Am. J. Trop. Med. Hyg., 86(6), 2012, pp. 972–979
doi:10.4269/ajtmh.2012.11-0652

Chagas Disease: Assessing the Existence of a Threshold for Bug Infestation Rate

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Abstract. To examine the existence of a possible threshold for the domestic infestation rate of *Triatoma dimidiata*, below which transmission becomes unlikely, a census was conducted in 59 Chagas disease endemic communities of El Salvador and Honduras. Entomological and serological tests were conducted targeting 4,083 households and 6,324 children between 6 months and 15 years of age. The overall domestic infestation rate of *Triatoma dimidiata* and seroprevalence among children were 12.9% and 0.49%, respectively. Communities with a domestic infestation rate at 8% or less consistently showed a seroprevalence of 0%. In communities with a domestic infestation rate above 8%, there was a wide range in seroprevalence. A domestic infestation rate of 8% could serve as the possible threshold below which transmission would become unlikely. The implementation of an 8% threshold for determining needs for universal insecticide spraying would lead to a 21% reduction in spraying-related costs.

INTRODUCTION

Today, neglected tropical diseases (NTDs) are a symptom of poverty and socioeconomic disadvantage.[1] Chagas disease, one of the 14 priority NTDs adopted by the World Health Organization (WHO), correlates closely with poverty[2] and costs US$ 1.2 billion per year in productivity loss,[3] because it is more prevalent among those living in poor housing structures where infestation with the Chagas vector bugs is frequent. It is also the world's fourth most critical parasitic disease in terms of the priority needs for disease elimination and control, after malaria, schistosomiasis, and intestinal worms.[4] Chagas disease was recently recognized not only as a Latin American endemic but also as a global epidemic, caused by transfusion-based transmission from serologically positive individuals who have migrated internationally[5] and mother-to-child transmission among migrants.[6,7] Nevertheless, vectorial transmission remains a major route of infection, accounting for more than 80% of the total number of transmissions.[3,8] This indicates that vector control in endemic areas continues to be a fundamental strategy for addressing the root causes of global spread of the disease.

To combat the spread of Chagas disease, seven Central American countries (Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, and Panama) support the Initiative of the Central American Countries for the Control of Chagas Disease (IPCA) launched in 1998, with the goal of eliminating transmission of Chagas disease by 2010. This goal was accordingly adopted at the 51st World Health Assembly.[9] One of the three specific targets of the IPCA is to realize the elimination of transmission through control of *Triatoma dimidiata* (*T. dimidiata*), a major vector of Chagas disease in Central America.[10] Significant progress in controlling *T. dimidiata* was recently achieved in El Salvador, Honduras,[11] and Guatemala,[12] through massive insecticide spraying campaigns. However, *T. dimidiata*, an indigenous species in Central America, cannot be entirely eliminated, because it infests not only housing structures but also peri-domestic and silvatic habitats.[10] On the other hand, *T. dimidiata* is a less effective vector than *Rhodnius prolixus* (*R. prolixus*), the other major vector in Central America.[13,14] The target for control of Chagas disease is therefore not complete elimination of *T. dimidiata*, but instead reduction in its domestic infestation rate to the levels below which transmission would become unlikely. This raises the question: *How far should we continue the use of spraying to reduce infestation by T. dimidiata?* The current common practice of controlling *T. dimidiata* in Central America is to reduce and maintain its domestic infestation rate (i.e., proportion of houses infested with a vector bug in a community) at 5% or less.[15] In other words, once the domestic infestation rate of a community is found to be 5% or higher, universal insecticide spraying is then practiced in the community. However, this target figure (5%) is not supported by rigorous evidences but rather derived from data on *Triatoma infestans* (*T. infestans*) in Brazil without scientific justification.[15] It is not clear to what extent the domestic infestation rate of *T. dimidiata* needs to be reduced and maintained to ensure a seroprevalence of 0%. Although 5% could continue to be used as the target figure, there is a critical need to scientifically explore the possible threshold below which a lower seroprevalence of Chagas disease would be achieved.

An evidence-based threshold of the domestic infestation rate, if it exists, may significantly affect the feasibility to achieve the IPCA target, either by saving resources or by requiring additional resources (e.g., insecticide, human resources for sprayings, households' costs associated with pre- and post-spraying activities). Therefore, in this study, we aimed to examine the existence of a possible threshold for a domestic infestation rate of *T. dimidiata,* below which transmission becomes unlikely and interruption of transmission is thus ensured or expected. If it is found to exist, then we further aimed to estimate the expected impact of application of the possible threshold such as cost saving through avoiding unnecessary spraying.

