Am. J. Trop. Med. Hyg., 106(1), 2022, pp. 192–198
doi:10.4269/ajtmh.20-1339

Zika Virus Seroprevalence in Two Districts of Chincha, Ica, Peru: A Cross-Sectional Study

Rodrigo Cachay,[1]* Alvaro Schwalb,[1] J. Gonzalo Acevedo-Rodriguez,[1] Xiomara Merino,[1,2] Michael Talledo,[1,2] Luis Suarez-Ognio,[3] Laura Pezzi,[4] Xavier de Lamballerie,[4] Humberto Guerra,[1] Thomas Jaenisch,[5,6] and Eduardo Gotuzzo[1]

[1]*Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru;* [2]*Unidad de Epidemiología Molecular, Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru;* [3]*Facultad de Medicina, Universidad Peruana Ciencias Aplicadas, Lima, Peru;* [4]*Unité des Virus Émergents, Aix-Marseille University, Marseille, France;* [5]*Heidelberg Institute of Global Health, Heidelberg University Hospital, Heidelberg, Germany;* [6]*Colorado School of Public Health, University of Colorado, Anschutz Medical Campus, Aurora, Colorado*

Abstract. In 2017, a major outbreak of Zika virus (ZIKV) infection took place in Chincha Province, Peru, where arboviral circulation had never been reported before. We conducted a cross-sectional survey (March–May 2019) in two districts of Chincha Province: Pueblo Nuevo and Chincha Baja. We included residents who were 20 to 40 years old and who had lived in these districts for at least 1 year. Serological testing combined screening with a commercial NS1 protein-based Zika IgG ELISA, and confirmation by a cytopathic effect-based virus neutralization test (VNT). Prevalence ratios (PRs) were calculated using Poisson regression with robust error variance. Four hundred participants, divided equally among districts, were enrolled. Anti-ZIKV IgG ELISA was positive for 42 participants (10.5%) and borderline for 12 (3%). Fifty-two of these 54 samples were confirmed positive by ZIKV VNT (13% of the total population). The Pueblo Nuevo district exhibited a greater ZIKV seroprevalence based on VNT results than the Chincha Baja district (23.5% versus 2.5%), with participants from the Pueblo Nuevo district being 9.4 times more likely to have a positive ZIKV VNT result. Average monthly income greater than the minimum wage and adequate water storage were found to be protective factors (PR, 0.29 and 0.24, respectively). In multivariate analysis, living in the Pueblo Nuevo district and a personal history of fever and rash were strong predictors of ZIKV positivity by VNT. The low ZIKV seroprevalence should prompt health authorities to stimulate interventions to prevent potential future outbreaks. In the Pueblo Nuevo district, the seroprevalence was greater but presumably not sufficient to ensure protective herd immunity.

INTRODUCTION

With the introduction of Zika virus (ZIKV) in the Americas, an important outbreak occurred in the Brazilian state of Rio Grande do Norte in 2015, which subsequently extended to the entire continent of South America.[1] In February 2016, the WHO designated the ZIKV epidemic as a Public Health Emergency of International Concern.[2,3] Zika infection was classified as an important re-emerging tropical disease with a potential impact on global health as a result of the teratogenic effects associated with ZIKV infection during pregnancy, which include a series of adverse fetal outcomes, the most visible being congenital microcephaly.[4–6]

In Peru, the first cases of ZIKV infection were reported in 2016 in the regions of Cajamarca and Loreto. Afterward, there were seasonal outbreaks associated with the rainy season, mainly involving the northern coastal region (Tumbes) and the northern jungle regions (Amazonas and Cajamarca).[7,8] The following year, 6,099 cases of ZIKV infection were notified nationwide by the Peruvian Center for Epidemiology, Prevention and Control of Diseases (CDC-Peru).[9]

ZIKV reached the Ica region, western Peru, in early 2017, with 4,317 reported cases and a cumulative incidence rate of 537.87 per 100,000 inhabitants.[7] The district of Pueblo Nuevo, in Chincha Province, was the most affected area, with 2,114 reported cases.[7] Before 2017, Chincha had never before been reported to be affected by arboviral infections, such as Dengue virus (DENV) and Chikungunya virus, according to previous epidemiological evidence. However, once ZIKV was introduced in the region, DENV was also reported at the same time.[7]

