

Autonomic Dysfunction and Risk Factors Associated with *Trypanosoma cruzi* Infection among Children in Arequipa, Peru

Natalie M. Bowman,* Vivian Kawai, Robert H. Gilman, Cesar Bocangel, Gerson Galdos-Cardenas, Lilia Cabrera, Michael Z. Levy, Juan Geny Cornejo del Carpio, Freddy Delgado, Lauren Rosenthal, Vivian V. Pinedo-Cancino, Francis Steurer, Amy E. Seitz, James H. Maguire, and Caryn Bern

University of North Carolina—Chapel Hill, Chapel Hill, North Carolina; The Johns Hopkins Hospital, Baltimore, Maryland; Asociación Benéfica PRISMA, Lima, Peru; The Johns Hopkins University, Bloomberg School of Public Health, Baltimore, Maryland; Arequipa Ministry of Health, Arequipa, Peru; University of Pennsylvania, Philadelphia, Pennsylvania; Fogarty International Center, National Institutes of Health, Bethesda, Maryland; Morgan Stanley Children's Hospital of New York, New York, New York; Centers for Disease Control and Prevention, Atlanta, Georgia; National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, Maryland; Harvard University School of Medicine, Boston, Massachusetts

Abstract. Chagas disease affects an estimated 8 million people in Latin America. Infected individuals have 20–30% lifetime risk of developing cardiomyopathy, but more subtle changes in autonomic responses may be more frequent. We conducted a matched case-control study of children in Arequipa, Peru, where triatomine infestation and *Trypanosoma cruzi* infection are emerging problems. We collected data on home environment, history, physical examination, electrocardiogram, and autonomic testing. Signs of triatomine infestation and/or animals sleeping in the child's room and household members with Chagas disease were associated with increased infection risk. Electrocardiogram findings did not differ between cases and controls. However, compared with control children, infected children had blunted autonomic responses by three different measures, the Valsalva maneuver, the cold pressor test, and the orthostatic test. *T. cruzi*-infected children show autonomic dysfunction, although the prognostic value of this finding is not clear. Sustained vector control programs are essential to decreasing future *T. cruzi* infections.

INTRODUCTION

Chagas disease affects an estimated 8 million people and causes more morbidity and mortality in Latin America than any other parasitic disease, including malaria.^{1,2} In Perú, an estimated 192,000 people are infected with *Trypanosoma cruzi* and 0.5 million residents of the city of Arequipa live in triatomine-infested districts, with potential risk of *T. cruzi* infection.¹ Acute *T. cruzi* infection usually causes detectable parasitemia and mild, nonspecific symptoms, and it resolves spontaneously over 4–8 weeks.³ Infected persons then enter the chronic phase of infection, with the majority remaining in the indeterminate form characterized by positive serology but no signs or symptoms. Up to 30% of infected individuals eventually progress to cardiac disease, with manifestations ranging from asymptomatic conduction abnormalities to lethal arrhythmias and dilated cardiomyopathy. Death may occur from congestive heart failure, ventricular arrhythmias, or high-grade heart block, and it is often sudden.⁴ Predictors of mortality in established cardiomyopathy include congestive heart failure, left ventricular systolic dysfunction on echocardiography, ventricular tachycardia, low QRS voltage, and male sex.^{5–7} However, there are no known indicators to predict which infected individuals will progress to cardiomyopathy.

Characteristic electrocardiogram (EKG) abnormalities, including right bundle branch block, left anterior hemiblock, and ventricular extrasystoles, are the most common early signs of Chagas cardiomyopathy and have been reported in otherwise asymptomatic *T. cruzi*-infected adults and children.^{8–10} In studies in Brazil in the 1980s, 11–14% of seropositive children had EKG abnormalities, most frequently right bundle branch block, supporting the notion that cardiac damage begins early in the disease process.^{8,9} Another early abnormality is para-

sympathetic dysfunction, usually manifested by blunting of the normal heart rate and blood pressure responses to deep breathing, Valsalva maneuver, orthostatic stress, and other stimuli.^{11–15} Vagal denervation is thought to play an important pathogenic role in gastrointestinal Chagas disease, and abnormal responses on autonomic stimuli were strongly associated with the digestive form of the disease in one study.^{16,17} However, the contribution of autonomic dysfunction to the pathophysiology of Chagas cardiomyopathy is less well-defined, and its significance in the absence of other cardiac findings is controversial.^{18,19}