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MATERIALS AND METHODS

To examine the existence of a possible threshold of a domestic infestation rate of *T. dimidiata*, a census was conducted targeting all the households and all the children between 6 months and 15 years of age in the selected Chagas disease endemic communities of El Salvador and Honduras, from September 2008 to April 2009. Because adult forms of *T. dimidiata* predominate during the dry season from April to June,[16,17] the domestic infestation rate of *T. dimidiata* during the rest of the year remains stable. Therefore, this relatively longer survey period from September to April does not affect the comparability of the domestic infestation rates measured over time.

Target area selection. Four steps were taken for selection of target communities. First, of 31 provinces in El Salvador and Honduras, 22 were identified as Chagas disease endemic provinces. A Chagas disease endemic province is defined as a province with any previous reported cases of presence of vector bugs and seropositivity identified in a household survey or at blood banks. Second, of the 22, 15 were further selected as the priority endemic provinces that require increased public health interventions, because they were reported to have both a *T. dimidiata* domestic infestation rate of 10% or greater during the past 1-year period from September 2007 to August 2008 and at least one seropositive case identified at local blood banks during the same period (Figure 1). Third, of the 13,861 communities in these 15 provinces, 13,488 were estimated as the communities free of *R. prolixus*. This step enabled the study to select communities with vector transmissions only through *T. dimidiata*, by excluding those infested with *R. prolixus*. Fourth, of the 13,488 communities, 59 were further selected by applying four criteria (Figure 2), i.e., communities: 1) with more than 25 households; 2) with infrequent population migrations; 3) within 90 and 110 km from provincial capitals respectively for El Salvador and Honduras; and 4) without insecticide spraying during the past 15 years. This step allowed the study to avoid having outliers of a seroprevalence and domestic infestation rate caused by smaller denominators, and to meet resources available for the survey; it also allowed the study to focus on seropositivity attributable primarily to local vector transmission through *T. dimidiata*. Selection of communities without spraying during the last 15 years enabled the study to assume all the target children between 6 months and 15 years of age in a community have been exposed to the same level of risk of transmission.

Entomological test. All the housing structures including public facilities in the 59 communities were visited to detect *T. dimidiata* infestation. The one-person-hour method[18] was used for the entomological test, as it is the standard method commonly practiced in both El Salvador and Honduras. A pair of two trained investigators searched for *T. dimidiata* bugs in both intra-domestic and peri-domestic locations, by spraying Aqua Resilin (Bayer Inc., Durham, NC), a flush-out insecticide sensitive to *T. dimidiata*. First, the pair attempted to search *T. dimidiata* bugs after flush-out spraying, by spending 40 person-minutes (20 minutes per investigator). When any *T. dimidiata* bugs were detected in the bedroom, the housing structure was judged to be intra-domestically infested. Second, after completing search for indoor bugs, the pair moved to small-scale, man-made facilities located in the peri-domestic areas (kitchen, latrine, bathroom, poultry house, and animal shed) to determine whether the house was peri-domesitcally infested. Twenty person-minutes (10 minutes per investigator) were spent in this investigation. When any *T. dimidiata* bugs were detected in the peri-domestic area, the housing structure was judged to be peri-domestically infested. A housing structure either intra-domestically or peri-domestically infested was judged to be domestically infested with *T. dimidiata*. Once a *T. dimidiata* bug was detected in the house, both investigators moved on to the peri-domestic investigation to improve time efficiency. The proportion of time spent between intra- and peri-domestic investigations was adjusted to housing structure and living environment. At public facilities such as churches and community centers, the most commonly used room and its outdoor surroundings were investigated.

Serological test. All children between 6 months and 15 years of age residing in the households examined for *T. dimidiata* infestation were selected as the targets for serological testing to detect transmission of *Trypanosoma cruzi* (*T. cruzi*). This age group is eligible to receive chemotherapy without significant side effects, once *T. cruzi* has been detected serologically. Enzyme-linked immunosorbent assay (ELISA) immunoglobulin G (IgG) was performed with the commercial kit Chagatest ELISA recombinant (Wiener Laboratory, Rosario, Argentina) in two stages as the Central American regional standard.[19,20] First, an ELISA IgG test was applied to blood samples absorbed into filter paper. Second, when the result was positive, another ELISA IgG test was then further applied to liquid blood of the relevant individuals. This study determined that those who were positive in both stages were serologically positive. The ELISA IgG tests were conducted at the National Chagas Disease Laboratory in El Salvador and Doctor Max Bloch Bacteriology Central Laboratory, Ministry of Health, in Honduras.

DATA ANALYSIS

The data obtained through entomological tests were field-checked and entered into a computer. Unique numbers were locally assigned to blood samples collected for serological tests. The results of ELISA IgG tests were then further incorporated into the dataset, and analyzed using SPSS for Windows, version 11.0 (SPSS Inc., Chicago, IL).