During intense ZIKV circulation in 2017 in Peru, the only approach available to monitor viral transmission was the use of passive surveillance systems. They require that ill individuals seek medical care, a clinician identifies the case as being caused by a suspected etiological agent, and then the case is reported to health authorities before or after laboratory testing. Passive surveillance systems are a cause of underreporting because they do not capture sub-clinical and mild infections, as well as ill people not reporting to health facilities. Given the high rate of asymptomatic ZIKV infections,[10] seroprevalence surveys are necessary to assess the actual viral burden and estimate population immunity.

To our knowledge, there are no previous seroprevalence studies conducted in Peru related to ZIKV transmission. The aim of this study was to evaluate the seroprevalence status in two districts of Chincha Province, Ica region, western Peru, and the associated risk factors related to ZIKV infection in relation to the 2017 outbreak.

METHODS

Study design and setting. We conducted a retrospective cross-sectional survey between March and May 2019 in two districts of Chincha Province (located in Ica region): Pueblo Nuevo and Chincha Baja, which reported the highest (24.4%) and the lowest (0.2%) ZIKV infection rates in the city, respectively, during the 2017 outbreak (Figure 1[11]).

Participants and inclusion criteria. The study population included residents from urban communities of the Pueblo Nuevo district and the Chincha Baja district, with an age range from 20 to 40 years, who had lived in these districts for at least 1 year. Those who had traveled outside of Chincha in the previous 6 months were excluded. Participants were enrolled by convenience sampling. Study personnel visited houses in the nearby towns and invited residents who

*Address correspondence to Rodrigo Cachay, Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru. E-mail: rodrigo.cachay.f@upch.pe

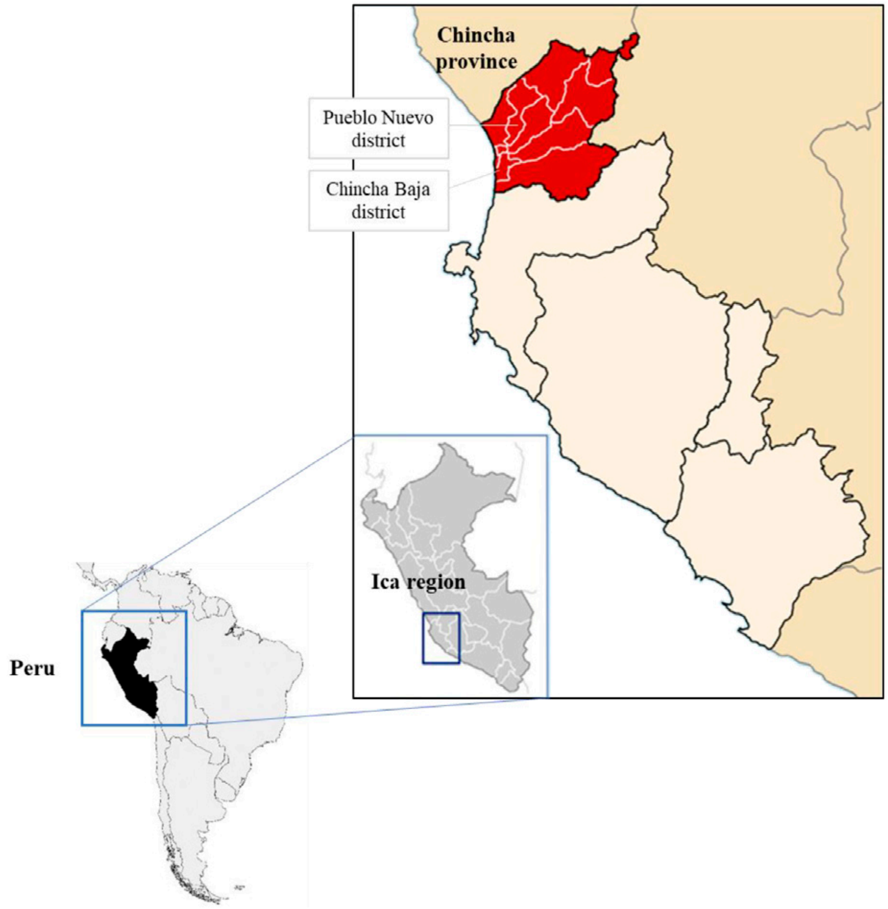

FIGURE 1. Study sites in Peru (Pueblo Nuevo and Chincha Baja districts) in Chincha Province, Ica region, western Peru. Map adapted from Wikipedia.[11] This figure appears in color at www.ajtmh.org.