Although risk factors for *T. cruzi* infection have been studied in a number of rural settings, there are few data from urban foci of transmission.^{20–24} Our group's investigations in periurban Arequipa showed increased risk of triatomine infestation associated with domestic animals, unplastered house walls, and animal pens, and showed the highest prevalence of human *T. cruzi* infection to be located on the densely populated hill-sides outside the city.^{25,26} In the present analysis, we examined risk factors for *T. cruzi* infection and compared EKG findings and four tests of parasympathetic autonomic function in infected children and matched uninfected control children.

METHODS

Study design and objectives. The study was designed as a matched case-control study to examine risk factors for *T. cruzi* infection and investigate electrocardiographic and autonomic findings in *T. cruzi*-infected children (cases) and uninfected controls.

Study site and population. The study site and participants are described in detail elsewhere.^{25–27} Most study children were recruited in public schools in periurban communities on the outskirts of Arequipa; 20% were recruited at their homes in Guadalupe, a community within the larger study area. We studied children, because most were born and likely infected with Arequipa, providing a better indicator of local transmission.

* Address correspondence to Natalie Bowman, Johns Hopkins University, Bloomberg School of Public Health, 615 N. Wolfe Street, Room W5515, Baltimore, MD 21205. E-mail: nbowman@post.harvard.edu

Ethical approvals and informed consent. The protocol was approved by the Institutional Review Boards of Johns Hopkins University Bloomberg School of Public Health, Centers for Disease Control and Prevention (CDC), Peruvian National Institutes of Health, and Asociación Benéfica PRISMA. The parents of all participants provided informed consent; participants 7 years or older provided informed assent before the study began. For parents or participants unable to read, the form was read aloud, and consent or assent was indicated by fingerprint.

Serosurvey data collection. Age, sex, and place of residence were recorded for each child. A 5-mL blood sample (3 mL for children under 5 years) was drawn. Blood was separated by centrifugation, and serum and cells were stored at -20°C in separate aliquots. Sera were screened for antibodies to *T. cruzi* with a commercial enzyme-linked immunosorbent assay (ELISA; Chagatek, Biomerieux, Marcy l'Etoile, France). The positive cutoff was set as 0.100 optical density (OD) units above the average OD of the negative control samples following instructions included in the kit. All positive samples and 10% of negative samples were tested by immunofluorescent antibody test (IFA) at CDC using a titer of 1:32 as the positive cutoff.²⁸ Specimens positive by both ELISA and IFA were considered to have confirmed *T. cruzi* infection.²⁹ Children positive by ELISA but negative by IFA were considered to have inconclusive results and were excluded from further analysis.

Clinical and cardiac studies. All children with confirmed *T. cruzi* infection were invited to participate in the case-control study. Two uninfected children were chosen at random, matched by age (± 3 years), sex, and neighborhood of residence, to act as controls. Alternate control children were also chosen and in some cases, underwent testing. When there were insufficient numbers of uninfected children meeting the matching criteria in a given community, controls were drawn from the closest town with similar geographic and entomological features.