Ethics. Ethical approval was obtained from the national authorities of both countries: the National Committee for Clinical Research Ethics Committee of El Salvador and the Ethics Committee for Biomedical Research (IRB), the National Autonomous University of Honduras. Verbal approval to conduct the study was provided by the community leaders in all 59 communities. Informed consent to participate in the study was verbally obtained before entomological tests from household heads. When conducting serological tests among children between 6 months and 15 years of age, informed consent was obtained from their parents. Those found to be positive for *T. cruzi* were either taken to the nearest health facilities where chemotherapy was available or encouraged to initiate chemotherapy.

RESULTS

All 59 eligible communities were included in the survey target area: 29 in El Salvador and 30 in Honduras (Table 1 and Figure 2). All of the 4,171 houses in those communities were

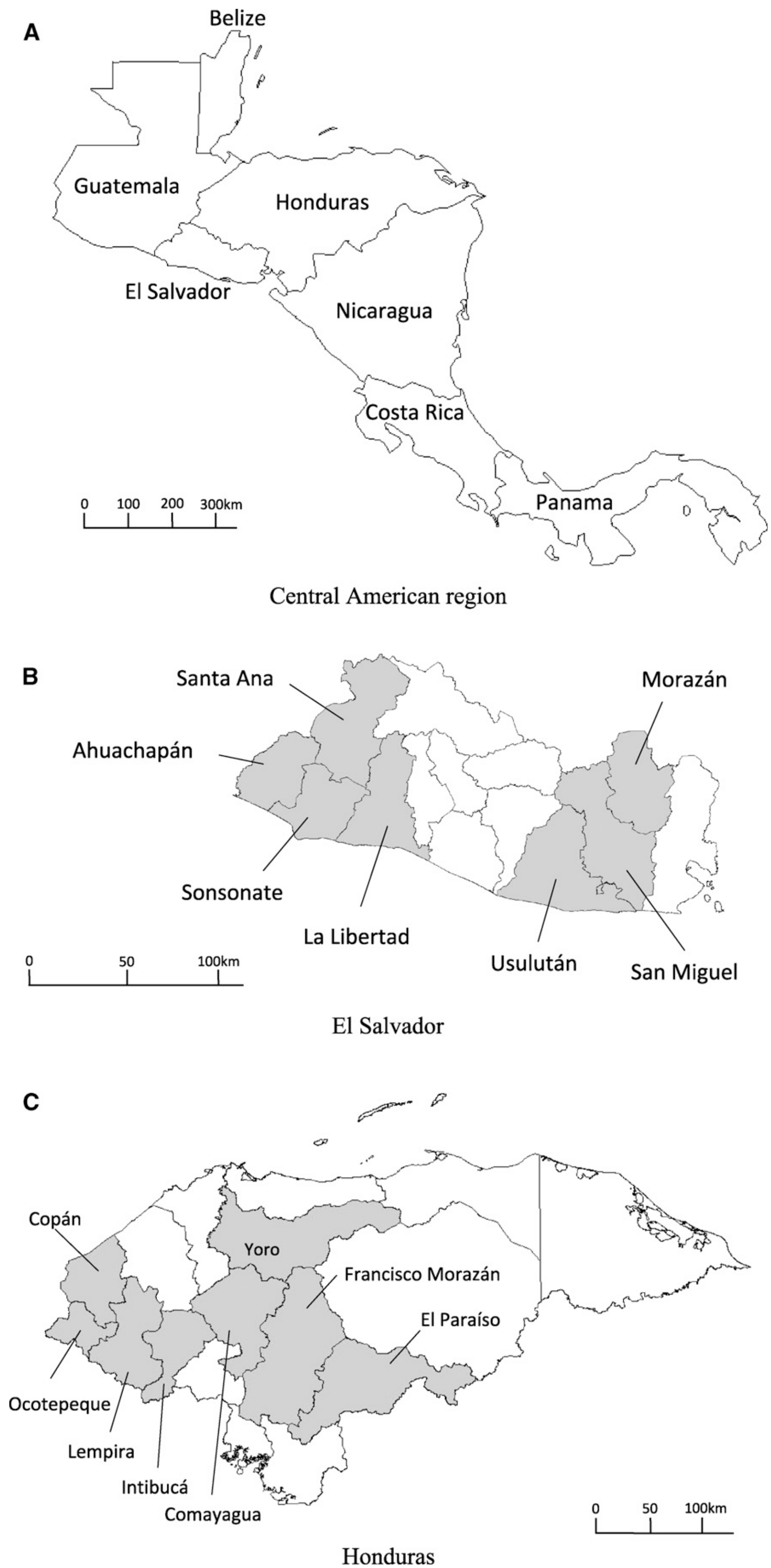

FIGURE 1. Fifteen endemic target provinces for the survey.