met all the eligibility criteria to participate in the study. A questionnaire was administered (duration, ≈20–30 minutes) and a blood sample was collected for serology testing after the informed consent form was signed.

The questionnaire covered information on demographics, and housing and living characteristics, including water source, access, and storage. Mosquito protection methods and the use of larvicides for stored water were also recorded. History of febrile illness with rash was evaluated for the participant, family members, and neighbors. The form also inquired about yellow fever immunization; respondents were asked to show their vaccination card as evidence.

Sample size calculation. The sample size was calculated for a population proportion, considering an accuracy of 5% and, because there has been no similar ZIKV seroprevalence studies in Peru, we considered a prevalence of 50%. A sample size of 400 participants was targeted, with a CI of 95%. The 2017 national population census reported that 44% of the population in Chincha was between 20 and 40 years old, 6% of whom lived in the Chincha Baja district and 29% lived in the Pueblo Nuevo district.[12]

Serology testing by ELISA. Serum samples were stored at –20°C until use. The serological analysis was performed using the EUROIMMUN anti-Zika IgG Virus ELISA, which detects IgG against ZIKV recombinant non-structural protein (NS1). The test was performed according to the manufacturer's instructions. Briefly, a dilution of the participant sera (1:101) was added to the wells, followed by the addition of anti-human IgG conjugated to horseradish peroxidase. A color reaction appears when the substrate (3,3',5,5'-tetramethylbenzidine) is added to the wells. The absorbance was read at 450 nm. Positive and negative controls, and a calibrator from the kit were used. Results were evaluated by calculating ratio values (RV): OD $\text{value}_{\text{sample}}/\text{OD}_{\text{calibrator}}$, where an RV of < 0.8 was considered negative, an of RV ≥ 0.8 to < 1.1 was borderline, and a RV of ≥ 1.1 was positive.

Confirmatory serology testing by ZIKV cytopathic effect-based virus neutralization test. Samples with positive and borderline results by EUROIMMUN anti-ZIKV IgG ELISA were characterized further using a ZIKV cytopathic effect-based (CPE) virus neutralization test (VNT) for confirmation. The VNT was adapted from a protocol described previously[13] and was performed in a BSL3 facility. The assay was performed in 96-well microtiter plates using Vero cells (E6). Briefly, sera were 2-fold diluted from 1:10 to 1:640 and were then mixed with an equal volume of 100 $TCID_{50}$ (tissue culture infective dose producing pathological change in 50% of the cells) of virus (H/PF/2013 strain from EVAg, Ref-SKU: 001v-EVA1545), incubated for 1 hour at 37°C, and inoculated onto 2×10^5 cells/well. After 7 days of incubation at 37°C with 5% carbon dioxide, the microplates were read under the microscope, and the absence (neutralization titer at 20, 40, 80, 160, 320, 640) or presence (no neutralization) of the cytopathic effect was noted. The cutoff value for positivity was set at titer 40, as described previously using the same technique.[13]

Statistical analysis. Descriptive and statistical analyses were conducted using Stata SE 15 (StataCorp). The data were expressed as frequencies for categorical variables, and medians and interquartile range for continuous variables. The χ^2 test was applied for comparison of proportions between two discrete variables; one-way analysis of variance was used when comparing multiple discrete variables. The Poisson regression with robust error variance was used to calculate prevalence ratios (PRs); for adjusted PRs, variables were chosen on the basis of significance by bivariate analysis.