Each participant underwent a structured medical history and noninvasive physical examination by a trained local study physician (G.G.-C.). A 12-lead EKG was performed on each participant using a portable Fukuda Cardiosuny 501B-III model EKG machine. Trained physicians and nurses performed the following tests of autonomic function: (1) deep breathing (breathing deeply and slowly, one breath approximately every 10 seconds, for 1 minute), (2) Valsalva (performing the Valsalva maneuver for 15 seconds by blowing against a closed mouthpiece made of a small cup followed by normal breathing for 45 seconds), (3) cold pressor (immersing one hand in ice water up to the wrist for 1 minute), and (4) orthostatic (actively moving from a lying to standing position; a tilt table was not used). The duration of each test was 1 minute; the participant rested at least 5 minutes before the first test and before each subsequent test. Testing was performed in the order described above for all patients. All tests were performed in the supine position unless otherwise noted, and children remained supine during the rest periods. Continuous EKG recording was conducted throughout all tests using lead II (or another limb lead if lead II was poor quality). Heart rate and blood pressure were recorded at baseline, 15 seconds (Valsalva and orthostatic), 30 seconds (deep breathing and cold pressor), and 1 minute. All clinical examinations, EKGs, and autonomic tests were performed at the local health posts. All seropositive children also underwent echocardiography at the Hospital Honorio Delgado in Arequipa. Parents were

encouraged to be present for all of the child's examinations. After collection of baseline clinical data, infected children were referred to the Ministry of Health for directly observed treatment with benznidazole for 60 days (10 mg/kg per day for children less than 10 years old; 5–7 mg/kg per day for children 10–15 years old).³⁰ Children were monitored by Ministry of Health physicians throughout treatment to assess for side effects of the medication.

Risk factor data collection. Risk factor data were collected for all consenting *T. cruzi*-infected children. Because of resource constraints, one control child per case was chosen at random from among the matched control children included in the clinical component. The study team visited the homes of each child. Data were collected concerning previous travel and places of residence, medical and family history, housing construction material, and number and types of domestic animals. Entomological data collected during vector control activities were provided by the Arequipa Ministry of Health.

Data analysis. EKG and autonomic studies were read and coded by two cardiologists blinded to the infection status of the participant; disagreements were resolved by a third physician with experience reading EKGs of patients with Chagas disease. The third reader categorized some EKGs as borderline, which was defined as abnormal but possibly normal variant and with no typical changes of Chagas disease; these were analyzed as abnormal studies. Statistical analysis was performed with STATA 8.0 (College Park, MD) and SAS 9.0 (Cary, NC). Univariate conditional logistic regression for matched samples was used to analyze clinical differences between groups and risk factors for *T. cruzi* infection. Continuous variables were compared with paired *t* test. Variables associated with *P* value less than 0.2 in univariate analyses were included in a stepwise backwards elimination process to construct the multivariable model. No variable pair with correlation coefficient greater than 0.4 was included in the same model.

RESULTS

Clinical, electrocardiographic, and autonomic findings. The serosurvey included 1,615 children, of whom 75 were confirmed seropositive for *T. cruzi*.^{25,26} A total of 200 of these children (68 infected and 132 uninfected) took part in the case-control study; 54 (79%) cases had two matched controls, whereas nine cases had only one control and five cases had three controls. Case and control children did not differ by age, sex distribution, heart rate, blood pressure, or body mass index for age (Table 1). No children had a history of rheumatic fever, arrhythmia, asthma, or diabetes, and no abnormalities were detected on cardiac examinations. A total of 39 children reported being hospitalized at least one time, most commonly for bone fractures or respiratory infections. However, one infected child had been hospitalized for Chagas disease, and two control children had been hospitalized for lymphocytic leukemia.

EKG data were available for 67 case and 126 control children. Compared with control children, infected children had a lower prevalence of sinus arrhythmia (physiological heart rate variation with respiration), longer corrected QT intervals (QTc), and higher prevalence of left anterior hemiblock, but none of these differences reached statistical significance (Table 2). Seropositive children underwent echocardiography as part of the clinical assessment recommended by the Ministry of Health. Of 65 echocardiograms, only one was abnormal, with

TABLE 1
Characteristics of 68 case children with confirmed *T. cruzi* infection and 131 uninfected control children in Arequipa, Peru

Characteristic	<i>T. cruzi</i> -infected children (N = 68)	Uninfected children (N = 131)*	P
Median age in years (range)	11.6 (6.1–16.4)	11.4 (6.0–15.8)	1.000
Male n (%)	34 (50%)	65 (49.6%)	0.561
Mean heart rate (range)	77.8 (62–92)	78.6 (62–100)	0.240
Mean systolic blood pressure (range)	97 (80–130)	95.7 (60–120)	0.681
Diastolic blood pressure (range)	64.5 (50–80)	63.0 (38–80)	0.650
BMI for age < -2 Z scores†	0	1 (0.89%)	1.000

* One control was lacking physical examination data.