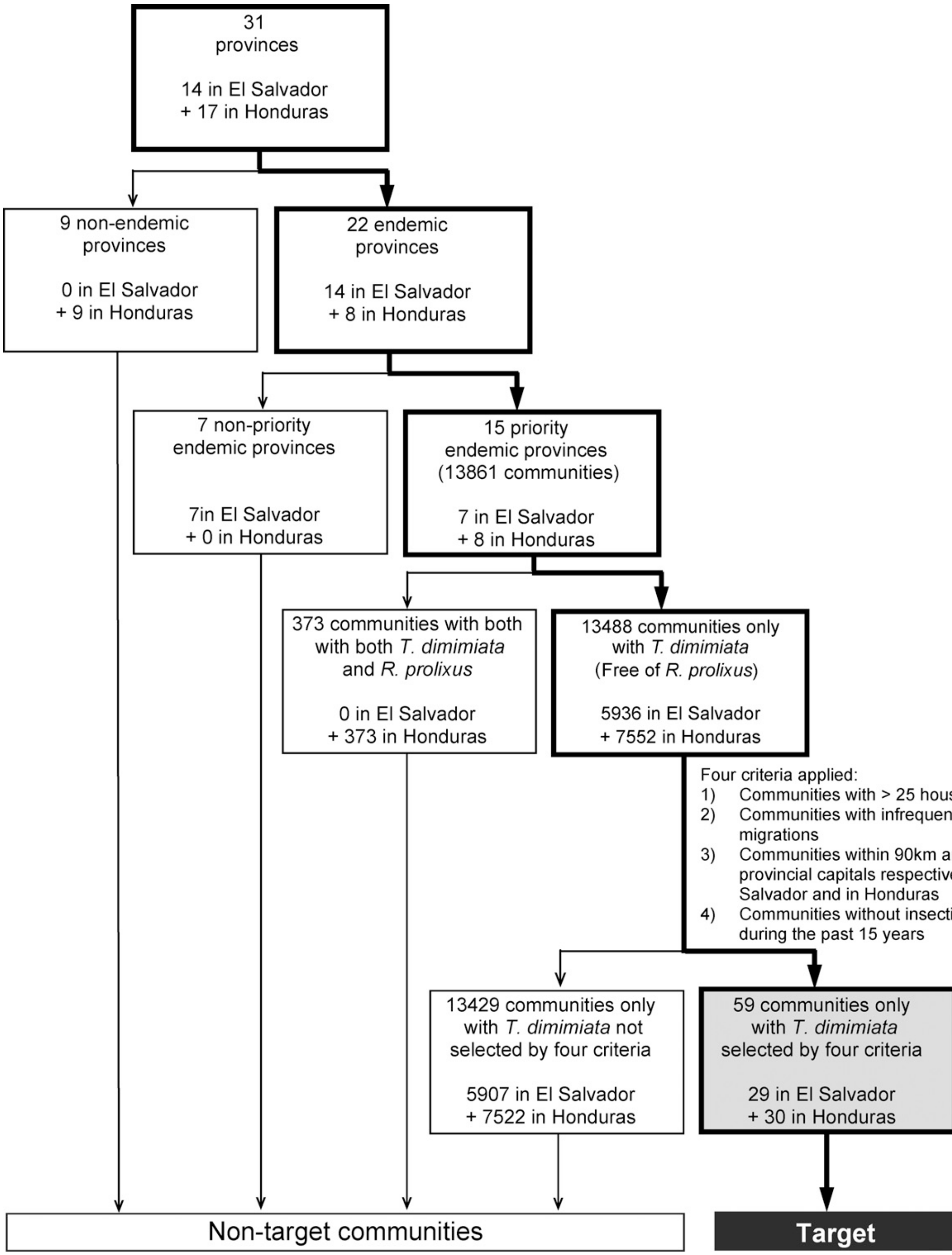

FIGURE 2. Selection of target communities for the study.

visited. Of them, 88 houses (2%) were absent during the study period or refused to participate in the study. As a result, 4,083 households (= 4,171 − 88) and 6,324 children between 6 months and 15 years of age who resided there participated.

The overall domestic infestation rate and seroprevalence were 12.9% and 0.49%, respectively. Figure 3 shows the relationship between the domestic infestation rate and seroprevalence, where each plot stands for a data-point community. The size of each plot in Figure 3 was adjusted in proportion to the total number of households in each community.

All communities with a domestic infestation rate of 8% or less consistently had a seroprevalence of 0%. Those with a domestic infestation rate above 8% had a highly variable seroprevalence (Table 1); this wide range of values complicated the creation of a weighted regression curve above 8%. For this reason, cross-tabulation of all communities was conducted for further analysis, by employing 8% as the cut-off point for the domestic infestation rate (Table 2).