Ethical considerations. The study was evaluated and approved by the Institutional Ethics Committee of the Universidad Peruana Cayetano Heredia (SIDISI 103488), and the project's implementation was authorized by the Regional Chincha Health Directorate. All participants signed an informed consent form before providing information and blood samples. Participants were anonymized prior to analyses.

Patient and public involvement. Patients or the public were not involved in the development of the research questionnaire, design, conduct, outcome measures, reporting, and dissemination plan of our research.

RESULTS

A total of 505 persons who met inclusion criteria from the two districts were contacted. Two hundred participants were enrolled for each district; enrollment stopped when the targeted study population size (400 participants) was reached. Among people who did not consent to participate, 63.8% were women and 59% were from the Pueblo Nuevo district. The main reasons for exclusion were refusal of phlebotomy and lack of time to answer the questionnaire (35.2% and 29.5%, respectively) (Figure 2).

Participants were distributed equally among districts, with 200 participants enrolled in each. Most (78.75%) of the enrolled participants were women; the mean age of participants was 28.6 (SD, 5.80). Half of the participants (52.91%) had a low socioeconomic status, reporting an average monthly income less than the minimum wage (900 PEN; ≈USD265). The most common methods to prevent mosquito bites were indoor repellents (65.15%), followed by personal repellents (30.61%) and bed nets (23.64%).

Results show a predominantly female and young population, especially in the Pueblo Nuevo district. There were no significant differences between districts with regard to household material, water source, and waste disposal. A greater number of participants with low socioeconomic status were enrolled from the Pueblo Nuevo district, where, accordingly, regular access to water was more difficult in comparison to the Chincha Baja district (34.5% having daily access to water in the Pueblo Nuevo district versus 93% in the Chincha Baja district). Nonetheless, water storage conditions were better in the Pueblo Nuevo district, as 88.64% of participants covered their water deposits instead of leaving them open (60.5% in the Chincha Baja district). Furthermore, the use of mosquito prevention methods was more common in the Chincha Baja district, whereas the use of larvicides in water deposits was more common in the Pueblo Nuevo district (Table 1).

CPE ZIKV VNT. We explored variables associated with ZIKV positivity using the VNT. Residents from the Pueblo Nuevo district were 9.4 times more likely to have anti-ZIKV antibodies by VNT compared with residents from the Chincha Baja district. Female gender was associated with ZIKV VNT-positive results (PR, 2.53; 95% CI, 1.04–6.18; P = 0.041), as well as personal and family history of fever and rash (PR, 4.89; 95% CI, 3.03–7.89; $P < 0.001$; and PR, 2.45; 95% CI, 1.45–4.14; $P = 0.001$, respectively). Participants who used larvicides in their water deposits were 1.92 times more likely to have been infected by ZIKV. Earning an average monthly income greater than the minimum wage and adequate water storage in covered deposits were both found to be protective factors (PR, 0.29; 95% CI, 0.15–0.57, $P < 0.001$; and PR, 0.24; 95% CI, 0.75–0.77; $P = 0.015$, respectively). No significant associations regarding the use of mosquito protection methods were found (Table 2). Of the 56 participants who reported prior yellow fever vaccination, none was ZIKV VNT positive. In the multivariate analysis, using the factors that showed association with ZIKV VNT positivity, personal history of fever and rash remained highly significant and were strong predictors of ZIKV VNT positivity (PR, 3.53; 95% CI, 2.16–5.77; $P < 0.001$), as was living in the Pueblo Nuevo district (PR, 5.41; 95% CI, 1.56–18.67; $P = 0.008$) (Table 3).

DISCUSSION

Several prevalence surveys conducted in South American ZIKV-affected countries in recent years have reported a wide range of seropositivity: 21.5% to 39% in Bolivia,[14] 42.2% in Suriname,[15] 63% to 73% in Brazil,[16,17] and 36% to 56% in Nicaragua.[18] However, most of these studies were conducted in specific populations, such as blood donors, hospital patients, university employees, and pediatric cohorts. Only one population-based survey is currently available from South America. Flemand et al.[19] reported a ZIKV seroprevalence of 23.3% in the general population of French Guiana, almost 2-fold higher than the overall seroprevalence based on the specific ZIKV VNT results of the two Peruvian districts analyzed in our study (13%).

