Chagas Disease in Southern Coastal Ecuador: Coinfections with Arboviruses and a Comparison of Serological Assays for Chagas Disease Diagnosis

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Abstract. Occurrence of Chagas disease and arbovirus coinfections is unknown, despite the vast co-endemic areas throughout the Americas. This study examined the proportion of individuals positive for *Trypanosoma cruzi* and coinfections with dengue, chikungunya, and Zika viruses in Machala, Ecuador (January 2014–December 2015). Chagas seropositivity was evaluated with five commercially available assays. Dengue infections were identified by nonstructural protein 1 rapid test and enzyme linked immunosorbent assay (ELISA), immunoglobulin M ELISA, and reverse transcription PCR (RT-PCR); chikungunya and Zika infections were identified by RT-PCR. Of 658 individuals, six were positive for *T. cruzi* (0.91%), including one *T. cruzi*/dengue coinfection and one *T. cruzi*/chikungunya/dengue coinfection. The clinical manifestations of coinfecting individuals corresponded to severe dengue and dengue with warning signs, respectively. We observed discrepant results by using the Hemagen Chagas kit and the rapid test Chagas Detect Plus (false positives: 3.9% and 15.4%), highlighting the need to assess diagnostic assays in geographic regions with distinct taxonomic units of *T. cruzi*.

Chagas disease and arboviral illnesses are co-endemic throughout the Americas, but their coinfection prevalence and implications on disease severity are largely unknown.¹ Chagas disease is caused by *Trypanosoma cruzi*, a protozoal parasite that comprises six distinct taxonomic units (DTU) Tc I–Tc VI.² *Trypanosoma cruzi* is transmitted by blood-sucking reduviid bugs of the subfamily Triatominae. Chagas disease acute phase involves mostly asymptomatic or self-limiting febrile illness; however, death can occur because of severe myocarditis or meningoencephalitis. The chronic phase includes the indeterminate form of the disease that is mostly asymptomatic and the determinate form with life-threatening cardiac, digestive, or cardio-digestive manifestations.³ Arthropod-borne viruses (arbovirus) such as dengue (DENV), chikungunya (CHIKV), and Zika (ZIKV) viruses cause the most rapidly spreading arboviral illnesses in the Western Hemisphere. These arboviruses are primarily transmitted to humans by *Aedes aegypti* and *Aedes albopictus* mosquitoes. Dengue, chikungunya, and Zika infections clinically present as febrile illness with similar manifestations; however, early recognition of dengue fever is crucial as it can quickly progress to a life-threatening condition.⁴ Dengue manifestations range from mild to severe febrile illness including hemorrhage, shock, and death.⁵ Although chikungunya is mostly asymptomatic, the febrile illness is characterized by severe arthralgias.⁶ Zika febrile illness is generally mild, however women of childbearing age and pregnant women with suspected ZIKV infection are at risk of birth defects in their offspring, including microcephaly and brain malformation.⁷

Coinfections with multiple pathogens affect more than one-sixth of the world population and remain a leading challenge for global public health because of their impact on

transmission, clinical progression, and control of multiple infectious diseases.⁸ In addition, coinfections are often under-diagnosed because of limited resources for diagnostic assays. Coinfecting pathogens can interact either directly with one another or indirectly through the host's immune system, and consequently alter disease progression. For instance, parasitic infections that dramatically favor a more antibody-mediated, humoral T helper 2 (Th2) immune response can counteract antiviral cellular mechanisms, promoting increased viral replication as seen in helminth and herpes virus coinfections.⁹ During DENV infection, an adaptive T helper 1 (Th1)-skewed immune response is established and Th2 polarization of the Th1/Th2 immune system determines the severity of clinical dengue.¹⁰ In this context, the Th2-polarized immune response during the chronic indeterminate form of Chagas disease could potentially worsen the severity of dengue fever.¹¹ Thus, understanding the nature and consequences of *T. cruzi*/arboviral coinfections on disease manifestation is necessary to elucidate adequate therapeutic interventions.