Of the 42 communities with 0% seroprevalence, 29 had a domestic infestation rate of 8% or less; the remaining 13 had

Table 1
Triatoma dimidiata domestic infestation rate and seroprevalence in communities

Country and community	Entomological test: number of households							Serological test: number of children 6 months to 15 years				Estimated cost saving through application of 8% as the threshold (US$)†
		(+)										
	(−)	Intra-domestically positive	Peri-domestically positive	Intra- or peri-domestically positive	Total	DIR: Domestic infestation rate (%)	No. of households in communities with 5–8% of DIR*	(−)	(+)	Total	Sero preva-lence (%)	
El Salvador	**1,410**	**399**	**70**	**430**	**1,840**	**23.4**	**139**	**2,663**	**30**	**2,725**	**1.11**	**7,410**
Calle Vieja al Capulin	95	15	4	17	112	15.2	(n.a.)	164	1	165	0.61	(n.a.)
Col Venicia	69	17	1	17	86	19.8	(n.a.)	174	2	176	1.14	(n.a.)
La Esperanza	80	19	2	20	100	20.0	(n.a.)	111	1	112	0.89	(n.a.)
Zacatal	81	20	5	22	103	21.4	(n.a.)	74	3	77	3.90	(n.a.)
Paraiso de Zacatal	79	7	1	8	87	9.2	(n.a.)	89	0	90	0.00	(n.a.)
Amatepec	106	24	1	25	131	19.1	(n.a.)	70	0	70	0.00	(n.a.)
Reynosa	57	13	8	17	74	23.0	(n.a.)	67	3	70	4.29	(n.a.)
Los carballo	34	5	4	7	41	17.1	(n.a.)	35	0	35	0.00	(n.a.)
El Aguacate	21	15	4	16	37	43.2	(n.a.)	55	1	56	1.79	(n.a.)
El Porvenir	22	5	3	8	30	26.7	(n.a.)	44	2	46	4.35	(n.a.)
El Mango	35	12	3	15	50	30.0	(n.a.)	71	4	75	5.33	(n.a.)
La Segovia	30	28	19	30	60	50.0	(n.a.)	141	0	141	0.00	(n.a.)
El Refugio	42	12	1	12	54	22.2	(n.a.)	111	0	111	0.00	(n.a.)
El Zapote	18	17	0	17	35	48.6	(n.a.)	76	0	76	0.00	(n.a.)
Cacho de oro	25	2	0	2	27	7.4	27	65	0	65	0.00	1,439
Centro de Siuhuapilapa	41	20	6	25	66	37.9	(n.a.)	72	0	72	0.00	(n.a.)
Apastepeque	77	16	0	16	93	17.2	(n.a.)	158	2	162	1.25	(n.a.)
Loma Pacha	106	16	1	17	123	13.8	(n.a.)	142	1	143	0.70	(n.a.)
Col. El Paraiso de La Torrecilla	59	12	0	12	71	16.9	(n.a.)	130	1	131	0.76	(n.a.)
La Bolsa	12	28	1	28	40	70.0	(n.a.)	88	2	90	2.22	(n.a.)
Los Canas	19	21	2	21	40	52.5	(n.a.)	79	1	80	1.25	(n.a.)
El Centro de del Boqueron	18	27	0	27	45	60.0	(n.a.)	66	2	68	2.94	(n.a.)
La Ermita	37	3	0	3	40	7.5	40	45	0	46	0.00	2,132
Los Vilatoros	86	9	2	11	97	11.3	(n.a.)	152	0	156	0.00	(n.a.)
Los Fernandez	26	1	0	1	27	3.7	(n.a.)	53	0	53	0.00	(n.a.)
Los Sanchez	67	4	1	5	72	6.9	72	102	0	104	0.00	3,838
Los Pinedas	18	15	0	15	33	45.5	(n.a.)	95	2	108	2.06	(n.a.)
Los Ventura	22	9	0	9	31	29.0	(n.a.)	55	2	58	3.51	(n.a.)
San Pablo	28	7	1	7	35	20.0	(n.a.)	79	0	89	0.00	(n.a.)
Honduras	**2,146**	**82**	**18**	**97**	**2,243**	**4.3**	**424**	**3,598**	**1**	**3,599**	**0.03**	**22,603**
Corralitos	66	0	0	0	66	0.0	(n.a.)	155	0	155	0.00	(n.a.)
Los Anices	73	0	0	0	73	0.0	(n.a.)	126	0	126	0.00	(n.a.)
Los Dos Rios	49	0	0	0	49	0.0	(n.a.)	115	0	115	0.00	(n.a.)
El Higuito	79	2	0	2	81	2.5	(n.a.)	92	0	92	0.00	(n.a.)
El Varillal	101	3	0	3	104	2.9	(n.a.)	205	0	205	0.00	(n.a.)
Rio Chiquito	76	0	0	0	76	0.0	(n.a.)	84	0	84	0.00	(n.a.)
Pana Blanca	154	17	0	17	171	9.9	(n.a.)	278	0	278	0.00	(n.a.)
San Joaquin	198	0	0	0	198	0.0	(n.a.)	336	0	336	0.00	(n.a.)
Agua Caliente	46	3	1	4	50	8.0	50	87	0	87	0.00	2,666
El Triunfo	77	0	0	0	77	0.0	(n.a.)	159	0	159	0.00	(n.a.)
Casitas	93	15	9	22	115	19.1	(n.a.)	114	0	114	0.00	(n.a.)
Frijores	62	5	1	6	68	8.8	(n.a.)	85	1	86	1.16	(n.a.)
Loma Verde	36	8	4	11	47	23.4	(n.a.)	55	0	55	0.00	(n.a.)
La Ceiba	98	6	0	6	104	5.8	104	154	0	154	0.00	5,544
San Nicolas	70	4	0	4	74	5.4	74	115	0	115	0.00	3,944
Esperanza de Centro de Valle de Angeles	74	0	0	0	74	0.0	(n.a.)	79	0	79	0.00	(n.a.)
Pozona de Centro de Valle de Angeles	49	0	0	0	49	0.0	(n.a.)	82	0	82	0.00	(n.a.)
Cebilla	90	0	0	0	90	0.0	(n.a.)	125	0	125	0.00	(n.a.)
Leoncito	36	0	0	0	36	0.0	(n.a.)	64	0	64	0.00	(n.a.)
El Paraiso de San Francisco	56	0	0	0	56	0.0	(n.a.)	117	0	117	0.00	(n.a.)
Catatao	72	0	0	0	72	0.0	(n.a.)	152	0	152	0.00	(n.a.)
Rancho Grande	38	4	0	4	42	9.5	(n.a.)	84	0	84	0.00	(n.a.)
San Jose del Alto	79	5	1	6	85	7.1	85	136	0	136	0.00	4,531
La Quesera	51	2	2	4	55	7.3	55	65	0	65	0.00	2,932
Las Delicias	44	1	0	1	45	2.2	(n.a.)	73	0	73	0.00	(n.a.)