† Body mass index for age Z scores based on the World Health Organization Child Growth Standards.

BMI = body mass index.

mild hypertrophy of the interventricular septum and posterior left ventricular wall. This child's EKG was normal, except for a prolonged QTc interval of 0.46 and a back-rotated axis consistent with the echocardiographic findings.

Infected children showed blunted responses in several autonomic tests (Table 3). Compared with control children, *T. cruzi*-infected children had statistically significantly lower ratios of longest to shortest RR interval for the Valsalva maneuver, cold pressor, and orthostatic tests. Infected children also showed statistically significantly smaller mean change in systolic blood pressure at 15 and 60 seconds into the Valsalva maneuver than control children. There was a trend in the same direction for heart rate and blood pressure responses in the cold pressor and orthostatic tests, but these did not reach statistical significance. There was no association found between autonomic dysfunction and EKG abnormalities.

Risk factors for infection by *T. cruzi*. Risk factor data were collected for 138 children (68 cases and 70 controls); one case and three controls lacked a match, yielding a total for this analysis of 67 cases and 67 controls. There were 34 female and 33 male pairs; the average age was 11.8 years (range = 6.12–19.6 years). Infected children were significantly more likely

TABLE 2
Electrocardiographic findings in 67 children with confirmed *T. cruzi* infection and 126 uninfected control children

EKG finding	<i>T. cruzi</i> -infected children (N = 67)*	Uninfected children (N = 126)*	P
Any EKG abnormality	17 (25.37%)	33 (26.19%)	0.786
Sinus arrhythmia†	4 (5.97%)	20 (15.87%)	0.086
Low voltage	2 (2.99%)	4 (3.17%)	0.886
QRS axis	+48.85	+52.67	0.311
Right-axis deviation	6 (9.23%)	6 (5.26%)	0.258
Left-axis deviation‡	6 (8.96%)	16 (12.70%)	0.326
Any conduction defect	6 (8.96%)	8 (6.35%)	0.451
PR interval in milliseconds	135	131	0.875
First-degree AV block	1 (1.49%)	1 (0.79%)	1.00
QRS interval (milliseconds)	76	78	0.992
Corrected QT interval (milliseconds)	424	370	0.158
Prolonged corrected QTc interval§	14 (20.90%)	22 (17.46%)	0.678
Left anterior hemiblock	3 (4.48%)	2 (1.59%)	0.175
Left posterior hemiblock	0	3 (2.38%)	1.00

* One case child and five control children were missing EKG data.

† Sinus arrhythmia defined as observed variability in heart rate with respiration.

‡ Axis deviation was scored based on age-related data in Peruvian children.⁴⁶

§ Corrected QT interval was calculated using the formula $QTc = QT/\sqrt{(60/\text{heart rate})}$.

AV = atrioventricular.

TABLE 3
Results of four tests of autonomic nervous system function in 67 case children and 126 matched control children