Despite effective prevention interventions, Chagas disease continues to pose a major burden on low-income rural populations in the Americas, where an estimated six to seven million people are infected with *T. cruzi*.¹² Simultaneously, the distribution and incidence of dengue fever in the region have increased dramatically over the past three decades; in 2016 alone, 2.3 million cases of dengue were reported.¹³ Recent outbreaks of CHIKV and ZIKV in the Americas resulted in more than two million cumulative cases of CHIKV and 712,167 cases of ZIKV.^{14,15} In Ecuador, nearly 200,000 people are infected with *T. cruzi* and 3.8 million are at risk of infection.¹⁶ *Trypanosoma cruzi* infections in Ecuador are mainly DTU Tc I.¹⁷ Over a 10-year period (2005–2014), 103,999 cases of dengue fever were reported in the country, with the greatest burden of disease in coastal urban areas. In 2015 and 2016, the first outbreaks of chikungunya and Zika fever were reported in Ecuador, respectively.

The aim of this study was to identify *T. cruzi* infections and coinfections with DENV, CHIKV, and ZIKV in Machala,

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Ecuador. Dengue virus prevalence rates in people living near positive DENV cases (< 200 m) in Machala range from 13% to 36%.¹⁸ Although a high burden of *T. cruzi* infection has historically been reported in Machala to date, active transmission is considered to be under control by the Ministry of Health (MoH); however, frequent population migration from nearby Chagas-endemic regions poses a significant risk of imported *T. cruzi* infection.

From January 2014–December 2015, individuals were recruited from a DENV surveillance study (described previously).¹⁸ The protocol was approved by institutional review boards at SUNY Upstate Medical University and the Luis Vernaza Hospital, and by the Ecuador MoH. Patients with suspected DENV infection (> 6 months) at MoH sentinel clinics were eligible for inclusion in the study (i.e., index cases). We obtained informed consent, recorded demographic information, and collected a blood sample that was tested for DENV using a nonstructural protein 1 (NS1) antigen rapid test. Up to four index cases that tested positive for DENV were selected each week (initiate index cases). We visited the homes of initiate index cases and recruited household members into the study (i.e., associate cases). Blood samples and demographic information were collected from up to four household members. This was repeated in four households within 200 m of the index household, the typical flight range of the *Ae. aegypti* mosquito.

We tested 658 serum samples for *T. cruzi* infection using the ORTHO *T. cruzi* ELISA test system (Ortho Clinical Diagnostics, Raritan, NJ), at SUNY Upstate Medical University in Syracuse, NY, and at the central hospital in Machala, Ecuador. All preliminary positive, equivocal, and 12 negative samples (matched by age, gender, housing type, and location) were reassayed by three conventional confirmatory tests at Johns Hopkins University in Baltimore, MD. We used the trypanomastigote excreted–secreted antigens (TESA) blot assay that measures the immunoreactivity to *T. cruzi* TESA, and two Food and Drug Administration (FDA)-approved ELISA tests, the Hemagen Chagas ELISA kit (Hemagen, Inc., Columbia,

MD) and the Chagatest ELISA recombinant v.3.0 (Wiener, Rosario, Argentina). In addition, the current FDA-approved rapid test Chagas Detect Plus (CDP) (InBios International, Seattle, WA) was also evaluated. Trypomastigote excreted–secreted antigens blot assay was carried out as previously described.^{19,20} ELISA optical density (OD) values and positive reactivity by the rapid test CDP were defined following the manufacturer's recommendations. Equivocal samples were reassayed three times, and concordant results considered as final. Chagas-positive diagnosis was determined by agreement in at least two conventional serological tests. Dengue virus–positive samples were defined using PanBio NS1 rapid strip, PanBio NS1 ELISA, PanBio IgM ELISA, or reverse transcription–PCR (RT-PCR). Samples from index cases in 2014 and index cases and associates in 2015 were also screened for CHIKV. Only samples from index cases and associates in 2015 were screened for ZIKV using RT-PCR. The arbovirus diagnostic protocol was described previously.¹⁸

Of the 658 samples, we found that 0.9% were positive for *T. cruzi* (6/658) by three confirmatory diagnostic tests (Table 1). Three of the six *T. cruzi*-positive cases were index cases, with a clinical diagnosis of dengue fever; three were associate cases; and all six were from different households distributed across the city of Machala. *Trypanosoma cruzi*-positive individuals were on average 63 years of age and 67% were female (Table 2). The gender distribution was similar to the overall study population (59% female); however, *T. cruzi*-positive individuals were older than the average of the study population (30 years of age). Of the three index cases that were *T. cruzi* positive, one was positive for DENV, one was positive for CHIKV and DENV (0.1%, 1/658), and one was negative for DENV/CHIKV/ZIKV. The three associate cases that were positive for *T. cruzi* were negative for DENV/CHIKV/ZIKV and did not display any arboviral symptoms (Tables 2 and 3). All *T. cruzi* cases had high signal-to-cutoff ratios and OD values in all ELISA tests; we found no differences between the *T. cruzi* mono-infection and *T. cruzi*/arbovirus coinfections (Table 3); however, further studies should evaluate this preliminary finding.