(Continued)

Table 1
Continued

Country and community	Entomological test: number of households (−)	(+) Intra-domestically positive	(+) Peri-domestically positive	(+) Intra- or peri-domestically positive	Total	DIR: Domestic infestation rate (%)	No. of households in communities with 5–8% of DIR*	Serological test: number of children 6 months to 15 years (−)	(+)	Total	Sero preva-lence (%)	Estimated cost saving through application of 8% as the threshold (US$)†
Los Encinos	26	0	0	0	26	0.0	(n.a.)	39	0	39	0.00	(n.a.)
Piedras Bonitas	57	0	0	0	57	0.0	(n.a.)	62	0	62	0.00	(n.a.)
Capulin	53	3	0	3	56	5.4	56	80	0	80	0.00	2,985
Guanchias Creek	90	4	0	4	94	4.3	(n.a.)	182	0	182	0.00	(n.a.)
Placido	53	0	0	0	53	0.0	(n.a.)	98	0	98	0.00	(n.a.)
Total	**3,556**	**481**	**88**	**527**	**4,083**	**12.9**	**563**	**6,261**	**31**	**6,324**	**0.49**	**30,013**

*All the households in communities with a domestic infestation rate between 5% and 8% will be additionally exempted from universal insecticide spraying.
†Crude cost saving through application of 8% is estimated, by multiplying the unit cost 53.31 (US$/household) by the number of households in communities.
n.a. = not applicable.

rates above 8%. This indicates that a domestic infestation rate of 8% might ensure 0% seroprevalence. The majority of communities with a domestic infestation rate > 8% showed a seroprevalence above 0% (17 communities). The χ^2 analysis showed this difference to be significant ($P < 0.001$). The same trend was confirmed in the number of children who resided in those communities (Table 2, bottom). Thus, it is suggested that the possible threshold for the *T. dimidiata* domestic infestation rate be set at 8%.

Of 2,680 households in the communities with a domestic infestation rate of 5% or higher, 563 households were located in communities with a domestic infestation rate of between 5% and 8%. Therefore, application of 8% as the criterion for universal spraying will lead to roughly a 21% reduction (= 563/2680 × 100) in the number of target households and for universal or selective spraying. This implies a 21% reduction in both financial and opportunity costs. The average direct financial cost of the survey including spraying was 53.3 (US$/household), composed of personnel costs, transport costs, and commodity costs. Note that personnel costs accounted for a major part of this average direct financial cost (87.5%), whereas transport costs and commodity costs accounted only for 2.6% and 9.9%, respectively. Multiplying this unit cost by the number of households in communities with a domestic infestation rate between 5% and 8% shows a saving of US$ 30,013 (= 53.31 × 563) with this survey.

