THE EPIDEMIOLOGY OF *TRYPANOSOMA CRUZI* INFECTION IN THREE PROVINCES OF RURAL ECUADOR

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ABSTRACT

CARLA BLACK: The Epidemiology of *Trypanosoma cruzi* Infection in Three Provinces of Rural Ecuador

(Under the direction of John R. Seed)

Trypanosoma cruzi, the causative agent of Chagas disease, is a protozoan parasite transmitted by insect vectors of the subfamily Triatominae. Human infection occurs throughout Latin America. The epidemiology of *T. cruzi* infection on Ecuador has not been widely studied. This is a cross-sectional study of *T. cruzi* seroprevalence and household risk factors for *T. cruzi* seropositivity in 14 rural communities in 3 provinces of Ecuador. 3,286 subjects from 997 households were included in the study. Seroprevalence of *T. cruzi* was 5.7%, 1.0%, and 3.6% in the sampled communities of the Manabi, Guayas, and Loja provinces, respectively. Seroprevalence increased with increasing age in the provinces of Manabi and Guayas, while in Loja the highest prevalence was seen in children younger than 10 years. In the coastal provinces of Manabi and Guayas, factors associated with seropositivity were living in a house with a palm roof (odds ratio, OR=2.63 [95% confidence interval, 1.61, 4.27]), wood walls (OR=5.75 [2.04, 16.18]) or cane walls (OR=2.81 [1.31, 6.04]), and the presence of firewood in the peridomicile area (OR=2.48 [1.54, 4.01]). Accumulation of trash outside the home was associated with a reduced risk of seropositivity (OR=0.25 [0.12, 0.51]). In the Andean province of Loja, living in a house with adobe walls and living in the same household as another seropositive individual were the only factors predictive of *T. cruzi* seropositivity. The risk of seropositivity was more than two times greater for an individual living in a household with another seropositive person in this region.

Clustering of seropositives within households was not observed in the coastal region after adjustment for known household risk factors for T. cruzi infection. In conclusion, risk factors for T. cruzi transmission in Ecuador varied by geographic region, likely due to differing behavior of the triatomine vector species in each region. These findings illustrate that an understanding of the transmission dynamics of T. cruzi in a particular area are necessary for the development of effective Chagas disease control strategies in those areas.

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LIST OF ABBREVIATIONS

ALR	Alternating Logistic Regression
CI	Confidence Interval
DAG	Directed Acyclic Graph
DF	Degree of Freedom
ELISA	Enzyme-linked Immunosorbent Assay
GEE	Generalized Estimating Equations
IHA	Indirect Hemagglutination
IIF	Indirect Immunofluorescence
OD	Optical Density
OR	Odds Ratio
PAHO	Pan-American Health Organization
POR	Pairwise Odds Ratio
RR	Risk Ratio
SD	Standard Deviation
SES	Socio-economic Status
WHO	World Health Organization

CHAPTER 1. REVIEW OF THE LITERATURE

Chagas disease, or American trypanosomiasis, is a parasitic disease caused by the protozoa *Trypanosoma cruzi*. Chagas disease occurs only in the Americas. As a zoonotic infection of wild mammals, *T. cruzi* is widespread from the Great Lakes of North America to southern Argentina, from approximately latitudes 42° N to 46° S. However, human infection mainly occurs in the rural and periurban areas of tropical and subtropical countries from Mexico to Argentina and Chile. (Moncayo, 1992; Ramsey and Schofield, 2003; Prata, 2001).

T. cruzi is transmitted to man and other mammals through the feces of blood-sucking insect vectors belonging to the order Hemiptera, family Reduviidae and subfamily Triatominae (WHO, 2000). Often, the insects defecate on the host while feeding, and the infected fecal droplets enter the bloodstream through lesions created by scratching of the bite due to itching or are inadvertently passed to the mucosa of the eye, nose, or mouth (WHO, 2000; Prata, 2001). Other routes of transmission include blood transfusion and congenital transmission from chagasic mother to child (Prata, 2001; Moncayo, 1992). Blood transfusion is the second most common route of transmission and accounts for approximately 10% of cases. The risk of congenital transmission to children of chagasic mothers ranges from less than 1% to 10%. (Prata, 2001)

Pathology of Chagas Disease

The course of Chagas disease in humans involves two stages--an acute phase and a chronic phase. The acute phase of disease begins when *T. cruzi* enters the body and is marked by a local reaction at the point of entry followed by general malaise, fever, tachycardia, lymphadenopathy, splenomegaly, and edema. During this phase, the parasite is disseminated and can be detected by direct blood examination. However, in the majority of infected persons the disease goes undetected due to a lack of clinical manifestations. Only an estimated 1-2% of all *T. cruzi* infections are recognized during the acute phase. Of those symptomatic during the acute phase, the majority of whom are children, 5-10% will die due to encephalomyelitis or severe cardiac failure. (Prata, 2001; WHO, 2000).

The acute phase of Chagas disease usually lasts 6-8 weeks, after which time parasitemia falls to undetectable levels, general symptoms and any clinical manifestations of myocarditis or meningoencephalitis disappear, and the individual enters the chronic phase of infection. During this phase, the patient appears healthy, and infection can be detected only by immunologic tests. This is often referred to in the medical literature as the indeterminant form of chronic Chagas disease, and the majority of infected individuals will remain in this condition for the remainder of their lives. While those with indeterminant chronic Chagas disease have a mortality rate equal to that of the general population and experience no overt adverse effects of infection, they act as a natural reservoir for *T. cruzi* and contribute to maintaining the life cycle of the parasite. (WHO, 2000; Prata, 2001).

Between 10 and 30 years after the chronic phase of Chagas disease has started, an estimated 10-40% of infected individuals, depending on the geographical area, will develop damage to various organs, mainly the heart and digestive system. The cardiac form of chronic Chagas disease is the most common clinical outcome of chronic *T. cruzi* infection. Epidemiological studies show that about 10-30% of individuals with positive serology for *T. cruzi* have some characteristic

changes in their electrocardiogram suggestive of cardiac damage. (WHO, 2000) Longitudinal studies in endemic areas have shown that approximately 2% of patients with the indeterminant form of Chagas disease progress to the cardiac form every year (Prata, 2001). Clinical manifestations of chronic chagasic cardiomyopathy range from mild or symptomless heart disease to heart failure, cardiac arrhythmias, and thromboembolism. Sudden death is frequent among patients with chronic cardiac Chagas disease. One study reports that 30% of Chagas disease patients with cardiac involvement experience sudden death with or without congestive heart failure. (Prata, 2001)

Approximately 6% of chagasic patients will develop the digestive form of Chagas disease (Moncayo, 1992). In these patients, *T. cruzi* infection causes the destruction of the autonomic enteric nervous system, leading to dysfunction of various organs of the digestive system, most frequently the esophagus and colon. Infected patients develop alterations of motility, secretion, and absorption in these organs and eventually progress to megaesophagus or megacolon. (Prata, 2001; WHO, 2000) Approximately 3% of patients with chronic Chagas disease experience involvement of the peripheral nervous system, with destruction of motor neurons and peripheral sensory nerve fibers (Prata, 2001; Moncayo, 1992).

Diagnosis of Infection

During the acute phase of Chagas disease the level of parasite circulating in the bloodstream is often high enough such that infection can be detected by microscopic observation of blood smears or indirect parasitological methods such as hemoculture and xenodiagnosis. While these methods are highly specific, the sensitivity of such tests is low, rarely exceeding 50%. (WHO, 2000; Carvalho et al, 1993; Wendel and Gonzaga, 1993) In the chronic phase, when levels of parasitemia are low, immunologic methods based on the detection of antibodies to *T. cruzi* are used for diagnosis of infection. The three conventional serologic tests most widely employed are

indirect hemagglutination (IHA), indirect immunofluorescence (IIF), and enzyme-linked immunosorbent assay (ELISA). The sensitivities of these assays reported from various validation studies are between 99%-100%. (Carvalho et al, 1993; Schattschneider et al, 1992; Wendel and Gonzaga, 1993; WHO, 2000) However, the specificity of these tests is low due to false positives generated by cross-reactivity with other parasites, mainly *Leishmania* species and *Trypanosoma rangeli* (WHO, 2000; Wendel and Gonzaga, 1993; Gomes et al, 1999). The latter is another trypanosome transmitted by the same vectors as *T. cruzi* but is not pathogenic to humans (Wendel and Gonzaga, 1993). The specificities of ELISA and IIF reported from validation studies range from 95%-98% and 99%-100%, respectively (Carvalho et al, 1993; Schattschneider et al, 1992; Wendel and Gonzaga, 1993). However, the sera used as negative controls against which the assays are evaluated are generally from persons from areas not endemic for *T. cruzi* and therefore also not endemic for *Leishmania* or *T. rangeli*. In the same validation studies, when tested on sera known to be positive for *Leishmania*, the IIF reacted positively to 13 of 13 samples in one study (Schattschneider et al, 1992) and 8 of 10 samples in another (Carvalho et al, 1993). Likewise, the ELISA showed cross-reactivity to 4 of 13 *Leishmania*-positive sera in one study (Schattschneider et al, 1992) and to between 10% and 80%, depending on the ELISA used, of 10 *Leishmania* samples in another study (Carvalho et al, 1993). Because of the lack of specificity, the Pan American Health Organization recommends the use of at least two methods for the positive diagnosis of *T. cruzi* infection (Carvalho et al, 1993).

Treatment of Infection

Two drugs, nifurtimox and benznidazole, are currently available for the treatment of *T. cruzi* infection. These drugs have demonstrated parasitological cure rates of up to 80% in patients treated in the acute phase of infection. However, both compounds have significant side effects and exhibit low antiparasitic activity in the chronic phase of the disease. For these reasons, treatment

of patients in the chronic stage of *T. cruzi* infection has been controversial. Nonetheless, data from recent studies suggest that patients treated with benznidazole, although not parasitologically cured, have a reduced occurrence of electrocardiograhic changes associated with clinical Chagas disease and a more favorable clinical course than untreated patients. Based on these findings, current WHO/PAHO guidelines recommend antiparasitic treatment for all seropositive individuals regardless of stage of infection. (Urbina and Docampo, 2003; WHO, 2000)

Prevalence of Infection

An estimated 16-18 million people in Latin America are infected with *T. cruzi* (Kirchhoff, 1993). In epidemiological studies in Central and South American countries from 1980-1985, the prevalence of *T. cruzi* seropositivity in the studied populations ranged from 3% in Venezuela to 24% and 30% in Bolivia and Colombia, respectively. It has been estimated that approximately 5% of the total population of Colombia is infected with *T. cruzi*. (WHO, 2000) In 1985, it was estimated that 100 million people, about 25% of all the inhabitants of Latin America, were at risk of contracting *T. cruzi*. However, with the success of the Southern Cone Initiative, a vector control program launched in 1992 in the southern cone countries of Argentina, Brazil, Chile, Paraguay, and Uruguay, the incidence of *T. cruzi* infection in the whole of Latin America has been reduced by over 65%. Incidence has fallen from an estimated 700,000 new cases in 1990 to less than 200,000 new cases in the year 2000. To date, Uruguay, Chile, and eight of the twelve endemic states of Brazil have been declared free of transmission of *T. cruzi*. (WHO, 2000)