TABLE 1
Chagas disease serology results in samples from Machala, Ecuador

Screening <i>Trypanosoma cruzi</i> results		ORTHO <i>T. cruzi</i> ELISA test system* (n = 658)				
Positive	n (%)	6/658 (0.9%)				
	Mean (SD)	4.9 (1.36)				
Equivocal†	n (%)	8/658 (1.2%)				
	Mean (SD)	0.9 (0.13)				
Negative	n (%)	644/658 (97.8%)				
	Mean (SD)	0.18 (0.10)				
Confirmatory <i>T. cruzi</i> results		ORTHO <i>T. cruzi</i> ELISA test system* (n = 26)	Hemagen Chagas kit ELISA‡ (n = 26)	Chagatest ELISA‡ recomb. v.0.3 (n = 26)	Trypomastigote excreted–secreted antigens blot (n = 26)	Rapid test Chagas Detect Plus (n = 26)
True positive	n (%)	6/26 (23.1%)	6/26 (23.1%)	6/26 (23.1%)	6/26 (23.1%)	6/26 (23.1%)
	Mean (SD)	4.9 (1.36)	1.3 (0.35)	2.14 (0.92)	NA	NA
False positive	n (%)	0/26 (0%)	1/26 (3.8%)	0/26 (0%)	0/26 (0%)	4/26 (15.4%)
	Mean (SD)	NA	1.3	NA	NA	NA
Equivocal†	n (%)	0/26 (0%)	1/26 (3.8%)	0/26 (0%)	0/26 (0%)	0/26 (0%)
	Mean (SD)	NA	0.2	NA	NA	NA
Negative§	n (%)	20/26 (76.9%)	18/26 (69.2%)	20/26 (76.9%)	20/26 (76.9%)	16/26 (61.5%)
	Mean (SD)	0.31 (0.10)	0.1 (0.05)	0.001 (0.001)	NA	NA

NA = not applicable.

* Mean and SD correspond to signal-to-cutoff ratio.

† Equivocal results were defined when discordant results were obtained in two independent measurements by the ORTHO *T. cruzi* ELISA test system, and Chagas Detect Plus, or when ELISA optical density (OD) values were 10% higher than the cutoff of the test by the Hemagen Chagas kit, and Chagatest ELISA recombinant v.3.0.

‡ Mean and SD correspond to OD.

§ Subset of samples matched to positive cases by age, gender, and geographical location.

TABLE 2

Demographic and clinical presentation of the six Chagas-positive cases

Demographics	No. case					
	1	2	3	4	5	6
Gender	F	F	M	M	F	F
Age (years)	77	42	58	58	73	69
Clinical presentation						
Clinically diagnosed with dengue fever	+	-	+	-	-	+
Hospitalized	-	-	+	-	-	-
Symptoms in previous 7 days						
Fever	+	-	-	-	-	+
Headache	-	-	-	+	-	+
Nausea	+	-	+	-	-	-
Muscle pain	+	-	-	-	-	+
Rash	-	-	-	-	-	+
Bleeding	-	-	+	-	-	-
Rhinorrhea	-	-	-	-	-	-
Vomiting	+	-	+	-	-	-
Drowsy	+	-	+	-	-	+
Cough	-	-	-	-	-	-
Abdominal pain	+	-	+	-	-	+
Diarrhea	-	-	+	-	-	+
Retro-orbital pain	+	-	-	-	-	+

Two index cases had *T. cruzi*/arbovirus coinfections (0.8%, 2/242 dengue-positive cases). Case 3 had a *T. cruzi*/DENV coinfection, with an acute secondary dengue infection, as defined by positive IgM and immunoglobulin G ELISA tests (Tables 2 and 3). The patient was hospitalized with characteristic severe dengue symptoms, including nausea, hemorrhage, vomiting, drowsiness, abdominal pain, and diarrhea. The patient was admitted for presumed upper digestive hemorrhage, which was confirmed by endoscopy as intense epithelial hemorrhage with signs of intense subepithelial hemorrhage of the stomach. Based on the 2009 WHO classification, this individual had dengue with warning signs.⁵ The severe clinical presentation is consistent with secondary dengue infections in this population.¹⁸ Case 6 had a *T. cruzi* and CHIKV/DENV coinfection, and presented an acute primary DENV infection with warning signs (abdominal pain and lethargy), but was not hospitalized. Although the clinical presentation/disease severity of the two *T. cruzi*/arboviral coinfections in our study corresponded with classical severe dengue and dengue with warning signs, respectively, the potential negative impact of coinfection on disease progression/outcome requires further study. Our findings provide the first evidence of *T. cruzi*/arbovirus coinfections in the area to our