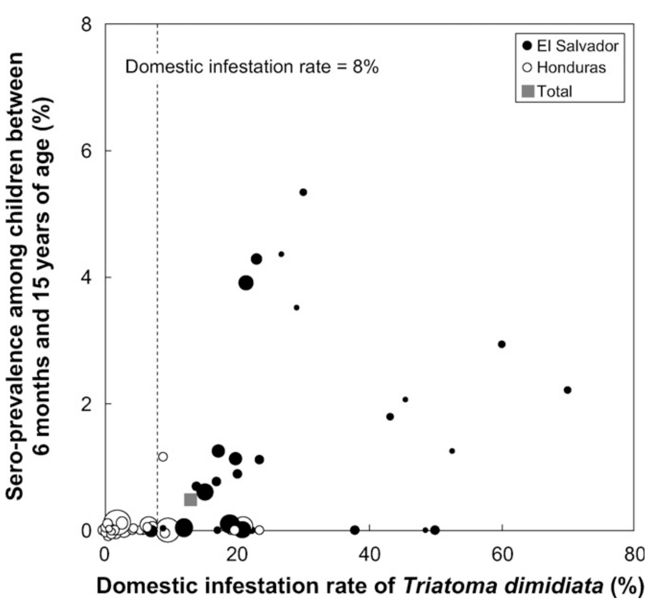

Figure 3. Relationship between domestic infestation rate and seroprevalence.

DISCUSSION

The results of this study support the possible existence of a domestic infestation rate threshold for the prevention of Chagas disease transmission. In all 29 communities with a domestic infestation rate of 8% or less, seroprevalence was 0%. Therefore, 8% could serve as a possible threshold, with potential room left for improvement and further refinement of this cutoff. Here are two rationales for piloting 8% as the possible threshold.

First, this possible threshold was identified through the analysis of a relatively large dataset composed of 4,083 households and 6,324 children between 6 months and 15 years of age, collected through a census in 59 *T. dimidiata*-infested communities of two countries. Taking into account there is no previous large-scale surveys focusing on the relationship between the domestic infestation rate of *T. dimidiate* and seroprevalence, the results of this study should be respected and adequately considered for a *T. dimidiate* control operation. For instance, an immediate application of 8% as the criterion for universal spraying could be recommended in resource-constrained settings. However, 5% may probably continue to be used in resource-rich settings to be on the safe side, as 5% is adequately below 8%. Note that there is room and need for conducting a similar survey targeting more remote communities infested exclusively with *T. dimidiata* in El Salvador and Honduras, which this study was unable to include. This complementary survey will contribute to increasing the precision and accuracy of the proposed possible threshold, by examining the potential difference from the results of this study and adjusting the proposed possible threshold.

Second, necessary resources should be urgently and efficiently mobilized to achieve the goals set by the IPCA, as transmission of Chagas disease was not eliminated by its target year 2010.[9] Our crude estimation of resource savings to be realized by shifting the threshold from 5–8% will draw sufficient attention from policymakers at ministries of health of Chagas disease endemic countries. Moreover, application of the proposed possible threshold will help not only save resources but also avoid unnecessary overspraying and opportunity costs spent at individual households (e.g., moving out furniture when spraying). Note that, though insecticide spray-

TABLE 2
Relationship between *Triatoma dimidiata* domestic infestation rate threshold 8% and seroprevalence

		Seroprevalence in communities = 0%	Seroprevalence in communities > 0%	Total
Number of communities				
T. dimidiata domestic infestation rate of communities	0–8%	29 (49.2%)	0 (0%)	29 (49.2%)
	> 8%	13 (22.0%)	17 (28.8%)	30 (50.8%)
	Total	42 (71.2%)	17 (28.8%)	59 (100%)
	Chi-square test			$P < 0.001$
Number of children between 6 months and 15 years of age				
T. dimidiata domestic infestation rate of communities	0–8%	3,250 (51.4%)	0 (0%)	3,250 (51.4%)
	> 8%	1,371 (21.7%)	1,703 (26.9%)	3,074 (48.6%)
	Total	4,621 (73.1%)	1,703 (26.9%)	6,324 (100%)
	Chi-square test			$P < 0.001$

ing is very expensive because of manpower, logistical costs, and supplies, it remains a major strategy for eliminating and controlling many NTDs.[21] Therefore, determining how cost-efficiently insecticide spraying can be operated is the key to the elimination of transmission of the disease.

Despite only a 3% difference between infestation cutoffs of 5% and 8%, the estimated cost savings by not spraying amounted to US$ 30,013 equivalent to 21% of total spraying-related costs. Because a relatively greater number and proportion of households (563 households) were in the borderline range of a domestic infestation rate between 5% and 8%, this substantial cost savings can be expected.