Transmission Patterns of Infection

Transmission of *T. cruzi* occurs in two cycles—a sylvatic cycle and a domestic cycle. Most species of triatomines are entirely sylvatic. Natural habitats include palm trees, rock piles, burrows and shelters of animals, tree hollows, beneath tree bark, and bird nests. Triatomines can feed on almost any vertebrate, though some species are highly adapted to a specific host. While all

mammal species are thought to be susceptible to *T. cruzi* infection, studies of *T. cruzi* prevalence in sylvatic vertebrate hosts most commonly find high infection rates in some species of opossums and edentates. Birds and reptiles are not susceptible to infection. Sylvatic triatomines do not fly to a moving host to take a blood meal, but rather infest the nesting sites of their vertebrate hosts. As long as the ecosystem remains stable, *T. cruzi* is transmitted between vertebrates in this sylvatic pattern with little significance to the epidemiology of human infection. (WHO, 2000; Miles et al, 2003; Ramsey and Schofield, 2003)

However, environmental changes that reduce populations of host vertebrates, such as drought, flood, deforestation, and urbanization, cause triatomines to migrate in search of food. Flying triatomines may be attracted to light or heat in human dwellings or may simply encounter a household by chance. Triatomines can also be passively transported into houses via palm leaves used in the construction of roofs. Once inside these manmade habitats, triatomines find an abundant blood supply in humans and domestic animals, as well as protection from predators and climatic extremes. These favorable conditions allow domiciliary populations of triatomines to reach higher densities than those seen in sylvatic environments. In this manner some species of Triatominae have become partially or completely domiciliated, either colonizing houses permanently or forming small, more transitory, intradomicialiary colonies. The species epidemiologically linked to human Chagas disease are those that have adapted to the human environment. (Ramsey and Schofield, 2003; WHO, 2000)

Over 130 species of Triatominae are known. Wild Triatominae species are found from the north of the United States to southern South America. However, only a few species of three genera, *Triatoma*, *Rhodnius*, and *Panstrongylus*, are important vectors of *T. cruzi* between domestic animals and humans. All three genera are widely distributed in Central and South America. (WHO, 2000) The three triatomine species of major epidemiological importance in Chagas disease are *Triatoma infestans*, found in Bolivia and the Southern Cone countries of Brazil,

Argentina, Chile, Paraguay, and Uruguay, and *Triatoma dimidiata* and *Rhodnius prolixus*, both found in Central America and northern South America. *T. infestans* is the most important vector of human Chagas disease. Except in its native Bolivia, where it is sometimes found in sylvatic environments, *T. infestans* is strictly domiciliated. This extreme adaptation to human dwellings has resulted in genetic fragility, making complete elimination of *T. infestans* possible. (WHO, 2000) *R. prolixus* and *T. dimidiata* are found in both domestic and wild ecotopes. The sylvatic habitat of *R. prolixus* is mainly the crowns of palm trees, and in domestic environments it is mainly captured in the straw roofs of houses. *T. dimidiata* is found naturally in the burrows of opossums, trunks of trees, and piles of rocks, and inside of houses it is associated with floors, where it covers itself with dirt as camouflage. (WHO, 2000; Schofield and Dujardin, 1997)

Risk Factors for Infection

Since transmission of *T. cruzi* generally occurs within households, much of the research regarding risk factors for infection with *T. cruzi* has focused on characteristics of the household that allow for infestation with the insect vectors of the disease. Few studies have been published in the English language literature that have assessed associations between household variables and *T. cruzi* seropositivity. In an early study from Brazil, Mott et al report that rates of seropositivity were twice as high among residents of unplastered mud-stick houses than among persons living in mud-brick houses or plastered mud-stick houses (Mott et al, 1978). However, since entomological studies have shown that different species of Triatominae prefer different habitats within human dwellings (Zeledon and Vargas, 1984), subsequent studies of factors related to *T. cruzi* infection have focused on more specific aspects of housing construction. One study conducted in Guatemala, where the main vectors of *T. cruzi* are *T. dimidiata* and *R. prolixus*, found that seropositivity was related to living in a house with a thatched roof but was not related to the type of materials used in the construction of the walls of the house (Greer et al, 1999). However, a later

study from Guatemala reports that type of roof (straw/palm vs other), walls (cane/stick/adobe vs other), and floor (dirt vs other) were all three associated with *T. cruzi* seropositivity. Living in a house with a thatched roof showed the strongest association, with a risk ratio of 2.2. (Rizzo et al, 2003) Like the Greer et al study, a study from Argentina, where *T. infestans* is the vector of *T. cruzi*, found that the likelihood of infection with *T. cruzi* was increased for persons living in houses with thatched roofs but was not related to the presence of cracks in walls (Gurtler et al, 1998b). In contrast to the Mott et al study, a recent study conducted among schoolchildren in an area of Brazil that had undergone a Chagas disease control program did not find an association between *T. cruzi* seropositivity and type of housing when houses with brick walls and tile floors were compared to those with mud walls and dirt floors (Carneiro et al, 2001).

Since household characteristics are related to *T. cruzi* transmission only in that they facilitate the presence of the disease vectors in an environment that puts the vectors in close contact with susceptible human hosts, many studies of the epidemiology of Chagas disease have focused on household infestation with triatomine insects as a study outcome rather than human infection with *T. cruzi*. A study conducted in Brazil found that the presence of *T. infestans* in human dwellings was associated with "incomplete house building," defined as those houses constructed of any combination of materials other than brick walls, cement floors, and asbestos roofs. However, when type of roof, walls, and floors were considered separately, only earthen floors remained a significant predictor of *T. infestans* infestation after adjustment for other household variables. The same study also identified indoor crop storage and the presence of rats in the house as independent risk factors for *T. infestans* infestation. (De Andrade et al, 1995) A study from Argentina that focused on the domestic density of *T. infestans* also found that neither type of roof nor cracks in walls was associated with the density of *T. infestans* after adjustment for other variables. Type of floor was not assessed in this study. (Cecere et al, 1998) A study from Costa Rica, where *T. dimidiata* is the major vector of *T. cruzi*, originally reported that colonies of triatomines inside

houses were found more frequently in houses with dirt floors (Zeledon and Vargas, 1984). However, the analyses of these data were limited to univariate associations. A subsequent paper in which the data were reanalyzed with multivariate techniques reports that the odds ratio associated with dirt floors was no longer statistically significant after adjustment for other variables, though the odds of infestation was still increased for houses with dirt floors (OR=1.7, 95% CI [0.8, 3.8]). This study also identified the presence of a tile roof as a risk factor for infestation (OR=2.4). Roof type was not considered in the initial analysis since *T. dimidiata* is rarely found more than one meter above the ground in Costa Rica. The increased risk identified in the reanalysis is probably due to the harboring of *T. dimidiata* in stacks of spare tiles next to the house rather than to the tile roofs themselves. (Starr et al, 1991)

Certain materials in the environment surrounding the house can provide habitats for triatomine vectors and can attract rodents and other mammals that serve as hosts for the vectors and reservoirs of *T. cruzi*. No studies have yet established a direct link between peridomicilliary factors and human infection with *T. cruzi*, though several have assessed their effect on household infestation with triatomine vectors. The presence of firewood outside of the house was associated with both domiciliary and peridomiciliary infestation of *T. dimidiata* in the Costa Rican study by Zeledon and Vargas (Zeledon and Vargas, 1984). The risk associated with the presence of firewood outdoors was not re-evaluated in the second analysis of these data, though the presence of firewood inside the house was included in the analysis and was not found to be associated with *T. dimidiata* infestation. (Starr et al, 1991) In a study from Mexico, the presence of junk piles in the yard was associated with intradomicilliary and peridomicilliary infestation by the insects of the species *Triatoma pallidipennis*. Interestingly, the presence of agricultural products in the yard was associated with intradomicially but not peridomicilliary infestation, though the authors note that having such products in the yard was likely an indicator that subjects stored agricultural products inside the house as well. Wood piles and stone piles in the yard were not associated with

household infestation in this study, despite the fact that *T. pallidipennis* have been found in rock piles in sylvan habitats. (Enger et al, 2004)

Domestic animals have been implicated in the transmission of *T. cruzi* since they serve as reservoirs for the parasite and sources of blood meals for the triatomine vectors. Most research has focused on dogs and chickens since these animals are commonly present in households in rural Latin America. Since dogs are a more attractive and accessible source of blood meals to triatomines than humans, and dogs infected with *T. cruzi* have a higher capacity to infect triatomine bugs than seropositive humans (Cohen and Gurtler, 2001; Gurtler et al, 1998a), dogs likely serve to both increase triatomine populations within households and increase the prevalence of *T. cruzi* infection among the triatomine population. Conversely, chickens and other birds are not susceptible to *T. cruzi* infection. Therefore, it has been proposed that the presence of chickens in a household could be both beneficial by decreasing the overall *T. cruzi* infection rate in triatomines or detrimental by supporting a larger bug population. (Gurtler et al, 1991; Gurtler et al, 1998a; Cohen and Gurtler, 2001) A proposed mathematical model of the household transmission of *T. cruzi* predicts that the infection rates of *T. cruzi* in both human and triatomine populations within a household decline as the number of chickens in a household increases and increase as the number of infected dogs in the household increases (Cohen and Gurtler, 2001). This model has been validated by an experimental study that showed *T. cruzi* infection rates among *T. infestans* populations in a household were 4.58 (2.94, 7.14) times higher when seroreactive dogs were present in the house, and vector infection rates showed a significantly increasing trend with the number of seroreactive dogs per house (Gurtler et al, 1991). However, in terms of triatomine population, studies have failed to show that the number of dogs in a household is related to domestic density of triaomines (Cercere et al, 1998) or that the presence of dogs in a household is related to house infestation with triaomines (De Andrade et al, 1995). Regarding chickens, one study showed, as predicted by the model, that triatomines that fed on chickens had a lower

infection rate than those that fed on dogs or humans and that the presence of chickens in bedrooms had a negative effect of the proportion of infected *T. infestans* but a positive effect on the number of infected bugs collected per house (Gurtler et al, 1998a). Another study also found a significant relationship between the presence of chickens in bedrooms and an increased domestic density of *T. infestans* (Cecere et al, 1998), but a study that examined the effect of presence of chickens and household infestation with *T. infestans* found no such relationship (De Andrade et al, 1995). Studies that have attempted to establish a link between the presence of dogs and chickens in a household to human infection with *T. cruzi* have produced mixed results. A 1998 study by Gurtler et al reports that the odds of *T. cruzi* seropositivity in humans increased with both the number of dogs in the house and the presence of chickens in bedroom areas (Gurtler et al, 1998b). However, two other studies failed to find an association between human seropositivity and the presence of dogs in the house (Carneiro et al, 2001; Greer et al, 1999). It should be noted that one of the studies had a sample size of only 11 cases (Carneiro et al, 2001), which is probably inadequate to detect such an association if one exists, and in the other dogs were present in 90% of the households (Greer et al, 1999).

A summary of the current literature regarding risk factors for infection with *T. cruzi* and household infestation with the triatomine vectors of *T. cruzi* is given in Table 1.1.