knowledge. Although we found a low proportion of *T. cruzi*/DENV and *T. cruzi*/DENV/CHIKV coinfections (0.1%) in a Chagas disease-controlled area of Ecuador, our result supports the need to define *T. cruzi*/arbovirus coinfection prevalence in high-risk populations to guide public health interventions.

Our data indicate that the proportion of individuals positive for *T. cruzi* in El Oro Province in this study is similar to those observed by other groups (1.0% prevalence) in the adjacent Guayas Province.²¹ Higher prevalence rates have been documented in Ecuador in Manabi Province (5.7%) and neighboring Loja Province (3.6%). Five of the six *T. cruzi* cases identified in this study were individuals who previously resided in nearby Chagas-endemic areas (the uplands of El Oro Province, and the Loja Province), where active transmission has been reported. Only one case is a presumable local infection, as the individual was born in urban Machala and had not visited endemic regions. These findings highlight the impact of population migration on the prevalence of Chagas disease and the occurrence of Chagas-arbovirus coinfection in transmission-controlled areas, such as the city of Machala.

With the recent globalization of Chagas disease due to imported *T. cruzi* infection in non-endemic areas, the validation of serological test accuracy is pivotal for successful control interventions. Chagas diagnostic test performance is primarily influenced by geographical differences in *T. cruzi* genotypes, among other factors. Although FDA approved, most of the commercially available rapid and ELISA assays' sensitivities and specificities are based on DTU Tc II, V, and VI infections present in the southern cone of Latin America.²² Most Chagas assays have not been validated previously in DTU I infections common in Ecuador. We compared five serological tests for Chagas disease in a subset of 26 samples, using the TESA blot assay as reference test. We observed discrepant results by the Hemagen Chagas kit and the rapid test CDP, with false-positive percentages of 3.9% (1/26) and 15.4% (4/26), respectively (Table 1). These false-positive results could be because of high analytical sensitivity, cross-reactivity with other agents (e.g., *Leishmania* parasites), and/or low positive predictive value in an area of low infection prevalence. In addition, the Hemagen Chagas kit and the OrthoELISA provided equivocal results with percentages of 3.9% (1/26) and 1.2% (8/658), respectively. Equivocal results by the OrthoELISA were determined as negative after reassaying the samples, and the mean OD changed from 0.9 (0.1 SD, 8/658) to 0.1 (0.2 SD, 8/8), respectively (Table 1). However, false-negative results were not observed with any of the tests.

Our preliminary observations suggest that the Chagatest ELISA recombinant v.3.0, the ORTHO *T. cruzi* ELISA system,

TABLE 3
Diagnostic results of the six individuals who were positive for Chagas disease

No. case	Chagas			Dengue				Chikungunya		Zika
	Ortho ELISA test system*	Hemagen Chagas kit ELISA†	Chagatest ELISA recomb. v.3.0†	Clinical diagnosis	Rapid test	Nonstructural protein1 ELISA	RT-PCR	IgM	IgG	RT-PCR
1	3.9	1.1	0.9	+	-	-	-	-	-	n/a
2	5.9	1.4	2.8	-	-	-	-	-	-	n/a
3	5.6	1.5	2.9	+	+	+	+	+	+	n/a
4	5.3	1.4	2.4	-	-	-	-	-	-	-
5	2.6	1.6	0.9	-	-	-	-	-	-	-
6	5.9	1.6	2.7	+	-	-	-	+	-	-

RT-PCR = reverse transcription-PCR.

* Signal-to-cutoff ratio.

† Optical density.

and TESA blot provide comparable diagnostic results in suspected DTU I infections (in our study population) to those previously reported in DTU II and V.¹⁹ However, our observation of false-positive results by the rapid test CDP (15.4%) contradicts recent reports and suggests that geographical variability of the CDP test requires further evaluation.²³ Chagas serology test validation in non-endemic areas, such as the United States, where most imported *T. cruzi* infections are from DTU I-prevalent areas, is crucial to facilitate accurate disease diagnosis.

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