Autonomic test	<i>T. cruzi</i> -infected children (N = 67)*	Uninfected children (N = 126)*	P
Deep breathing test			
Mean ratio of longest to shortest RR interval	1.193	1.204	0.418
Valsalva maneuver test			
Mean ratio of longest to shortest RR interval	1.575	1.645	0.013
Mean change in HR from 0 to 15 seconds†	+11.21%	+12.85%	0.292
Mean change in HR from 15 to 60 seconds	-16.14%	-17.35%	0.307
Mean change in SBP from 0 to 15 seconds‡	+6.69%	+9.42%	0.011
Mean change in SBP from 15 to 60 seconds	-5.04%	-7.01%	0.034
Cold pressor test			
Mean ratio of longest to shortest RR interval	1.322	1.377	0.003
Mean change in HR from 0 to 60 seconds	-1.03%	+1.96%	0.031
Mean change in SBP from 0 to 30 seconds	+4.69%	+7.20%	0.015
Mean change in SBP from 0 to 60 seconds	+8.26%	+11.49%	0.019
Orthostatic test			
Mean ratio of longest to shortest RR	1.464	1.560	0.000
Mean change in HR from 0 to 15 seconds	+11.37%	+14.07%	0.065
Mean change in HR from 15 to 60 seconds	-5.89%	-6.71%	0.756
Mean change in SBP from 0 to 15 seconds	+3.21%	+5.16%	0.252
Mean change in SBP from 15 to 60 seconds	-1.64%	-3.74%	0.117

* One case child and four control children were missing autonomic examination data; the respective control for the infected child was also excluded from this analysis.

† HR = heart rate; 0 seconds signifies pretest value.

‡ SBP = systolic blood pressure.

to report the presence of triatomines in the home or having been bitten by a triatomine (Table 4). Additional variables associated with *T. cruzi* infection included having a household member previously diagnosed with Chagas disease, parents born outside of the province of Arequipa, signs of triatomine infestation (feces on the wall or shed exoskeletons) in the child's room, the presence of animals in the home, especially in the child's bedroom, and the use of loose stone or woven mats in household walls. The presence of fully plastered walls in the home was protective against infection. Documented capture of triatomines in the house or peridomestic area during Ministry of Health vector control activities was not significantly associated with infection. In multivariable model, three variables were significantly associated with increased risk of infection: reporting a relative or household member with Chagas disease, the presence of dead triatomines or triatomine feces in the child's bedroom at the time of interview, and an animal sleeping in the child's room at night (Table 5).

DISCUSSION

The most interesting finding in our data was consistent blunting of cardiovascular autonomic responses in *T. cruzi*-infected children compared with their matched controls. Ours are the first such data for children with chronic *T. cruzi* infection, but similar findings have been reported in infected adults.^{13–15,31}

TABLE 4

Risk factors for *T. cruzi* infection based on univariate conditional logistic regression of data from 67 *T. cruzi*-infected children and 67 matched control children

	Odds ratio (95% confidence interval)	P
Family characteristics		
Mother born outside Arequipa	4.0 (1.5–10.7)	0.006
Father born outside Arequipa	3.4 (1.3–9.2)	0.016
Both parents born outside Arequipa	5.3 (1.8–15.3)	0.002
Relative or household member with Chagas disease	17 (2.6–127)	0.006
Entomological characteristics of the home		
Any triatomines captured in house or yard*	1.2 (0.6–2.3)	0.613
Any triatomines captured inside house*	1.1 (0.57–2.2)	0.732
Risk per additional vector captured in house or yard*	1.0 (0.97–1.1)	0.304
Risk per additional vector captured inside house*	1.1 (0.97–1.2)	0.163
Triatomines in house†	16.5 (2.2–123)	0.006
Child bitten by triatomine†	18.1 (4.3–76)	< 0.001
Characteristics of child's room		
Dead triatomines or triatomine feces	20 (2.7–149)	0.003
Plastered ceiling	0.36 (0.12–1.1)	0.083
Cracks in the walls or ceiling	2.00 (0.75–5.3)	0.166
Dog sleeps in room	3.3 (0.9–12.1)	0.067
Cat sleeps in room	2.8 (0.9–8.6)	0.083
Any animal sleeps in room	3.8 (1.2–11.3)	0.019

* Based on routine Ministry of Health vector collection at the time of indoor residual insecticide application.

† Reported by respondent in study interview.