Table 1.1. Literature review summary of risk factors for transmission of *Trypanosoma cruzi*

Reference	Exposure	Outcome	Result[1]	Comments
	Overall house construction			
Mott et al, 1978	Mud-stick vs mud-brick house	Seropositivity	Prevalence of seropositivity twice as high in mud-stick houses	Univariate analysis only; sampled by household, did not account for non-independence of subjects
Carneiro et al, 2001	Mud walls and dirt floor vs brick walls and tile floor	Seropositivity	OR = 0.90 (0.25, 3.29)	Univariate analysis based on 11 cases
De Andrade et al, 1995	Any other combination vs brick walls, cement floor, asbestos roof	Infestation	OR = 2.5 (1.5, 4.1)	
	Type of Roof			
Gurtler et al, 1998b	Thatched vs simbol[2]	Seropositivity	OR = 1.87 (0.97, 3.62)	
Greer et al, 1999	Thatched vs synthetic	Seropositivity	18.5% vs 9.1% seropositive (p=0.014)	Sampled by household, did not account for non-independence of subjects
Rizzo et al, 2003	Straw/palm vs other	Seropositivity	RR = 2.20 (1.54, 3.15)	Univariate analysis only; sample included only schoolchildren
Grijalva et al, 2003	Open vs closed	Seropositivity	OR = 1.89 (1.30, 2.76)	Sampled by household, did not account for non-independence of subjects
Starr et al, 1991	Tile vs galvanized metal	Infestation	OR = 2.4 (1.1, 5.4)	
De Andrade et al, 1995	Mud vs asbestos	Infestation	OR = 2.7 (0.7, 10.4)	Univariate analysis only
Cecere et al, 1998	All simbol vs none or partly simbol	Infestation	64% vs 80% infestation (p=1.00)	Univariate analysis only; no association with triatomine density in households
	Type of walls			
Gurtler et al, 1998b	Many cracks vs few cracks	Seropositivity	OR = 0.9 (0.3, 2.4)	Univariate analysis only; sample included only adults
Greer et al, 1999	Plant vs earthen	Seropositivity	No association	Sampled by household, did not account for non-independence of subjects
Rizzo et al, 2003	Cane/bajareque[3]/adobe vs Adobe	Seropositivity	RR = 1.36 (1.02, 1.82)	Univariate analysis only; sample included only schoolchildren
Grijalva et al, 2003	Open vs closed Mixed vs closed	Seropositivity Seropositivity	OR = 2.84 (0.69, 11.69) OR = 6.03 (1.41, 25.75)	Sampled by household, did not account for non-independence of subjects

Table 1.1. Literature review summary of risk factors for transmission of *Trypanosoma cruzi* (cont.)

Reference	Exposure	Outcome	Result[1]	Comments
	Type of walls (cont.)			
Starr et al, 1991	Earthen vs other	Infestation	OR = 1.7 (0.9, 3.0)	Univariate analysis only
De Andrade et al, 1995	Other vs brick	Infestation	OR = 1.4 (0.8, 2.3)	
Cecere et al, 1998	No or some cracks vs many	Infestation	76% vs 94% infestation (p=1.00)	Univariate analysis only; no association with triatomine density in households
Enger et al, 2004	Adobe vs all other	Infestation	OR = 1.75 (1.06, 2.89)	Inradomicilliary infestation
	Type of floor			
Rizzo et al, 2003	Dirt vs other	Seropositivity	RR = 1.63 (1.26, 2.12)	Univariate analysis only; sample included only schoolchildren
Zeledon and Vargas, 1984	Dirt vs concrete	Infestation	Of infested houses, 58% (29) had dirt floors, 12% (6) had concrete floors (p<0.001)	Univariate analysis only; based on 50 positive houses; did not look at presence of dirt floors in uninfested houses
Starr et al, 1991	Dirt vs other	Infestation	OR = 1.7 (0.8, 3.8)	Reanalysis of data from Zeledon, 1984 using multivariate techniques
De Andrade et al, 1995	Earth vs cement	Infestation	OR = 2.4 (1.4, 4.1)	
	Peridomicilliary factors			
De Andrade et al, 1995	Presence of palm trees	Infestation	OR = 1.7 (0.9, 3.2)	
Zeledon et al, 1984	Firewood outdoors	Infestation	Of infested houses, 56% (28) had firewood, 4% (2) did not (p<0.001)	Univariate analysis only; based on 50 positive houses; did not look at distribution of firewood in uninfested houses
Starr et al, 1991	Firewood indoors	Infestation	OR = 1.0 (0.4, 2.1)	Reanalysis of data from Zeledon, 1984 using multivariate techniques
Enger et al, 2004	Wood piles Stone piles Junk piles Agricultural products	Infestation Infestation Infestation Infestation	OR = 0.79 (0.49, 1.26) OR = 0.92 (0.56, 1.50) OR = 1.62 (1.00, 2.62) OR = 2.78 (1.29, 6.00)	Intradomicilliary infestation Intradomicilliary infestation Intradomicilliary infestation Intradomicilliary infestation

Table 1.1. Literature review summary of risk factors for transmission of *Trypanosoma cruzi* (cont.)

Reference	Exposure	Outcome	Result[1]	Comments
	Dogs			
Gurtler et al, 1998b	Number of dogs	Seropositivity	OR = 5.95 (1.24, 28.52)	No. of dogs transformed to $\log_{10}(x+1)$
Carneiro et al, 2001	Dog(s) present vs absent	Seropositivity	OR = 2.6 (0.29, 26.62)	Univariate analysis based on 11 cases
Greer et al, 1999	Dog(s) present vs absent	Seropositivity	15.6% vs 6.3% seropositive (p=0.09)	At least one dog present in 90% of households
De Andrade et al, 1995	Dog(s) present vs absent	Infestation	OR = 1.0 (0.6, 1.9)	Univariate analysis only
Cecere et al, 1998	Number of dogs	Household density of triatomines	Linear regression coefficient=0.19 (p>0.05)	No association with household infestation with triatomines
Gurtler et al, 1991	Dog(s) present vs absent	*T. cruzi* infection in triatomines	RR for infection in bugs = 4.58 (2.94, 7.14)	Total of 505 triatomines in 25 houses
Gurtler et al, 1998a	*T. cruzi* infection in dogs	*T. cruzi* infection in triatomines	OR for infection in bugs per unit increase in infected dogs = 6.5 (3.2, 13.1)	Total of 22 houses; number of triatomines not given
	Proportion of triatomines that fed on dogs	*T. cruzi* infection in triatomines	OR for infection in bugs per unit increase in the proportion of bugs that fed on dogs = 7.9 (2.7, 22.8)	
	T. cruzi infection in dogs	Number of *T. cruzi* infected triatomines per house	Regression coefficient for 1 unit increase in infected dogs = 1.34 (p<0.001)	
	Proportion of triatomines that fed on dogs	Number of *T. cruzi* infected triatomines per house	Regression coefficient for 1 unit increase in proportion of bugs that fed on dogs = 1.01 (p<0.001)	

Table 1.1. Literature review summary of risk factors for transmission of *Trypanosoma cruzi* (cont.)

Reference	Exposure	Outcome	Result[1]	Comments
	Chickens			
Gurtler et al, 1998b	Number of chickens (0-10 vs 31-100)	Seropositivity	OR = 2.6 (1.1, 6.1)	Univariate analysis among adults only; authors state that there was significant negative effect of number of chickens in adjusted model (data not shown) and also a positive significant effect of chickens in bedroom areas (data not shown), so results contradictory
De Andrade et al, 1995	Chickens present vs absent	Infestation	OR = 1.5 (0.9, 2.3)	Univariate analysis only
Cecere et al, 1998	Chickens nesting indoors	Infestation Household density of triatomines	No association Regression coefficient = 0.42 ($p<0.01$)	No association between number of chickens and density of infestation
Gurtler et al, 1998a	Proportion of triatomines that fed on chickens	*T. cruzi* infection in triatomines Number of *T. cruzi* infected triatomines per house	OR for infection in bugs per unit increase in the proportion of bugs that fed on chickens = 0.4 (0.1, 1.1) Regression coefficient for 1 unit increase in proportion of bugs that fed on chickens = 0.72 ($p<0.01$)	

[1] Results presented are adjusted associations as presented by authors unless otherwise noted; Numbers in parenthesis are 95% confidence intervals unless otherwise specified

[2] A long-leaved grass arranged in compact bundles

[3] Mixture of sand and straw that covers a stick wall

Epidemiology of *T. cruzi* in Ecuador

Prevalence of Infection

An estimated 120,000 to 200,000 people in Ecuador are infected with *Trypanosoma cruzi*, with 2.2 to 3.8 million people (25% of the entire population of Ecuador) estimated to be exposed to the risk of *T. cruzi* transmission (Aguilar et al, 1999). The presence of human infection with *T. cruzi* was documented from the urban area of Guayaquil in the province of Guayas as early as 1927, and in the 1940s and 1950s disease foci were reported from the provinces of Guayas, Manabi, Los Rios, and the temperate areas of Loja, Azuay, and Bolivar. At present the main endemic areas of *T. cruzi* in Ecuador are considered to be El Oro in the southern coastal region and Guayas and Manabi in the central and northern Pacific coast. However, the prevalence of *T. cruzi* in many parts of Ecuador is unknown due to a lack of systematic countrywide surveys. Recent reports suggest that active transmission of *T. cruzi* is occurring in the northern Amazon provinces of Sucumbios, Napo, and Orellana as well (Aguilar et al, 1999; Chico et al, 1997; Grijalva et al, 2003). The prevalence rates of *T. cruzi* that have been reported from various studies in various areas of the country are given in Table 1.2. However, results in this table should be interpreted with caution because in many instances the sample size and the population from which the samples were drawn are unknown. (Aguilar et al, 1999)

Table 1.2. Prevalence rates of infection with *Trypanosoma cruzi* reported from various studies in different areas of Ecuador

Author, year	Province	Prevalence (%)	Sample size
Montalvan J, 1950	El Oro	29	696 total
	El Oro	13.3	"
	Guayas	3.1	"
	Guayas	11.8	"
	Manabi	3.8	"
	Manabi	5.8	"
INH, 1949-1957	Coastal region	13.9	3,333
Espinoza L, 1955	El Oro	8.2	Not given
	Guayas	3.5	Not given
	Guayas (urban)	1.9	Not given
	Loja	2	Not given
	Los Rios	1.5	Not given
Rodriguez JD, 1959	Guayas (urban)	24	Not given
	Guayas	4	Not given
	El Oro	7.6	Not given
	El Oro	7	Not given
	Manabi	4	Not given
	Manabi	3	Not given
	Loja	2	Not given
	Esmeraldas	4	Not given
	Los Rios	1.5	Not given
Gomez LLF, 1968	Coastal region	3	2,160
Andrade A et al, unpublished	Manabi	17	521
Momori T et al, 1985	Guayas	4.3	Not given
	El Oro	3.9	Not given
SNEM-TDR, 1986	El Oro	17.1	Not given
	El Oro	14.6	Not given
	El Oro	10.1	Not given
	El Oro	2.3	43
	El Oro	7.3	41
	Guayas (urban)	2.6	2,078
Racines VJ et al, 1994	El Oro	1.8	1,514
Guderian R et al, 1994	El Oro	7.2	Not given
	El Oro	6	Not given
	El Oro	11.4	Not given
Chico et al, 1997	Napo/Orellana	6	1,011

Table 1.2. Prevalence rates of infection with *Trypanosoma cruzi* reported from various studies in different areas of Ecuador (cont.)