Seroprevalence rates reported throughout Latin American countries can vary not only with the study design, but also with the testing approach used. In fact, anti-ZIKV ELISA, a widely used serological technique for ZIKV seroprevalence studies, can be affected by cross-reactivity between antibodies against ZIKV and against other closely related flaviviruses. In particular, previous immunity against DENV can cause false-positive results in ZIKV ELISA testing, which can be excluded by performing more specific tests such as neutralization assays.

Chincha is an interesting area for seroprevalence surveys because no arboviral outbreaks have been reported before 2017 and the population was mostly immunologically naive for flavivirus infections.[10] Then, ZIKV and DENV were introduced in this region at nearly the same time. According to CDC-Peru, the first DENV case was reported 1 week before the introduction of ZIKV.[7] This represents a stark contrast to other areas in Latin America where DENV or Chikungunya virus transmission was well established before ZIKV

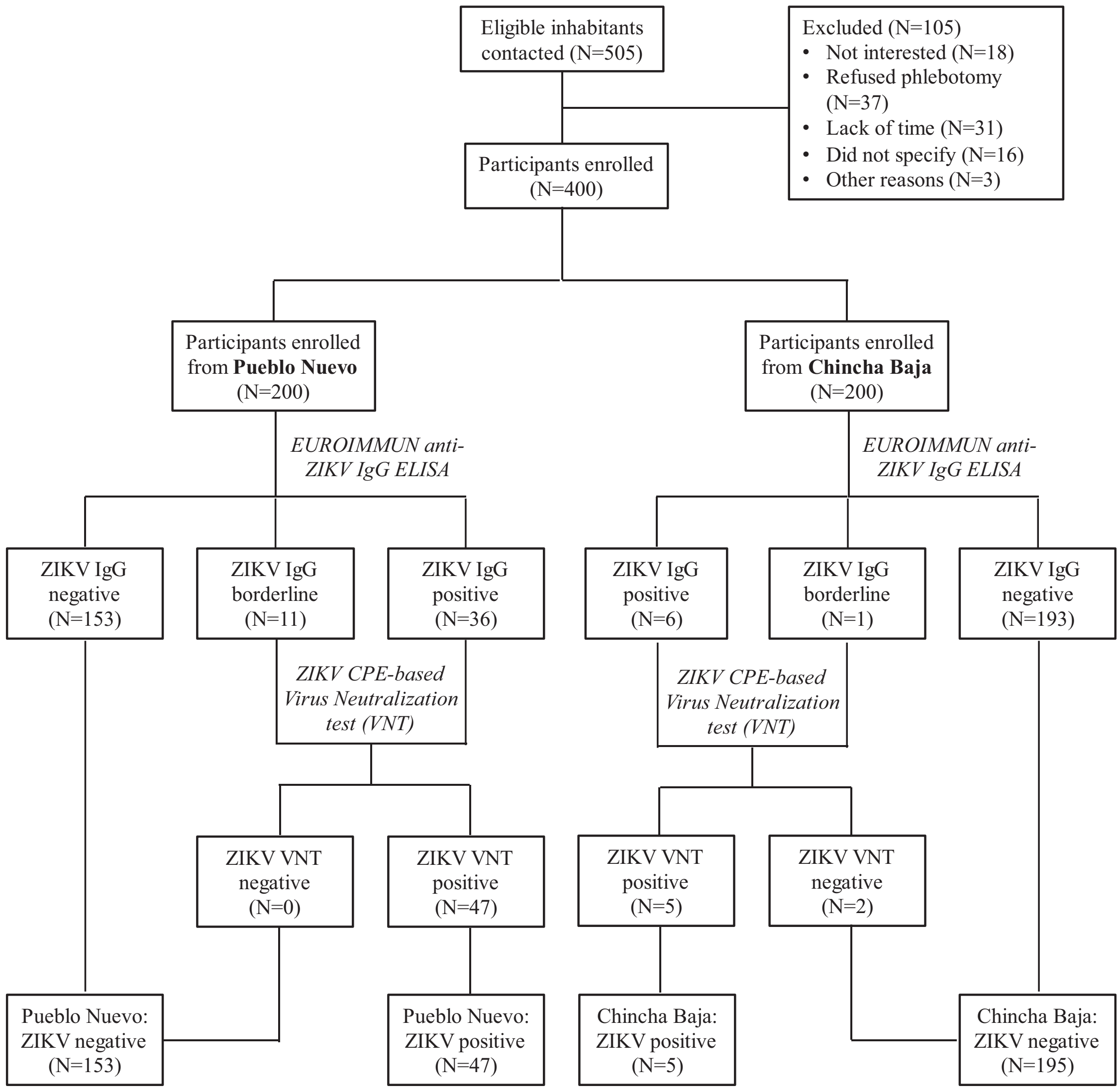

FIGURE 2. Flowchart of participants, including screening, enrollment, and testing by anti-Zika virus (ZIKV)IgG ELISA and ZIKV virus neutralization test.

circulation. Because of the recent DENV introduction in Chincha, it is unlikely that DENV seroprevalence is high in the province. Considering, also, the low level of yellow fever vaccination among the study population (Chincha is not an endemic area and vaccination is not mandatory), ZIKV testing on the samples collected for these study has probably been affected poorly by cross-reactivity with other flaviviruses. Moreover, the NS1 protein-based Zika IgG ELISA + CPE VNT combination strategy used in our study has already been described as a convenient tool to expedite ZIKV seroprevalence studies, with sensitivity and specificity values for ZIKV or more than 98%, even in settings with a strong DENV circulation.[13]

The overall ZIKV seroprevalence observed in Chincha is low. This may be a result of the fact that in this semi-desert coastal area, baseline climatic and ecological conditions limit entomological activity and make this region modestly amenable to arbovirus transmission. Moreover, infection control interventions were implemented rapidly by the Ministry of Health after the first ZIKV cases appeared, achieving control of the outbreak, especially in the Pueblo Nuevo district, which had the highest infection rate. Seroprevalence reached in the Pueblo Nuevo district is not expected to be sufficient to ensure protective herd immunity. In a previous outbreak study, it was shown that the seroprevalence rate established using the same tests used in our study was more than 40% before the epidemic was controlled.