One may argue that the one-person-hour method lacks sensitivity in detecting bug infestation. In fact, a previous study in Guatemala reported that the method detected only 7% (= 42 of 604) of the total number of *T. dimidiata* bugs collected through demolition of mud walls.[22] Needless to say, counting the number of the bugs through demolition of mud walls provides the most accurate infestation rate. Yet, it is absolutely unrealistic to demolish mud walls of every target housing structure inhabitants currently live in, for both an economic reason (i.e., financial and temporal resource limitations in inclusion of replacement wall rebuilding in the routine surveillance activity components) and a cultural reason (i.e., potential emotional attachment to mud wall at specific households). We rather suggest that a practically measurable infestation rate (e.g., the one-person-hour method) be systematically used for the two reasons. First, a standardized method must be commonly used between a routine surveillance and the threshold identification, to ensure the data comparability between an observed infestation rate and the threshold. Second, a simplified bug detection method and its reporting system encourage communities to participate in bug detection activities and thereby help sustain a routine surveillance system.[23]

The relationship between infestation of the species other than *T. dimidiata* and seropositivity among inhabitants has been sufficiently analyzed. These studies reported the significant association between types of materials of walls, infestation density, and seropositivity[24–26]; they serve as useful evidence for determining insecticide spraying and house improvement as the necessary interventions at households infested with those other species. Yet, they provide neither a clue on the relationship between the infestation of *T. dimidiata* and seropositivities nor a reference value that triggers scaling up of the interventions in an entire community. Thus, exploring and identifying the possible threshold of the *T. dimidiata* infestation rate is clearly an urgent task, despite its possible technical challenges.

Note that this 8% was identified in an evidence-based manner, whereas use of the 5% cutoff is an insensible application of the threshold for *T. infestans*, a different species, without scientific justification. The relationship between the domestic infestation rate and seroprevalence is less clear when domestic infestation rates are beyond 8%. For instance, seroprevalence was 0% in 13 communities with infestation rates > 8% (Figure 3 and Table 2). This indicates that the possible threshold is expected to serve as the minimum requirement that ensures nearly 0% seroprevalence and implies an interruption of Chagas disease through *T. dimidiata*.

This study has several limitations. First, because of financial and temporal constraints, only a limited number of *T. dimidiata*-infested communities within 90–110 km from provincial capitals were assessed. This was an agonizing but necessary choice, to ensure that a census could be conducted in each community. Without assessing all the households and all the children between 6 months and 15 years of age in each target community, the domestic infestation rate and seroprevalence would have been neither representative enough nor meaningful enough to be interpretable in Figure 3. Yet, this study is obviously unable to estimate the degree to which this potential community selection bias might have contributed to the difference between the proposed possible threshold and the "*true*" threshold. As discussed earlier, to increase precision and accuracy of the proposed possible threshold, a complementary survey needs to be conducted in more remote communities by employing the same methods. Second, the proposed possible threshold is based on a cross-sectional study. To further increase precision and accuracy of the threshold, incidence should be used rather than prevalence by analyzing the pattern of a cohort group. Taking into account several challenges in estimating the incidence of Chagas disease,[27] we are planning to perform another census by targeting the same households and children to increase the precision of the proposed possible threshold by examining the incidence. Third, a complementary test of randomly sampled ELISA-negative sera may need to be conducted to further increase the precision and accuracy of the results of the serological test. Fourth, there is significant room for improving the estimation of the cost reduction effect caused by less frequent spraying. A more detailed cost analysis will help policymakers appropriately translate its recommendations into operational planning and budgeting.

CONCLUSIONS

Although acknowledging the potential for improvement, a domestic infestation rate of 8% could serve as the possible threshold below which transmission would become unlikely. This possible threshold is based on the analysis of a large dataset collected from a census in 59 communities in two countries exclusively infested with *T. dimidiata*. This study may ensure the existence of a suggested threshold of the infestation rate. The implementation of an 8% threshold should lead to a 21%

reduction in spraying-related costs. A crude estimate further indicates a savings of US$ 30,013 with this survey, by avoiding unnecessary spraying and its accompanied interventions.

Received October 19, 2011. Accepted for publication March 20, 2012.

Acknowledgments: We gratefully thank Christopher Schofield (London School of Hygiene and Tropical Medicine), Carlos Ponce (Honduran ministry of health), Hiroki Nakatani, Lorenzo Savioli, Jean Jannin, Pedro Albajar Viñas, Kazuyo Ichimori (WHO, Geneva), Yoshie Yamashita and Ryotaro Oda (JICA, Tokyo) for their valuable technical support. We also thank the Japan International Cooperation Agency for its financial support to this study. This work is sincerely dedicated to those who live in Chagas disease-endemic areas, particularly in Central America.

Financial support: This study was funded by the National Chagas Disease Programs of Salvadorian and Honduran ministries of health, with technical and financial support from the Japan International Cooperation Agency (JICA).

Disclosure: Ethical approval was obtained from the national authorities of both El Salvador and Honduras: i.e., *the National Committee for Clinical Research Ethics Committee of El Salvador* (approval no. IRB0005660) and *the Ethics Committee for Biomedical Research* (*IRB*)*, the National Autonomous University of Honduras* (approval no. IRB00003070).

Disclaimer: We declare that we have no conflict of interest.

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