Author, year	Province	Prevalence (%)	Sample size
Racines & Grijalva, 1999	Manabi	1	203
	Manabi	1.9	628
	Guayas	0.6	178
	Guayas (urban)	1.8	2,604
	Guayas	1.1	94
	Sucumbios	2.3	493
	Sucumbios	1.3	1,232
	Sucumbios	0	263
	Napo/Orellana	0.4	1,796
	Napo/Orellana	0	105
	Napo/Orellana	0.3	311
	Napo/Orellana	0.6	167
	Napo/Orellana	1.6	186
	Napo/Orellana	1.6	495
	Napo/Orellana	0	40
	Napo/Orellana	0.2	1,050
	Pastaza	0.4	227
	Cotopaxi	0.4	501
	Cotopaxi	0.2	404

Adapted from Aguilar et al, 1999

Vectors of T. cruzi

Sixteen triatomine species have been reported in Ecuador, 13 of which are actual or potential vectors of *T. cruzi*. *Triatoma dimidiata* and *Rhodnius ecuadoriensis* have the widest range of distribution and are the main Chagas disease vectors in the country. *T. dimidiata* occurs only in the low, dry areas of the coast. (Abad-Franch et al, 2001) Although *T. dimidiata* is found in wild ecotopes in other parts of Latin America (WHO, 2000; Schofield and Dujardin, 1997), the presence of sylvatic colonies of this species has never been documented in Ecuador (Abad-Franch et al, 2001). However, it is frequently found in peridomestic environments.

Rhodnius ecuadoriensis is found in central and southern Ecuadorian provinces west of the Andes. In the central coastal region, *R. ecuadoriensis* is known to invade and colonize human habitats and is also found in sylvatic habitats, primarily associated with *Phytelephas aequatorialis* palm trees. A 2000 survey of 64 *P. aequatorialis* palms in three provinces of the Pacific slope of

the Andes found 27% to harbor breeding colonies of *R. ecuadoriensis*. (Abad-Franch et al, 2001) A household survey conducted in the Manabi province in 2004 found 14% of 353 households to be infested with *R. ecuadoriensis* in domiciliary and peridomicile areas. The majority of the triatomines were captured in the peridomicile in wood piles, chicken nests, and guinea pig pens. (Grijalva, unpublished data) Sylvatic populations of *R. ecuadoriensis* have not been reported in southern Ecuador. Two studies conducted in the southern province of Loja, where palm trees are completely absent, report domestic infestation of *R. ecuadoriensis*. In both studies, *R. ecuadoriensis* was found in peridomiciliary environments only in association with chicken nests and inside houses in beds and cracks in walls. (Abad-Franch et al, 2001; Grijalva et al, 2005).

Triatoma carrioni is found in the temperate valleys and highlands of the Andean mountain range in southern Ecuador, where it has only been reported from human habitats. However, there is one report of a nymph belonging to this species captured in an epiphytic bromeliad in the canopy of a primary cloud forest in a northern province. (Abad-Franch et al, 2001) The epidemiological significance of *T. carrioni* as a vector of *T. cruzi* has not been reported in the literature. However, a recent entomological survey in the province of Loja found 7% of 138 households to be infested with *T. carrioni*. The insects were found solely in the intradomicile within cracks in walls and in bedding. A follow-up survey conducted one year later found intradomiciliary infestation with nymphs of *T. carrioni* in five houses in a community where *T. carrioni* was not found in the previous year. This suggests the ability of *T. carrioni* to infest new dwellings in a short period of time and the possibility that domestic environments are becoming colonized by wild *T. carrioni* populations in this area. (Grijalva et al, 2005)

Although *T. dimidiata* and *R. ecuadoriensis* are reported to be the main vector species in the coastal region of Ecuador (Abad-Franch et al, 2001; WHO, 2000), a recent entomological survey of 353 houses in the province of Manabi found a household infestation rate of 8.5% with insects of the species *Panstrongylus howardii*. The majority (95%) of the 154 *P. howardii* captured were

found in the peridomicile in piles of wood or bricks (Grijalva, unpublished data). These insects were initially classified as *T. dimidiata* as their appearance is very similar to that of *P. howardii*. *T. dimidiata* have been reported from Manabi in peridomestic terrestrial bromeliads and periurban rubbish dumps (Abad-Franch et al, 2001), though insects may have been incorrectly speciated in previous reports as well. The significance of *P. howardii* as a vector of *T. cruzi* in the Manabi province is unknown.

While the province of Guayas is historically considered to be endemic for *T. cruzi*, a survey of 476 households conducted in six rural communities of Guayas in 2003 found evidence of triatomine vectors in only one domicile, where five insects of the species *R. ecuadoriensis* were found inside the house and in a tree in the nest of a bird. (Grijalva, unpublished data)

Risk Factors for Transmission of T. cruzi

Until recently, no studies addressing risk factors for the transmission of *T. cruzi* in Ecuador appeared in the scientific literature. A recently published study conducted in the Amazon region of Ecuador reports that *T. cruzi* seropositivity in this area was associated with older age, being a lifelong resident of the Ecuadorian Amazon provinces, and living in a house with a thatch roof or open wall construction (Grijalva et al, 2003). Data previously collected in the Manabi province in conjunction with the current study indicate that individuals residing in houses with wood board floors were twice as likely to be seropositive when compared to those living in houses with cement floors. These data also indicate that accumulation of vegetal matter around the house, which includes palm tree leaves, agricultural products, or agricultural refuse, was protective against seropositivity, a finding that has never been reported in previous literature. A significant clustering effect of seropositives within households was also observed, even after adjustment for other household variables. (Grijalva, unpublished data) A recent study from the Loja province evaluating risk factors for domiciliary infestation with triatomine vectors of *T. cruzi* found that the

only household variables associated with triatomine infestation were the absence of some form of toilet or latrine (odds ratio=2.8) and insecticide spraying of the household (odds ratio=0.4). However, the lack of variability in housing construction materials in this area precluded the investigation of housing materials as potential risk factors for triatomine infestation. The number of dogs or chickens in the household was not related to the number of triatomines captured per house. (Grijalva, unpublished data)

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CHAPTER 2. STATEMENT OF SPECIFIC AIMS

Specific Aims

1. To determine the prevalence of infection with *Trypanosoma cruzi* in rural areas of the Manabi, Loja, and Guayas provinces of Ecuador.

2. To evaluate the relationship between housing characteristics and *Trypanosoma cruzi* seropositivity. Housing characteristics of interest include: construction materials of walls, roof and floor; presence of palm trees around house; and accumulation of firewood, lumber, rocks, trash, or organic matter around house.

3. To determine if the association between *T. cruzi* and housing characteristics is different in different geographic regions.

4. To evaluate the clustering effect of *Trypanosoma cruzi* seropositivity within households.

Rationale

The first aim of this project involves determining the prevalence of *Trypanosoma cruzi* in selected rural communities in the Manabi, Guayas, and Loja provinces of Ecuador. Manabi and Guayas are both historically considered to be endemic for *T. cruzi* infection, and many estimates of the prevalence of *T. cruzi* in various localities in these provinces exist in the literature (Table 1.2). However, these estimates vary widely, ranging from 1% to 17% in Manabi and 0.6% to 24% in Guayas. (Aguilar et al, 1999) The samples from which these estimates were obtained varied and were not necessarily representative of the populations from which they were drawn, possibly explaining the variation in prevalence estimates. The lowest estimates of prevalence in both Manabi and Guayas were obtained from surveys conducted in 1999. Prior to the 1999 survey, the most recent estimates of *T. cruzi* prevalence in these provinces were reported in 1986. Whether the low prevalence observed in 1999 represents a true decline in prevalence or is a result of differences

in survey methodology merits further investigation. A recent entomological survey conducted in three rural communities in Manabi found 20.7% of 353 houses to be infested with triatomine insects that could potentially serves as vectors of *T. cruzi*. In contrast, a 2002 survey conducted in five rural communities in the Guayas province found only one of 476 houses to harbor any triatomine insects. (Grijalva, unpublished data) The province of Loja is usually not included in the list of areas of Ecuador known to be endemic for Chagas disease. Only two serological studies of *T. cruzi* infection have been reported from Loja. Both were conducted among schoolchildren in the 1950s, and both report a seropositivity of 2%. However, a recent entomological survey revealed that 35% of surveyed households were infested with triatomines that could potentially transmit *T. cruzi*, with an average of 52 bugs per infested house. *Trypanosoma cruzi*-like organisms were found in the feces or hindgut of 5% of a subset of the captured insects, suggesting that transmission of *T. cruzi* to humans is likely occurring in the Loja province. (Grijalva et al, 2005)

The remaining aims of the project focus on evaluating household risk factors for the transmission of *T. cruzi*. An understanding of the relationship between domiciliary and peridomiciliary characteristics and the risk of *T. cruzi* infection is necessary for the development of appropriate intervention strategies in endemic areas of Ecuador. Previously published studies have identified materials used in the construction of roofs, walls, and floors of homes as well as other characteristics of the peridomicile area such as accumulation of firewood outside the house and the presence of palm trees as factors associated with both infection with *T. cruzi* and household infestation with triatomine vectors of *T. cruzi*, but results have been inconsistent across studies. Many prior studies that assessed the relationship between household variables and *T. cruzi* seropositivity have methodological flaws. A recent study by Rizzo et al reported that materials used in the construction of walls, roofs, and floors were all related to *T. cruzi* seropositivity, but the authors conducted only univariate analyses and did not evaluate the independent associations of each of these factors controlling for the effects of the others (Rizzo et al, 2003). In two other

studies, subjects were sampled in households but the data were not analyzed with techniques that accounted for the non-independence of the subjects, thus making it more likely for the authors to report as significant associations that truly were not (Greer, 1999; Mott, 1978). An additional study had a sample size of only 44 subjects, yielding very imprecise estimates (Carneiro et al, 2001). The majority of the remaining studies evaluated household infestation with triatomine vectors as the study outcome without establishing a direct link between the studied factors and human infection with *T. cruzi* (Starr et al, 1991; De Andrade et al, 1995; Cecere et al, 1998; Enger et al, 2004)

Risk factors for *T. cruzi* transmission in a particular area depend on regional living habits and the behavior of local vector species. Therefore, risk factors that were identified in studies conducted in other countries or in the Amazon region of Ecuador may not be risk factors in the coastal and Andean regions of Ecuador. All of the previous studies of both *T. cruzi* infection and house infestation have been conducted in Brazil (Mott et al, 1978; Carneiro et al, 2001; De Andrade et al, 1995), Argentina (Gurtler et al, 1998a, 1998b; Cecere et al, 1998), Guatemala (Greer et al, 1999; Rizzo et al, 2003), Costa Rica (Starr et al, 1991; Zeledon and Vargas, 1984), and the Ecuadorian Amazon (Grijalva et al, 2003). In Brazil and Argentina, the main or only vector of *T. cruzi* is *Triatoma infestans*, a species that is strictly domiciliated. The main species of triatomine vectors in Ecuador are *T. dimidiatia* and *R. ecuadoriensis*, which can be domiciliary, peridomiciliary, or sylvatic.