[20] Because the Pueblo Nuevo district and the Chincha Baja district had the lowest positive ZIKV VNT rates, both districts should be prepared for potential future outbreaks through vector-control activities and promoting preventive practices among the population.

Although only 30 km separate Pueblo Nuevo and Chincha Baja, ZIKV seroprevalence between these two districts was significantly different (23.5% in the Pueblo Nuevo district versus 2.5% in the Chincha Baja district, $P < 0.05$), which is consistent with another study in Nicaragua that reported a large difference in seropositivity in two areas only 3 km apart.[18] The participants enrolled in the two districts in Chincha Province had similar characteristics, except that the

TABLE 1
Sociodemographic characteristics of study population ($N = 400$) by districts

Variable	*n* (%)	PN (*n* = 200), *n* (%)	CB (*n* = 200), *n* (%)	*P* value
Female	315 (78.75)	168 (84.00)	147 (73.50)	0.010
Age, y; median (IQR)	28 (23–33)	27 (23–31)	30 (25–34.5)	< 0.001*
Income less than minimum wage (*n* = 378)	200 (52.91)	164 (87.23)	36 (18.95)	< 0.001†
Closed water storage (*n* = 258)	236 (91.47)	164 (96.47)	72 (81.82)	< 0.001†
Larvicide use	234 (58.50)	127 (63.50)	107 (53.50)	0.042†
Mosquito protection method (*n* = 399)	330 (82.71)	157 (78.89)	173 (86.50)	0.045†
House material (*n* = 399)				0.085†
Adobe	35 (8.77)	12 (6.03)	23 (11.50)	–
Brick	307 (76.94)	155 (77.89)	152 (76.00)	–
Wood	36 (9.02)	20 (10.05)	16 (8.00)	–
Straw	21 (5.26)	12 (6.03)	9 (4.5)	–
Water source (*n* = 399)				0.753‡
Water system	374 (93.53)	184 (92.46)	190 (95.00)	–
Tank	18 (4.51)	13 (6.53)	5 (2.5)	–
Well	7 (1.75)	2 (1.00)	5 (2.5)	–
Water access				< 0.001‡
Daily	255 (63.75)	69 (34.50)	186 (93.00)	–
Every other day	131 (32.75)	120 (60.00)	11 (5.5)	–
Weekly	12 (3.00)	11 (5.5)	1 (0.50)	–
Biweekly	2 (0.50)	0 (0.00)	2 (1.00)	–
Waste disposal system (*n* = 398)				0.483‡
Sewerage	357 (89.70)	182 (91.45)	175 (87.93)	–
Septic tank	28 (7.04)	4 (2.01)	24 (12.06)	–
Homemade tank	13 (3.27)	13 (6.53)	0 (0.00)	–
Yellow fever vaccination	56 (14.00)	4 (2.00)	52 (26.00)	< 0.001‡