Because the absolute differences in performance on the autonomic tests between groups were small and all children were well, we cannot interpret the clinical significance of this finding. Pathological studies of Chagas disease show marked parasympathetic denervation, both in human autopsy specimens and animal models.³² The neuronal damage is thought to occur during the acute phase of the infection and is presumed to be permanent.¹² The occurrence of autonomic dysfunction in children as well as adults is consistent with this hypothesis. What is less clear is how autonomic dysfunction relates to the pathogenesis of Chagas cardiomyopathy.^{12,19} Some investigators postulate that these are independent phenomena.¹⁷ Because this study was not longitudinal in design and none of our subjects had any evidence of clinically significant cardiac dysfunction, we could not draw any conclusions about the relationship between autonomic abnormalities and the development of Chagas cardiomyopathy. Although we found no association between autonomic test results and EKG findings, we found few EKG abnormalities and may have lacked the statistical power to detect such an association.

In contrast to earlier studies showing a substantial prevalence of conduction system disease in infected children in highly affected areas,^{8,9} we found no significant difference in

the prevalence of EKG abnormalities between seropositive and seronegative children. Indeed, we found a low frequency of the conduction system abnormalities (right bundle branch block and left anterior hemiblock) and ventricular arrhythmias typical of Chagas disease in our case children. This finding may reflect lower intensity of parasite exposure, lower parasite burdens, and/or strain differences in Arequipa compared with other study sites. The lower infection prevalence in Arequipa suggests a lower force of infection compared with study sites in Brazil, Argentina, or Bolivia,^{33–36} whereas the relatively flat age prevalence curve supports the hypothesis that the parasite was only introduced into the periurban communities of Arequipa in the early 1990s.²⁶ Investigators have shown that repeated infection increases cardiac pathology in animal models^{37,38} and have suggested that vector control, by decreasing reinfection rates, may be responsible for the milder disease now seen in formerly endemic communities.³⁹ Similarly, the children in our study may have had few repeated infections after their initial exposure. Another hypothesis, not mutually exclusive, is that the *T. cruzi* strain in Arequipa is less virulent than strains found in other parts of South America. Our previous work shows that the immunological responses to whole epimastigote lysate and recombinant antigens are substantially lower than in specimens from Bolivia.⁴⁰

The risk factors that we identified for urban transmission of Chagas disease were quite similar to those found in rural epidemiological studies.^{20,22,41} Not surprisingly, household members' reports of triatomine infestation as well as traces of triatomine exuvia or feces in the child's room were very strong predictors of infection. However, household infestation rates based on bugs collected by the Ministry of Health spray teams were not strongly associated with the child's risk of infection. Heavy infestation was associated with increased risk in studies in Argentina, and vector density was shown previously to be an independent risk factor for infection in children in Guadalupe (one community within the current study area).^{25,41} Reports from families and the presence of triatomine exuviae and feces may preferentially indicate heavier infestations, which more strongly elevate the risk of *T. cruzi* infection. Other risk factors for *T. cruzi* infection in the child included the reported presence of Chagas disease in a relative or household member, a factor that could reflect both shared exposures and the possible role of the infected household member as a reservoir of the parasite.

In studies from Argentina, the presence of dogs was strongly associated with infection risk.^{20–24} In our data, the presence of a dog per se did not significantly increase risk of infection; the majority of households owned dogs. However, allowing an animal to sleep in the child's bedroom was associated with significantly increased *T. cruzi* risk; this factor was associated with increased bedroom vector infestation in our earlier analysis.²⁵ Building materials, in particular those used in the child's room, also altered the risk of *T. cruzi*. In Arequipa, most shantytown homes are built of basalt stones, brick, or sillar that are either stacked or held together with cement and corrugated metal or brick roofs. Cracks in the walls and unmortared bricks increased risk, whereas plastering the walls and ceiling with stucco was protective, presumably by decreasing triatomine hiding places and eliminating crevices protected from insecticide application.^{25,42}

Our study had a number of limitations. Risk factor studies for *T. cruzi* suffer unavoidably from the chronic character of