The Southern Cone Initiative was successful in interrupting transmission of *T. cruzi* by eliminating domestic colonization of triatomines through systematic spraying with residual insecticides. This approach worked in this area because the triatomines were completely domiciliated. This same approach might not be as successful in Ecuador because the presence of peridomestic and sylvatic vectors introduces the possibility that houses can be recolonized by vectors from peridomestic or sylvatic habitats once the domestic vectors are eliminated and also

that exposures to *T. cruzi* can occur outside of the household. For these reasons, household characteristics and characteristics of the peridomicile are included as exposures in the current study.

A previous study by our group in another population in Manabi found that having trash and "vegetal matter", which includes palm tree leaves, agricultural products, or agricultural refuse, in the peridomicile area were protective against seropositivity. This finding has not been previously reported and is contrary to a study from Mexico that found the presence of junk piles in the yard to be associated with intradomicilliary and peridomicilliary infestation with triatomine insects of the species *Triatoma pallidipennis*. In this same study, the presence of agricultural products in the yard was associated with intradomicilliary but not peridomicilliary infestation, while stone piles and wood piles were not associated with any infestation. (Enger et al, 2004) Our finding of a protective effect against seropositivity could be a spurious finding or an indication that vectors in this area have colonized peridomiliciliary habitats rather than become domiciliated. The conflicting results of these two studies merit further investigation of the relationship between characteristics of the peridomicile and *T. cruzi* infection. Materials accumulated in the peridomicile area that could provide habitats for triatomine insects, including firewood, trash, organic matter, lumber, and rocks, have been included as exposures in the present study.

Since the vectors of *T. cruzi* and the lifestyles of the people are different in the different regions of Ecuador, risk factors might not be consistent across the three study areas. Therefore, interactions between risk factors and geographic area were investigated.

In a paper published in 1976, Mott et al state "Since transmission of Chagas' disease largely occurs within households, description and analysis of the characteristics of household clustering of seropositivity to *T. cruzi* are of particular interest." (Mott et al, 1976) Knowledge of the extent to which *T. cruzi* clusters within households can provide information about the extent to which an individual's risk of infection is affected by living in close proximity to another infected person,

who can serve as a reservoir of *T. cruzi*. Additionally, the persistence of clustering within households after adjustment for other known household risk factors for *T. cruzi* infection may indicate that other as yet unknown factors are involved in the transmission of *T. cruzi* within households. Mott used a technique to estimate the presence of household clustering by comparing the observed versus expected distribution of seropositive individuals per household, but could not quantify the degree of clustering within households nor control for the effects of other household factors. (Mott et al, 1976) Only one paper since then has addressed the issue of clustering. Gurtler et al employed a random effects model, (Gurtler et al, 1998b) which can estimate the residual household effect on the probability of being infected after adjustment for other household variables, but this parameter does not have an easily understandable interpretation in terms of the magnitude of the clustering, nor do other household parameters in the model have a logical interpretation since the odds ratios produced by the random effects model relate the change in risk of seropositivity for individuals within a household due to changes in covariates for that household (Preisser et al, 2003; Hu et al, 1998; Handley et al, 2003). For example, Gurtler et al report a significant association between type of roof and *T. cruzi* seropositivity. The interpretation of the odds ratio associated with type of roof is "within a household, an individual with a thatched roof has 5 times the odds of being infected than an individual with a roof made of simbol." This interpretation is illogical since persons living in the same household will naturally have the same type of roof.

While Mott et al report significant clustering of *T. cruzi* seropositivity within households (Mott et al, 1976), Gurtler et al found no effect of clustering after adjustment for other covariates (Gurtler et al, 1998b). In the present study, alternating logistic regressions (ALR) were used to quantify the degree of clustering within households. Alternating logistic regressions provide pairwise odds ratios of association of the outcome, in this case *T. cruzi* seropositivity, within clusters while also taking into account the dependence of the outcome on individual and cluster-

specific covariates (Carey et al, 1993; Katz et al, 1993). The pairwise odds ratios obtained from ALR have a more natural interpretation for quantifying the magnitude of clustering within households than the variance component obtained from a random effects logistic model, and, since ALR is a "population-averaged" approach, the interpretation of the coefficients of the other covariates in the model is not restricted to persons residing within the same household.

Hypotheses

1. Individuals living in houses with palm roofs are more likely to be seropositive than those living in houses with roofs composed of closed materials (concrete, asbestos, zinc, tile).

2. Individuals living in houses with cane, wood, or adobe walls are more likely to be seropositive than those living in houses with cement or brick walls.

3. Individuals living in houses with dirt, wood, or cane floors are more likely to be seropositive than those living in houses with cement or tile floors.

4. Individuals living in houses near palm trees are more likely to be seropositive then those living in households with no palm trees.

5. There is an association between seropositivity and living in households with firewood, lumber, rocks, trash, or organic matter accumulated in the peridomicile area.

6. Seropositivity will cluster within households.

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CHAPTER 3. METHODS

Overview of Methods

This is a cross-sectional study conducted during the years 2001-2003 in rural communities in three provinces of Ecuador: the coastal provinces of Manabi and Guayas and the province of Loja, located in the Andean highlands. All residents of 14 selected communities were invited to participate in the study. All subjects were tested for serologic evidence of infection with *T. cruzi*, and were designated as cases or controls based on a positive or negative test result. Demographic information on each subject was obtained by personal interview. Additionally, study personnel visited each household to collect information about the construction materials of the house and other relevant household exposures. The overall project is a collaborative effort between Ohio University, Catholic University of Ecuador, and the non-governmental organization Plan International. Informed consent for participation in the study was obtained from all adults and parents or guardians of minor subjects in accordance with the Institutional Review Board of Ohio University and the Catholic University of Ecuador Ethical Committee policies and procedures. Approval to conduct secondary analyses on the collected data was granted by the Institutional Review Board of the University of North Carolina School of Public Health.

The prevalence of seropositivity to *T. cruzi* was calculated for each province overall and for each community within the provinces. Prevalence was defined as the number of seropositives divided by the total number of subjects. Age-specific prevalence in each of the provinces was estimated with Poisson regression. The shape of the age-specific prevalence curves was used to make predictions about the current state of *T. cruzi* transmission in each province.

Another objective of the study was to examine the relationship between *T. cruzi* seropositivity and household risk factors. Specifically, exposures of interest were the type of material used in the construction of the roof, walls, and floor of the house, the presence of palm trees near the house, and the accumulation of firewood, lumber, rocks or bricks, trash, and organic matter (defined as palm tree leaves, agricultural products or agricultural refuse) in the peridomicile area. Each variable was evaluated as a unique exposure, and for each exposure a model was constructed containing the appropriate confounding and interaction terms. Variables to be considered as potential confounders for each exposure were initially assessed through the use of a directed acyclic graph (DAG) illustrating the effect of household exposures on seropositivity to *T. cruzi* based on the theory of causal diagrams proposed by Greenland, Pearl, and Robins (Greenland et al, 1999; Hernan et al, 2002). Once a potential adjustment set of variables was identified for each exposure, each variable in the adjustment was further evaluated as a confounder by assessing if the variable was associated with seropositivity independent of the exposure of interest and if the variable was associated with the exposure (Rothman and Greenland 1998, p. 123). When these two criteria were met then the variable was included along with the exposure in a multivariate model. The outcome was modeled as the log odds of seropositivity for an individual given a certain household exposure. Because the exposures of interest are household characteristics and therefore all members of a household necessarily have the same values for all exposures, thus violating the assumption of independence necessary for standard logistic regression analyses, the outcome was modeled with generalized estimating equations (GEE) to account for the correlation of individuals within households.

Since the species of triatomine vectors and their behavior vary by geographic region, there was a priori speculation that the risk of seropositivity associated with each exposure differed by geographic region. In order to test if such an effect modification existed, an interaction term was created for each exposure consisting of the exposure and an indicator variable for region. Region

was dichotomized as either "coastal" for the Manabi and Guayas provinces or "highlands" for the province of Loja. The significance of the interaction term was tested in each exposure model with the Wald test.

Also of interest was to quantify the extent to which *T. cruzi* infections aggregate within households after adjustment for known household risk factors of *T. cruzi* transmission. This was done with the use of alternating logistic regressions (ALR), which can estimate the association of disease within households in the form of a pairwise odds ratio adjusted for the effect of other household exposures on the risk of seropositivity. The variables identified in the previous analyses as risk factors for *T. cruzi* seropositivity were included for adjustment in the ALR model.

Sensitivity analyses were conducted in order to quantify the potential bias in the estimates of each exposure-seropositivity relationship due to misclassification of disease or exposure status. To correct for misclassification, 2x2 tables were constructed for each exposure-seropositivity relationship and an odds ratio (OR) for each exposure was calculated using these observed data. The data in the tables were reclassified using the sensitivities and specificities of each exposure measurement as determined in a validation study. These partially corrected data were recategorized again using estimates of the sensitivity and specificity of the serologic tests as reported in the literature. From the resulting 2x2 representing the "true" distribution of this population regarding exposure and disease status, an OR was calculated from these reclassified data. This "true" OR was compared to the observed OR to estimate the magnitude of bias due to misclassification.

In addition to the sensitivity analyses, regression diagnostics were performed on each exposure model to ensure that the observed estimates of effect were not due to one or more households with extreme data values that exhibited undue influence on the overall results.

Study Design

Source Population

The study population consisted of all residents of selected communities in rural areas of the Manabi, Loja, and Guayas provinces of Ecuador. Manabi and Guayas are both coastal regions with a tropical climate and an average annual rainfall of approximately 750 mm, while the Loja province is located in the southern highlands of Ecuador and has a temperate climate with an average annual rainfall of 400 mm. Sampled communities in Guayas, Manabi and Loja had altitudes ranging from 20-40, 5-316 and 825-2200 meters above sea level, respectively. The participating communities were Cruz Alta, Pinpigausi, and Pasaje in Manabi province, Pindo Alto, Jacapo, Bramaderos, Playas, and Naranjo Dulce in Loja province, and Los Angeles, Puerto Rico, Lomas de Colimes, San Antonio, La Alegria, and Macul in Guayas province. Study communities were identified by local branches of Plan International, a non-governmental health organization, based on the logistical ability to carry out the proposed study in the community and the community having a prior relationship with Plan International.

Selection Criteria

All residents of the selected communities were invited to participate in the study. In order to be included in the study, the head of the household to which the subject belonged must have consented to participate in the household interview, and the subject must have attended a study-sponsored medical clinic. The clinics were conducted in conjunction with Plan International and the Ecuadorian Ministry of Health. At the clinic, each participant donated a blood sample for serologic testing for infection with *T. cruzi* and participated in an interview during which demographic information and information on personal risk factors for *T. cruzi* infection was collected. Each subject was also given a free medical exam by a physician from the Ministry of Health and received treatment for medical conditions that could be treated on-site and appropriate

referrals for more serious ailments. Subjects were excluded from the analyses in the present study if they did not provide a blood sample or if the blood sample provided was not suitable for serologic testing.