CB = Chincha Baja district; IQR = interquartile range; PN = Pueblo Nuevo district. The number of participants analyzed was 400 unless specified otherwise. Overall, anti-Zika virus (ZIKV) IgG EUROIMMUN ELISA provided a positive result for 42 participants (10.5%) and a borderline result for 12 (3%). The Pueblo Nuevo district exhibited a higher anti-ZIKV IgG prevalence according to ELISA in comparison to the Chincha Baja district (18% vs. 3%, respectively). Positive and borderline samples by ELISA ($n = 54$) were tested using the ZIKV virus neutralization test as a confirmatory serological method. Fifty-two samples proved to have a neutralizing antibody against ZIKV (13% of the total population) and thus were considered true positives: 47 (23.5%) from the Pueblo Nuevo district and 5 (2.5%) from the Chincha Baja district (Figure 2).

* Mann-Whitney test.
† χ^2 test.
‡ Analysis of variance.

TABLE 2
Bivariate analysis of factors associated with Zika virus seropositivity by virus neutralization test

Variable	ZIKV VNT+	ZIKV VNT–	PR	95% CI	*P* value*
District					
Pueblo Nuevo	47	153	9.40	3.81–23.16	< 0.001
Chincha Baja	5	195	Ref.	–	–
Gender					
Male	5	80	Ref.	–	–
Female	47	268	2.53	1.04–6.18	0.041
Income					
Less or equal to minimum wage	38	162	Ref.	–	–
More than minimum wage	10	168	0.29	0.15–0.57	< 0.001
Water storage					
Open, no lid	3	65	Ref.	–	–
Closed, lid	43	193	0.24	0.75–0.77	0.015
Larvicide use					
No	14	152	Ref.	–	–
Yes	38	196	1.92	1.07–3.44	0.027
Mosquito protection method					
No	9	60	Ref.	–	–
Yes	43	287	0.99	0.51–1.95	0.998
Personal history of fever and rash					
No	38	334	Ref.	–	–
Yes	14	14	4.89	3.03–7.89	< 0.001
Family history of fever and rash					
No	36	301	Ref.	–	–
Yes	16	45	2.45	1.45–4.14	0.001
Fever and rash reported in neighborhood					
No	46	325	Ref.	–	–
Yes	6	21	1.79	0.84–3.81	0.131

PR = prevalence ratio; VNT = virus neutralization test; ZIKV = Zika virus. For some variables (income, water storage, mosquito protection method, family history of fever and rash, fever and rash in neighborhood), the sum of participants is not 400 because some did not provide the information in the questionnaire.

* Poisson regression with robust error variance.

TABLE 3
Multivariate analysis of factors associated with Zika virus seropositivity by virus neutralization test

Variable	PR	95% CI	aPR	95% CI	*P* value
Resident of PN	9.40	3.81–23.16	5.41	1.56–18.67	0.008
Female gender	2.53	1.04–6.18	2.25	0.78–6.52	0.132
Income more than minimum wage	0.29	0.15–0.57	0.93	0.48–1.77	0.828
Closed water storage	0.3	0.10–0.97	0.42	0.16–1.10	0.078
Personal history of fever and rash	4.89	3.03–7.89	3.53	2.16–5.77	< 0.001
Familial history of fever and rash	2.45	1.45–4.14	0.96	0.52–1.78	0.917

aPR = adjusted prevalence ratio; PN = Pueblo Nuevo district; PR = prevalence ratio.