TABLE 5

Risk factors for infection with *T. cruzi* based on a multivariable conditional logistic regression model of 67 matched case-control pairs

	Odds ratio (95% confidence interval)	P
Relative or household member with Chagas disease	16.2 (0.7–357)	0.077
Dead triatomines or triatomine feces in child's bedroom	44.2 (3.2–612)	0.005
Any animal sleeps in child's bedroom	14.8 (1.4–162)	0.027

this infection: the factors measured now may not indicate the situation 5 or 10 years ago when children were initially infected. The youngest study children were not always able to perform some of the autonomic tests, particularly, deep breathing and Valsalva maneuver, exactly as directions; the Valsalva maneuver might also have been better standardized if a pressure gauge mouthpiece had been available. Most tests were done in the afternoon after school, but a proportion was performed in the morning, introducing the possibility of diurnal variation and differences in food and caffeine intake, which may have affected hemodynamic measures and autonomic responsiveness. Furthermore, there are few data describing autonomic function in healthy children, and normal limits have never been defined; our study may be the largest pediatric group with autonomic testing reported to date. Finally, our data are cross-sectional and therefore, cannot address the question of the prognostic value of autonomic dysfunction to predict the development of cardiomyopathy.

Our risk factor data highlight the importance of household insecticide application programs to interrupt domestic vector-borne transmission of *T. cruzi*. Long-term success will require surveillance for and effective responses to reinfestation.⁴³ Meanwhile, advice to keep animals from sleeping in bedrooms and appropriate location-specific housing improvements may help to decrease infestation and infection risk. Targeted screening strategies may improve the efficiency of programs to detect infected children and facilitate early treatment.²⁷ Antitrypanosomal drug treatment is indicated for all *T. cruzi* infected individuals younger than 18 years, with a recent trend to treat adults as well,^{44,45} introducing important ethical considerations when considering the possibility of longitudinal cohort studies to investigate the prognostic value of autonomic dysfunction to predict development or progression of cardiomyopathy.

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Authors' addresses: Natalie M. Bowman, Division of Infectious Diseases, University of North Carolina—Chapel Hill, Chapel Hill, NC; and Department of Internal Medicine, The Johns Hopkins Hospital, Baltimore, MD, E-mail: nbowman@post.harvard.edu. Vivian Kawai and Vivian V. Pinedo-Cancino, Asociación Benéfica PRISMA, Lima, Peru, E-mails: vivian_kawai@yahoo.com and vivi_vane@hotmail.com. Robert H. Gilman, Asociación Benéfica PRISMA, Lima, Peru; and The Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, E-mail: rgilman@jhsph.edu. Cesar Bocangel, Juan Geny Cornejo del Carpio, and Freddy Delgado, Arequipa

Ministry of Health, Arequipa, Peru, E-mails: c_bocangel@yahoo.com, juanepi50@hotmail.com, and fredeldi@hotmail.com. Gerson Galdos-Cardenas, The Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD; and Arequipa Ministry of Health, Arequipa, Peru, E-mail: gersongaldos@gmail.com. Lilia Cabrera, Asociación Benéfica PRISMA, Lima, Peru, E-mail: lcabrera@peruresearch.com. Michael Z. Levy, University of Pennsylvania, Philadelphia, PA; and Fogarty International Center, National Institutes of Health, Bethesda, MD, E-mail: mzlevy@mail.med.upenn.edu. Lauren Rosenthal, Morgan Stanley Children's Hospital of New York, New York, NY, E-mail: laurenbroenthal@yahoo.com. Francis Steurer and Caryn Bern, Centers for Disease Control and Prevention, Atlanta, GA, E-mails: fjs1@cdc.gov and cxb9@cdc.gov. Amy E. Seitz, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, MD, E-mail: Amy.Seitz@nih.hhs.gov. James H. Maguire, Harvard University School of Medicine, Boston, MA, E-mail: jmaguire@partners.org.

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