Identification of Cases/Controls

All subjects with serologic evidence of infection with *T. cruzi* were considered cases. All subjects without serologic evidence of infection with *T. cruzi* served as controls. Serologic testing was performed in the following manner:

Serum Samples. Blood was collected via venous puncture from all participants using Vaccutainer separator tubes. Blood was allowed to clot, centrifuged, and serum was transferred to individually labeled criovials and stored at -20^{o} C until use. Human chagasic sera from clinically diagnosed patients were provided by the Instituto Izquieta Perez de Higiene y Medicina Tropical (Quito, Ecuador) for use as positive controls. Endemic negative controls were obtained from students of the Universidad Catolica del Ecuador in Quito, and nonendemic negative controls were obtained from healthy laboratory volunteers from the Instituto Nacional de Higiene y Medicina Tropical in Quito.

Serological Testing. For all three study sites, initial screening was performed by enzyme-linked immunosorbent assay (ELISA) using detergent extracted *T. cruzi* epimastigote antigens as previously described (Grijalva et al, 1995). The optical density (OD) values of positive and negative controls were analyzed and used to define the limits for seropositivity and seronegativity of the assay. OD values within 2.5 standard deviations (SDs) of the OD average for the positive controls were considered seropositive and all OD values within 2.5 SDs of the OD average for negative controls were considered negative. Sera previously confirmed as anti-*T. cruzi* seropositive served as positive controls. Samples that did not fulfill the criteria for positive or negative were classified as borderline. Positive and borderline samples were assayed at least two

more times. Samples that were positive at least three times were considered positive. Samples with repeated borderline results were considered negative. All tests were based on a single blood sample.

For samples collected in the Manabi province in 2001, all samples positive by the initial ELISA were confirmed by immunofluoresence at the Centers for Disease Control and Prevention (Atlanta, GA). All samples collected in the Loja province in 2002 that were positive by the initial ELISA and approximately 50% of the negative samples were subsequently tested with two commercially available ELISA kits (Chagatest ELISA recombinante V 3.0, Wiener Labs, Rosario, Argentina; Chagas Serum Antibody Detection Assay Microwell ELISA, IVD Research Inc, Carlsbad, CA) and a commercial indirect hemagglutination test (Chagatest HAI, Wiener Labs, Rosario, Argentina). All samples collected in Guayas province were also tested using Chagatest ELISA recombinante V 3.0 and Chagatest HAI (Wiener Labs). These tests were performed according to the manufacturer's instructions. Samples from these provinces that were positive by the recombinant ELISA or by at least two of the other tests were considered positive.

Classification of Exposures

Exposure information was ascertained through the use of surveys. Household surveys were conducted by trained study personnel fluent in Spanish who visited each household. Household characteristics, including construction materials, vegetation around the house, and presence of trash, firewood, etc around the house were observed and noted by the interviewer. Other information, such as the number of people that sleep in the house and ownership of domestic animals, was asked of the head of the household. Household exposures were classified in the following manner:

Type of roof was classified as concrete, asbestos, zinc, tile, palm or straw, or other. A hierarchy of materials was constructed based on ability to harbor triatomine vectors, ranked in the above order, and roofs composed of more than one type of material defaulted to the lowest material for analysis purposes.

Type of wall was classified as cement, brick, or block, wood, adobe, cane reinforced with paper, cane reinforced with clay, cane alone, or other. Materials were ranked in the above order based on ability to harbor triatomines, and walls composed of more than one type of material defaulted to the lowest material for analysis purposes.

Type of floor was classified as cement, parquet, tile or vinyl, wood board, cane, dirt, or other. Materials were ranked in the above order based on ability to harbor triatomines, and floors composed of more than one type of material defaulted to the lowest material for analysis purposes.

A house was considered to have palm trees if at least one palm tree was observed within 20 meters of the house.

A house was considered to have firewood, trash, organic matter, construction materials, or rocks and bricks accumulated outside if the interviewer noted the presence of any of these materials piled outside of the house.

A house was considered to have dogs or chickens if the head of the household responded that he/she owned at least one of these animals.

Quality Control

Digital photographs were taken of all houses in the study and their surrounding peridomicile. A validation study was conducted using the photographs of 100 randomly selected houses from each province. The sensitivity and specificity of the survey instrument in correctly classifying each exposure was calculated by validating that the values recorded in the database for each exposure match the characteristics shown in the photos of the houses. The sensitivity of each exposure measurement was defined as percentage of households with the characteristic that were correctly classified as having that characteristic, and the specificity was defined as the percentage of households without the characteristic that were correctly classified as not having that characteristic. These sensitivities and specificities were later used in sensitivity analyses to estimate the extent to which the estimates of the associations between *T. cruzi* seropositivity and each exposure could be incorrect due to misclassification of the exposures.

Other quality control measures included conducting each serologic test on each sample in duplicate and requiring that a sample be positive in at least two different tests to be considered a

positive result, thus decreasing the likelihood of a false positive test, and checking the database for implausible or outlying values for any variable. When a suspect value was found, the original questionnaire was sought to ensure that the data had been entered correctly. If the value entered on the original questionnaire was implausible then the data point was deleted and considered a missing value.

Data Analysis

Prevalence of T. cruzi *Seropositivity*

Prevalence of *T. cruzi* seropositivity was defined as the number of subjects with a positive serology for *T. cruzi* divided by the total population. The prevalence of *T. cruzi* in the study population is presented by province and by community within each province for descriptive purposes. Age-specific *T. cruzi* seroprevalence by province was estimated from these data by a Poisson regression model containing predictor variables for age and province. Several models were constructed in an effort to fit the most parsimonious model that conferred the best fit to the data. Age was divided into 10-year categories and was tested in models as both an ordinal variable for category of age and as a series of indicator variables for each 10-year age interval. Squared and cubic forms of the ordinal age variable as well as various combinations of age by province interactions were added to the models and tested for significant improvement of model fit. Goodness of fit was assessed by the deviance of the models, which follows an approximate chi-squared distribution with degrees of freedom (DF) equal to $n - k$, where n is the number of observations and k is the number of parameters in the model. Heirarchical models were compared to one another by taking the difference in the deviances between the two models, which is equivalent to a likelihood ratio test statistic with DF equal to the difference in degrees of freedom between the two models (Kleinbaum et al, 1998). A summary of the different models tested and assessment of goodness of fit for these models is given in Appendix 1.

The final selected model upon which estimates of prevalence (Y/N) are based is given as:

$$\text{Log}(E[Y]) = \alpha + \beta_1\text{agecat} + \beta_2\text{agecat}^2 + \beta_3\text{loja} + \beta_4\text{guayas} + \beta_5\text{agecat*loja} + \beta_6\text{agecat*guayas} + \beta_7\text{agecat}^2\text{*loja} + \beta_8\text{agecat}^2\text{*guayas} + \log(N)$$

Where Y = *T. cruzi* cases
N = Population size
Agecat = 0 if age 0-9
1 if age 10-19
2 if age 20-29
3 if age 30-39
4 if age 40-49
5 if age 50-59
6 if age 60-69
7 if age ≥ 70
Loja = 1 if province of Loja; 0 if province of Manabi or Guayas
Guayas = 1 if province of Guayas; 0 of province of Manabi or Loja

The number of subjects in each age category in each province was included in the model as the offset term.

Because subjects in the study population were sampled by household, the estimates of *T. cruzi* seroprevalence will be less precise than if individuals were sampled at random if *T. cruzi* seropositivity is correlated between individuals within households. The amount by which the variance must be inflated to account for the clustering of disease is the design effect (Φ), which is equal to the ratio of the variance obtained with cluster sampling to that obtained with simple random sampling (Snedecor and Cochran, 1989). If there is no correlation between individuals within households, the design effect will equal 1. (Katz and Zeger, 1994) The design effect was estimated according to the method of Katz and Zeger using the within-household pairwise odds ratio of seropositivity calculated with the use of alternating logistic regression. The estimation of the pairwise odds ratio with alternating logistic regression is outlined later in the section titled "Assessment of Clustering Effect." The formula for the design effect is as follows (Katz and Zeger, 1994; Katz et al, 1993):

$$\Phi = 1 + \{(p_{11}-p^2)/[p(1-p)]\}\{[(m-1/N]s^2+\bar{u}-1\}$$

Where p = prevalence of disease
m = the number of households
N = the total sample size
$\bar{u}$ = the mean of the household sizes
s^2 = the variance of the household sizes
α = within household pairwise odds ratio
p_{11} = the probability that two subjects chosen at random both have disease

$$= \frac{1 - 2p(1-\alpha) - \{[1 - 2p(1-\alpha)]^2 - 4\alpha p^2(\alpha-1)\}^{1/2}}{2(\alpha-1)}$$

The age-specific prevalences in each province were displayed graphically (age on the x-axis and prevalence on the y-axis), and the graphs were used to make predictions about the state of transmission of *T. cruzi* in each province. For example, assuming all ages are equally susceptible to infection and infection is life-long, the predicted shape of the graph if infection has been endemic in the province for a long period of time is a linearly-increasing diagonal line, while a straight horizontal line is indicative that infection has recently been introduced into the province.

Identification of Risk Factors for T. cruzi *Seropositivity*

The relationship between *T. cruzi* seropositivity and the following household exposures was evaluated: type of material used in the construction of the roof, walls, and floor of the house, presence of palm trees near the house, and the accumulation of firewood, lumber (and other materials used for construction), rocks and bricks, trash, and organic matter (defined as palm tree leaves, agricultural products, or refuse from agricultural products) in the peridomicile area. Each of these exposures was evaluated separately, and for each one a logistic regression model was constructed containing the adjustment variables and interaction terms appropriate for that particular exposure. In these analyses, variables for housing construction materials were categorized in the following manner:

Type of roof was divided into three categories. Cement, asbestos, and zinc were grouped together as the referent category. "Tile" was one category and "palm" was one category.

Type of walls was divided into four categories. Cement was the referent category. "Adobe" was one category, "wood" was one category, and cane reinforced with paper, cane reinforced with clay, and cane alone were grouped into a single "cane" category.

Type of floor was divided into four categories. Cement, tile, and parquet were grouped together as the referent category. "Wood boards," "cane," and "dirt" were each a single category.

For all three classes of variables "other" was included with the referent category.

The log of the odds of seropositivity among individuals in households with the characteristic of interest relative to the odds among individuals in households without was modeled with logistic regressions with the use of generalized estimating equations (GEEs) to account for the correlation of subjects within households. The GEE approach is an extension of generalized linear models that estimates population-averaged estimates while accounting for the dependency between the correlated subjects. The correlation between subjects in a cluster (in this case a household) is taken into account by robust estimation of the variances of the regression coefficient. GEEs treat the within-cluster correlation as a nuisance parameter, and a "working" correlation matrix is specified for the vector of observations from each cluster to account for the dependency among the observations. An exchangeable working correlation, which assumes uniform correlations across clusters, was specified for all GEE models. (Hu et al, 1998; Stokes et al, 2000)

Evaluation of Effect Modification. Since risk factors for seropositivity could differ by geographic region due to differences in human behaviors and the behavior of the triatomine vectors specific to each region, the first step in the model building strategy was assessment of effect modification by geographic region for each exposure-seropositivity relationship. An interaction term between exposure and geographic region was created and significance of this term was tested in each exposure model with a Wald test. An alpha level of 0.10 was used to determine the significance of the interaction term. Geographic region was dichotomized as "coastal," which includes the provinces of Manabi and Guayas, and "highlands," which includes the province of Loja. Manabi and Guayas are contiguous provinces with the same insect vectors of *T. cruzi*

reported from both locations, while Loja is geographically distinct with different triatomine vector species.