population in the Pueblo Nuevo district reported lower incomes and had water access every other day. Despite reporting greater use of larvicides and closed water containers, the lack of regular access to running water in the Pueblo Nuevo district increases the risk of improper water storage, which may explain why this was the most affected area. One study described that the one-time use of Temefos (Abate®), applied to the water reservoirs during the Chincha outbreak, did not have the expected effect because the water reservoirs were not eliminated.[21] The use of aerosols had a greater area of coverage (1,913 households reached with larvicides versus 117,908 households reached with aerosols), which emphasizes that combined methods for vector control must be applied to ensure protection.[21]

Interestingly, the cemetery of Chincha Province is located in the Pueblo Nuevo district. Cemeteries and their artificial containers (flower vases) are important mosquito breeding sites.[22] A recent study from Nicaragua reported seroprevalence rates ≈10% to 15% higher in neighborhoods near the cemetery than in other neighborhoods.[18] Closeness to the cemetery could explain in part the higher seroprevalence rates in the Pueblo Nuevo district compared with the Chincha Baja district.

The past medical history of the participants, especially fever and rash during the outbreak, as well as having a member of the family with those same symptoms, were the main predictive factors for ZIKV antibody by VNT. In this study, 38 of 52 (73.1%) VNT-positive participants did not report any rash and fever episode, which is consistent with a previous estimated prevalence of asymptomatic ZIKV infections ranging from 29% to 82% according to the study populations.[23]

Our study has some limitations. The participants may not be representative of the entire population of the two districts, because convenience sampling was used. Moreover, the sample size should have been calculated separately for each district using previous CDC-Peru prevalence rates to ensure the adequate number of enrolled participants per district. In addition, because this study was designed retrospectively and performed 2 years after the ZIKV outbreak, questions about presence or absence of symptoms could have been influenced by recall bias and may lead to an overestimation of ZIKV asymptomatic infections. Last, questions regarding the use of larvicides may not have been addressed correctly because public interventions were implemented during and after the outbreak.

This study represents the first estimation of ZIKV seroprevalence in Peru. The low seroprevalence observed in Chincha Province suggests that most of the population is still susceptible to ZIKV infection, which highlights the need for implementation of active surveillance systems and vector-control activities to detect and respond to future outbreaks promptly.

Received October 15, 2020. Accepted for publication July 9, 2021.

Published online November 22, 2021.

Financial support: This project, including the EuroImmun® anti-Zika Virus ELISA IgG kits and the UVE in-house ZIKV Virus Neutralization Test, was supported by the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement N.734548. We also thank the Faculty of Medicine Alberto Hurtado, Universidad Peruana Cayetano Heredia for its partial support to cover the publication cost.

Authors' addresses: Rodrigo Cachay, Alvaro Schwalb, J. Gonzalo Acevedo-Rodriguez, Humberto Guerra, and Eduardo Gotuzzo, Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru, E-mails: rodrigo.cachay.f@upch.pe, alvaro.schwalb@upch.pe, juan.acevedo@upch.pe, humberto.guerra@upch.pe, and eduardo.gotuzzo@upch.pe. Xiomara Merino and Michael Talledo, Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru, and Unidad de Epidemiología Molecular, Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru, E-mails: xiiomyjean@gmail.com and michaeltalledo@yahoo.com. Luis Suarez-Ognio, Facultad de Medicina, Universidad Peruana Ciencias Aplicadas, Lima, Peru, E-mail: luissurezognio@gmail.com. Laura Pezzi and Xavier de Lamballerie, Unité des Virus Émergents, Aix-Marseille University, Marseille, France, E-mails: laura.pezzi3@studio.unibo.it and xavier.de-lamballerie@univ-amu.fr. Thomas Jaenisch, Heidelberg Institute of Global Health, Heidelberg University Hospital, Heidelberg, Germany, and Colorado School of Public Health, University of Colorado, Anschutz Medical Campus, Aurora, CO, E-mail: thomas.jaenisch@uni-heidelberg.de.

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