times the odds of having a positive RDT compared to samples collected in states with annual temperatures less than 26.9°C (95% CI: 1.13-14.90). Samples collected in states with a population density greater than 145 people per kilometer squared were associated with about 2.6 times the odds of having a positive RDT compared to samples collected in states with a population density of fewer than 145 people/km$_2$ (95% CI: 1.06 -6.31). This model yielded a Likelihood test statistic of 17.08 (p=0.0002).

$$Model\ 2: log\ (odds\ of\ positive\ RDT) = -3.7766 + 1.4114 * Temp + 0.9514 * Density$$

Table 5. Odds ratio estimates for Model 2. at significance level a=.05 (n=280)

	Point estimate	95% Confidence Interval
Mean annual temperature < 26.9°C	Ref	Ref
Mean annual temperature >= 26.9°C	4.10*	1.13 – 14.90
Population density <145 /km2	Ref	Ref
Population density >= 145 /km2	2.59*	1.06 – 6.31

*significant at .05 level

Discussion

This study provides further evidence of the presence of Zika virus infections in Nigeria, specifically in the context of Yellow fever endemic communities. The highest percent positivity for Zika IgG/ IgM antibodies found in this study was 20.5%, estimated in the state of Benue. The local government area (LGA) Oju, with 5 positive samples, contributed the most to the significantly larger number of positive cases in Benue. Nigeria Centre for Disease Control's outbreak response team considers people with acute onset of fever followed by jaundice within 2 weeks of onset symptoms as a suspected case of Yellow fever. It is notable that Oju also had 87 suspected cases of YF, the second-highest among all study locations. Two states with only 1 suspected case of Yellow fever (Osun and Ondo) had 0 ZIKV positive samples. Pearson correlation showed that the number of suspected Yellow fever cases in an LGA was correlated with a positive RDT result for ZIKV by 55% (p<0.0001). These results are parallel with the knowledge of the shared vector between Zika virus and Yellow fever. Given this knowledge, Zika virus should be included on the list of NCDC's monitored diseases.

One potential concern with the design of this study is the accuracy of the rapid diagnostic tests, as there are known circulation of other arboviruses such as dengue and chikungunya as well as circulation of arboviruses that we may not be aware of. This is relevant to this research

because ZIKV and dengue are highly homologous. Specifically, DENV has been found to be cross-reactive with ZIKV and confound serological testing. A study by Priyamvada et al. reported E proteins of DENV type 2 and ZIKV share about 53% of the same amino acid sequence identity[22]. Still, the extent to which the dengue group will bind antigenically to the ZIKV complex is not fully understood. Therefore, high accuracy of the RDTs is necessary to distinguish ZIKV and DENV IgG/ IgM antibodies or there is the potential that the prevalence of ZIKV in this study is inflated. Moreover, other evidence supports that antibodies from primary ZIKV infections that occur in areas with flavivirus infection endemicity, such as Yellow fever, more often recognize E proteins from other flaviviruses[23]. Only including Yellow fever negative samples in this study could have negated these effects.

When interpreting the results of this research, it is also important to consider the integrity of stored serum samples. Ideally, an enumerated list of all Yellow fever negative samples stored no longer than 3 months would serve as the sampling frame, as IgM antibodies are most likely to be detected within 12 weeks of infection and IgG antibodies can be detected from months to years[18]. Though, within 10 days of reported ZIKV symptoms, ZIKV has been reported to clear from the blood. Manufactures of the Artron One Step Zika IgG/IgM Antibody Test confirmed that stability of ZIKV antibodies is dependent on the stored condition and it will degrade over time, reducing the positive signal. In this study, the median number of days between sample collection and storage was 17 days. We interpret this as another driver of the underestimation of the prevalence of Zika virus found in this study.

Future research that aims to study the epidemiology of Zika virus in Nigeria should consider including additional demographic data such as level of education and history of contraceptive use in predictive models. Environmental data should be included at the city or LGA level in order to better differentiate study sites. In a broader context, we note that the

severity of microcephaly cases has shown to vary across contexts. Therefore, additional research to study the relationship between infection history and infant outcomes is necessary. We would like to highlight the context of this study in that WHO and NCDC guidelines to prevent Zika virus transmission were not sufficient to protect the general public. Future NCDC outbreak responses for Yellow fever, dengue, and other flaviviruses should anticipate positive Zika virus results and routinely test for ZIKV. Additional research to better understand the drivers and likelihood of Zika virus infection in the context of prior Yellow fever infection and endemic settings is needed.

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