Materials used in the construction of homes are highly specific to region. Tile roofs are found almost exclusively in Loja while palm roofs are found almost exclusively in Manabi and Guayas. Similarly, adobe walls are found only in Loja while cane walls are absent in Loja but are abundant in Manabi and Guayas, and cane floors are found only in Manabi and Guayas but are not present in Loja. The addition of a term for region would create collinearity between region and the variables for roof type, wall type, and floor type; therefore, an interaction term between region and exposure was not added to the models for roof type, wall type, and floor type. For these exposures, two separate analyses were conducted: one in which the population included only subjects from Manabi and Guayas and one in which the population included only subjects from Loja.

Evaluation of Confounding. A potential set of confounders for each exposure was initially identified based on the theory of causal diagrams proposed by Greenland, Pearl, and Robins (Greenland et al, 1999), which is a formal mechanism for making decisions in data analysis based on a priori knowledge about the underlying biologic mechanisms of the disease under study. The basis of this theory is the directed acyclic graph (DAG), a diagram that links variables by arrows that represent direct causal effects of one variable on another (Hernan et al, 2002; Greenland et al, 1999). A DAG illustrating the effect of household exposures on infection with *T. cruzi* is shown in Figure 3.1. For each exposure of interest, the potential set of confounders identified for that exposure according to the rules of causal diagrams is listed in Table 3.1.

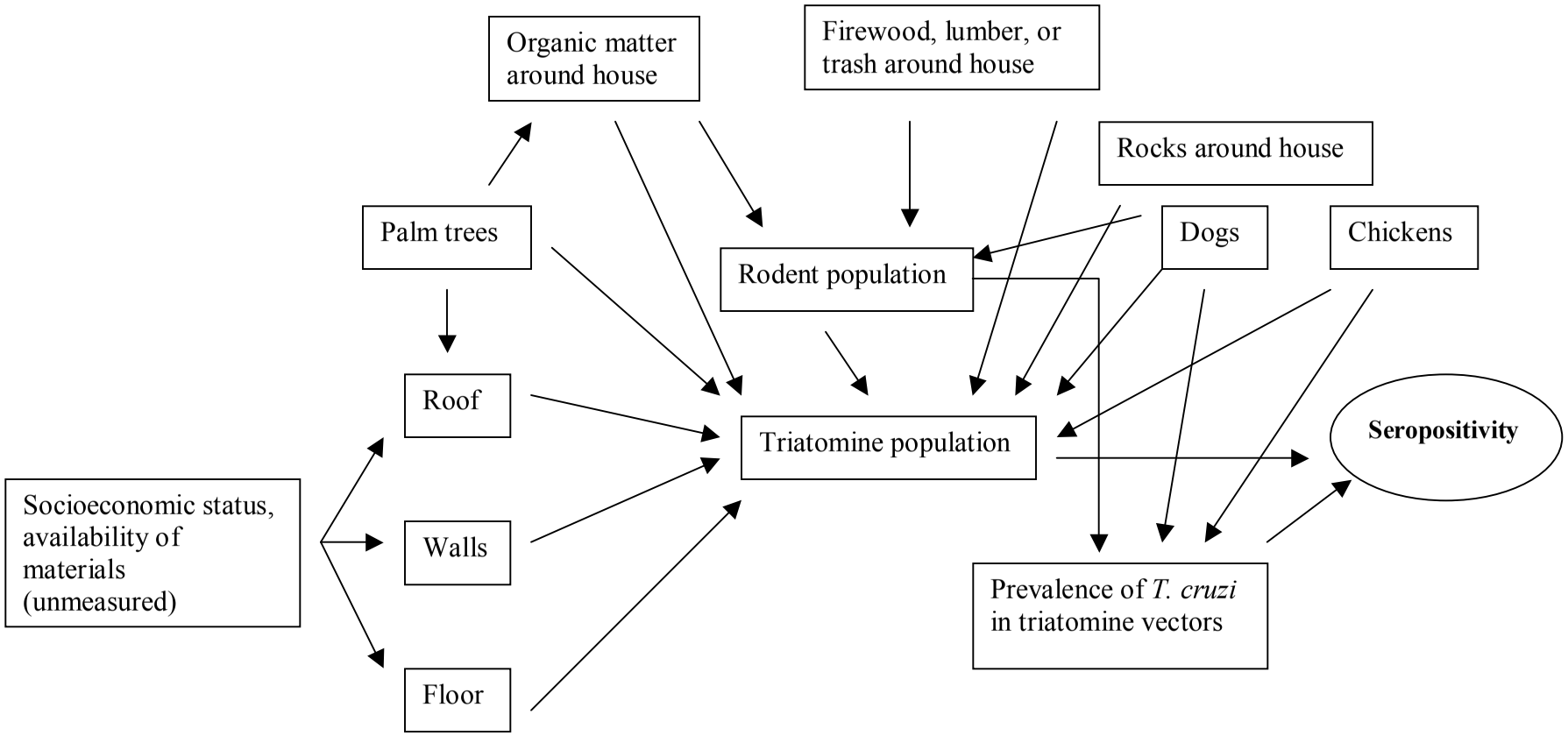

Figure 3.1. Directed acyclic graph illustrating the effect of household exposures on seropositivity with *Trypanosoma cruz*

Table 3.1. Potential adjustment variables for each exposure necessary to eliminate confounding in the exposure-seropositivity relationship

Exposure	Adjustment set	Comments
Type of roof	Walls, floor, palm trees	Walls, floor associated with roof through unmeasured factors (i.e, SES)
Type of walls	Roof, floor, palm trees	By adjusting for roof, an association between walls and palm trees is created; therefore, palm trees must be included as adjustment variable
Type of floor	Roof, walls, palm trees	By adjusting for roof, an association between floor and palm trees is created; therefore, palm trees must be included as adjustment variable
Palm trees	None – univariate analysis sufficient	Roof and organic matter are intermediates on the palm trees-serology pathway
Organic matter	Palm trees	All other factors on pathway are descendants of organic matter
Firewood	None – univariate analysis sufficient	All other factors on pathway are descendants of firewood
Rocks	None – univariate analysis sufficient	All other factors on pathway are descendants of rocks
Trash	None – univariate analysis sufficient	All other factors on pathway are descendants of trash
Lumber	None – univariate analysis sufficient	All other factors on pathway are descendants of lumber

The variables in the adjustment set for each exposure were further evaluated as confounders by establishing that the variable met the following criteria: 1) variable was associated with the exposure in the source population and 2) variable was associated with the outcome independent of the exposure of interest (Rothman and Greenland, 1998). As can be seen in Table 3.1, no potential confounders were identified for the variables palm trees, firewood, rocks, trash, and lumber. For the variables, no further evaluation of confounding was necessary and the univariate analyses are presented. Since many exposures had the same variables identified as confounders, the adjustment sets for the remaining exposures reduced to only two full models. For the exposure "organic matter," the model evaluated contained the variables organic matter and palm trees. For the exposures roof type, wall type, and floor type, the same full model was identified. This model contained the variables roof type, wall type, floor type, and palm trees.

To test for the first of the confounding criteria, that the potential confounder was associated with the exposure, the following combinations of variables were tested for their association:

Organic matter-Palm trees
Roof type-Wall type
Roof type-Floor type
Wall type-Floor type
Roof type-Palm trees
Wall type-Palm trees
Floor type-Palm trees

The associations were evaluated by calculating an odds ratio and corresponding 95% confidence interval for each pair. The household was the unit of analysis for these calculations. In these analyses, the variables for roof, walls, and floor were dichotomized into open/closed in the following manner:

Open roof = tile, palm, other / Closed roof = cement, asbestos, zinc

Open walls = adobe, wood, any form of cane, other / Closed walls = cement or brick

Open floor = boards, cane, dirt, other / Closed floor = parquet, tile, cement

The magnitude of the odds ratio and the width of the confidence interval were used in determining if an association exists between the variables. This analysis also served to evaluate for collinearity between the above variables, since the type of materials used in the construction of one part of a house might be highly correlated with the materials used in the construction of another part. An odds ratio of greater than 5 was considered indicative of collinearity. When collinearity existed between any two variables those variables were not used in the same model. Also, having palm trees outside one's house could be highly correlated with having a roof composed of palm leaves; therefore, the roof type-palm tree odds ratio was also calculated with roof type dichotomized as palm versus all others to check for collinearity between these two variables. As an additional check for collinearity, the aforementioned associations were also tested among only those households that contain at least one seropositive case, as high correlation among the cases could

introduce collinearity into the models even if the two variables are not highly correlated in the entire sample.

For the evaluation of the second criterion for confounding, that the potential confounding variable was associated with the outcome independent of the exposure of interest, the outcome was *T. cruzi* seropositivity, and each variable was evaluated for its association with seropositivity among those unexposed to the exposure of interest. Since this criterion for confounding requires that the association between the potential confounder and seropositivity be present among those unexposed to the exposure of interest, the populations being evaluated differed for each exposure. Table 3.2 gives the associations that were tested and the populations included in the analyses for each exposure.

Table 3.2. Associations between potential confounders and seropositivty to be tested for each exposure

Exposure	Population included in analysis	Relationships to be tested
Roof type	Subjects in households with closed roofs	Seropositivity-wall type Seropositivity-floor type Seropositivity-palm trees
Wall type	Subjects in households with closed walls	Seropositivity-roof type Seropositivity-floor type Seropositivity-palm trees
Floor type	Subjects in households with closed floors	Seropositivity-roof type Seropositivity-wall type Seropositivity-palm trees
Organic matter	Subjects in households with no organic matter	Seropositivity-palm trees

The magnitude of the odds ratio and the width of the corresponding 95% confidence interval were be used in determining if a relationship existed between each variable and seropositivity.

Construction of Final Models. The full models evaluated for each exposure based on the variables identified from the DAG as potential confounders and the a priori speculation of possible interactions between exposures and geographic region are shown below. Variables for housing construction materials were categorized as indicator variables as previously described. All other variables are dichotomous (yes/no). The final models for each exposure after evaluation of effect modification and confounding are shown in Chapter 4, Table 4.2.

Exposure	Full model
Type of roof[1]	$\text{logit}(Y) = \alpha + \beta_1\text{rooftile} + \beta_2\text{roofpalm} + \beta_3\text{walladobe} + \beta_4\text{wallwood} + \beta_5\text{wallcane} + \beta_6\text{floorwood} + \beta_7\text{floorcane} + \beta_8\text{floordirt} + \beta_9\text{palms}$
Type of walls[1]	$\text{logit}(Y) = \alpha + \beta_1\text{rooftile} + \beta_2\text{roofpalm} + \beta_3\text{walladobe} + \beta_4\text{wallwood} + \beta_5\text{wallcane} + \beta_6\text{floorwood} + \beta_7\text{floorcane} + \beta_8\text{floordirt} + \beta_9\text{palms}$
Type of floor[1]	$\text{logit}(Y) = \alpha + \beta_1\text{rooftile} + \beta_2\text{roofpalm} + \beta_3\text{walladobe} + \beta_4\text{wallwood} + \beta_5\text{wallcane} + \beta_6\text{floorwood} + \beta_7\text{floorcane} + \beta_8\text{floordirt} + \beta_9\text{palms}$
Palm trees	$\text{logit}(Y) = \alpha + \beta_1\text{palms} + \beta_2\text{region} + \beta_3\text{palms*region}$
Organic matter	$\text{logit}(Y) = \alpha + \beta_1\text{organic} + \beta_2\text{palms} + \beta_3\text{region} + \beta_4\text{vegetal*region}$
Firewood	$\text{logit}(Y) = \alpha + \beta_1\text{firewood} + \beta_2\text{region} + \beta_3\text{firewood*region}$
Rocks	$\text{logit}(Y) = \alpha + \beta_1\text{rocks} + \beta_2\text{region} + \beta_3\text{rocks*region}$
Trash	$\text{logit}(Y) = \alpha + \beta_1\text{trash} + \beta_2\text{region} + \beta_3\text{trash*region}$
Lumber	$\text{logit}(Y) = \alpha + \beta_1\text{lumber} + \beta_2\text{region} + \beta_3\text{lumber*region}$

[1] Separate analyses done for coastal and highland populations

Where Y = 1 if seropositive, 0 if seronegative
logit (Y) = ln[probability(Y=1)/probability(Y=0)]
α = intercept term

Assessment of Clustering Effect

The magnitude of household clustering of *T. cruzi* seropositivity was estimated in the form of pairwise odds ratios (ORs) of the association of seropositivity within households. Pairwise odds ratios were estimated with the use of alternating logistic regressions (ALR), which fit a model for the within-household odds ratio while simultaneously adjusting for the effect of other covariates on the risk of seropositivity. (Katz et al, 1993) The pairwise OR between individual *j* and individual *k* within household *i* is defined as

$$\psi_{ijk} = \frac{\text{pr}(Y_{ij} = 1, Y_{ik} = 1)\ \text{pr}(Y_{ij} = 0, Y_{ik} = 0)}{\text{pr}(Y_{ij} = 1, Y_{ik} = 0)\ \text{pr}(Y_{ij} = 0, Y_{ik} = 1)}$$

where Y = 1 if subject is seropositive; otherwise Y = 0
and $j \neq k$. (Carey et al, 1993)

The ALR algorithm involves the simultaneous estimation of two logistic regression models: one for the within-household pairwise odds ratios, given by $\log(\psi_{ijk}) = \alpha$, and one for the probability of seropositivity, given by $\text{logit pr}(Y = 1) = \beta_0 + \beta_1 x_1 + \ldots + \beta_p x_p$ where $x_1 \ldots x_p$ is a set of p explanatory variables associated with the risk of seropositivity and the βs are the log odds ratios for the risk of seropositivity associated with the respective covariates. The α and βs were estimated with the GENMOD procedure in SAS, which iterates between two steps until convergence:

1) Given the current estimate of α, a generalized estimating equation logistic regression is performed to obtain an updated estimate of the βs.

2) Given the current estimates of α and β, an offset logistic regression relating each outcome in a cluster to all other outcomes in that cluster is used to obtain an updated estimate of α.

Thus, alternating logistic regressions are being applied, one to estimate α and one to estimate the βs. An exchangeable structure for α was specified in the GENMOD procedure, which assumes that *j* and *k* are two randomly chosen individuals from the same household and the association between individuals is constant across all households. (Katz et al, 1993; Stokes et al, 2000)

The variables included in the β model were those factors that were shown in the previous analyses to be independently associated with *T. cruzi* seropositivity. Although the presence of dogs and chickens in the households were not previously evaluated as exposures of interest, these variables have been reported as possible risk factors for infection with *T. cruzi*. Therefore, these exposures were evaluated for their association with seropositivity for possible inclusion in the β model but were not found to be related to *T. cruzi* seropositivty in this population.

Power Calculations

Power was calculated using the Episheet Spreadsheets for the Analysis of Epidemiologic Data (Rothman, 2004). The potential sample sizes were generated using an estimated seroprevalence of *T. cruzi* ranging from 1.5% to 4%. In order to account for the correlation of individuals within households, the actual sample size was divided by the design effect in order to create an effective sample size upon which the power calculation was based. A design effect of 1.2 was used, calculated according to the method of Katz and Zeger (Katz and Zeger, 1994) using data from a previous study conducted in the Manabi province where the seroprevalence of *T. cruzi* was 4.1% and the within-household pairwise odds ratio was 3.21 (Grijalva, unpublished data). The power to detect an exposure odds ratio of 2.0 for a range of estimates of sample size and prevalence of exposure among non-cases is given in Table 3.3.

Table 3.3. Power to detect an odds ratio of 2.0 for various given sample sizes and prevalences of exposures among non-cases

		Prevalence of exposure among non-cases				
Sample size	**Effective sample size**	10%	20%	25%	30%	40%
65	54	0.5056	0.6484	0.6831	0.7030	0.7103
85	71	0.5913	0.7477	0.7837	0.8045	0.8158
100	83	0.6437	0.8020	0.3865	0.8563	0.8682
120	100	0.7075	0.9607	0.8912	0.9083	0.9194
150	125	0.7825	0.9183	0.9415	0.9537	0.9620
170	142	0.8228	0.9436	0.9620	0.9713	0.9775

Sensitivity Analyses

Selection Bias. While the heads of 1293 households consented to participate in the housing survey, residents of only 997 households presented to the medical clinics for further participation in the study. Therefore, serologic information was not available for residents of 296 (22.9%) houses, and the total number of inhabitants of these houses is unknown. However, the characteristics of these houses are known, so the characteristics of those houses in which the residents participated in the study were compared to the characteristics of the houses of the non-participants. Likewise, for those subjects that presented to the clinic but were excluded from the analysis due to a missing serology result, information regarding their household exposure status as well as other demographic information is known. Household and other relevant demographic information such as age and gender were compared between those subjects that were included in the sample population and those that were excluded. The generalizeability of the prevalence estimates may be affected if differences in exposure status and factors such as age exist between participants and non-participants and included and excluded subjects. However, because neither the subjects nor the investigators knew any of the serology results at the time of the surveys, serologic status is unlikely to have influenced whether or not a subject chose to participate in the

study. Therefore, any selection bias should be non-differential with respect to disease status, and the OR estimates for the household exposures should be unbiased.

Sensitivity Analysis of Misclassification. Since both the serologic tests used to determine the case or control status of the subjects and the survey instrument used in collecting household exposure data are imperfect, the possibility of bias in the estimates exists due to misclassification of the outcome and the exposure status. Sensitivity analyses were conducted to estimate the potential magnitude of the bias of each exposure-seropositivity relationship due to these misclassifications.

For each exposure, the following table was constructed with the observed classification of diseased and exposed subjects:

	Exposed	Unexposed	Total
Case	A_1*	A_0*	M_1*
Control	B_1*	B_0*	M_0*
Total	N_1*	N_0*	T

An observed odds ratio (OR*) for the association between each exposure and seropositivity was calculated as $OR^* = (A_1^*)(B_0^*)/(A_0^*)(B_1^*)$.

The true odds ratio using correctly classified data was estimated by first re-classifying the data with regard to exposure status using a range of plausible sensitivity and specificity values for each exposure measurement, including those determined by the validation study, and then applying the sensitivity and specificity of the serologic tests to this semi-corrected table to make the final reclassification due to disease status.

The table correctly classified by exposure status is given as:

	Exposed	Unexposed	Total
Case	eA_1	eA_0	M_1*
Control	eB_1	eB_0	M_0*
Total	eN_1	eN_0	T

The cells in the table were calculated with the following formulae:
$eA_1 = (A_1^* - Fp\ M_1^*) / (Se + Sp - 1)$

$eA_0 = M_1^* - eA_1$
$eB_1 = (B_1^* - Fp\ M_0^*) / (Se + Sp - 1)$
$eB_0 = M_0^* - eB_1$

where Se = sensitivity of the exposure measurement
Sp = specificity of the exposure measurement
Fp = false positive probability = 1 – Sp

In this step of the correction process the disease status is assumed to be classified correctly, so the total number of cases and controls after reclassification due to exposure status is equal to the observed total number of cases and controls (M_1^* and M_0^*). Sensitivity and specificity are assumed to be non-differential with regard to disease status.

The final correctly classified table is given as:

	Exposed	Unexposed	Total
Case	A_1	A_0	M_1
Control	B_1	B_0	M_0
Total	N_1	N_0	T

Since the correct total numbers of exposed and unexposed subjects were determined in the previous step, $N_1 = e\ N_1$ and $N_0 = e\ N_1$.

The remaining cells will be calculated as follows:
$A_1 = (eA_1 - FpeN_1) / Se + Sp - 1$
$B_1 = eN_1 - A_1$
$A_0 = (eA_0 - FpeN_0) / Se + Sp - 1$
$B_0 = eN_0 - A_0$

where Se = sensitivity of the serologic test
Sp = specificity of the serologic test
Fp = false positive probability = 1 – Sp

A series of correctly classified tables was constructed using a range of values for the sensitivity and specificity of the serologic tests as reported in the literature. Thus, a range of potential "true" ORs was calculated as $OR_{true} = A_1B_0 / A_0B_1$.

This method of sensitivity analysis has several limitations. Namely, the "true" ORs calculated here are not adjusted for confounding by other variables and do not take into account the non-independence of subjects due to clustering within households. Thus, they are not directly comparable to the odds ratios estimated by the GEE models in the risk factor analysis. However,

the "true" ORs can be compared to the observed univariate odds ratios (OR*) to estimate the degree to which the observed measures of effect for each exposure could be biased due to misclassification of disease and exposure status.

Regression Diagnostics. Regression diagnostics were performed on the models for each exposure to ensure that observed estimates of effect were not due to one or more household with extreme data values that conferred undue influence on the overall estimates. Two measures were used to estimate the influence of a particular cluster of data (in this case data from a single household) on the fitted regression models: *DFBETA* and Cook's D. Formulae for these diagnostic measures have been described for GEEs which are similar to those employed in linear regression (Preisser and Qaqish, 1996). The *DFBETA* is a measure of the change in the regression coefficient of a parameter due to the deletion of any cluster. Cook's D is a measure of the influence of any cluster on the overall fit of the model. *DFBETA*s were used to identify influential households on the odds ratios for exposures that were estimated from models without interaction terms. For models that contained an interaction term and thus the OR for a given exposure could not be calculated from a single parameter estimate, Cook's D was used as a measure of influence from a particular household.

For each exposure, the five households with the highest *DFBETA* associated with the model parameter for that exposure, or the five households with the highest Cook's D for the model that included the exposure of interest, whichever was the applicable case, were identified. These households were sequentially removed from the dataset (i.e. the household with the highest value of *DFBETA* was removed, then the two households with the highest values were removed, and so on), and the models were re-implemented. The ORs obtained after removal of the influential households were compared to those obtained from the full dataset. All regression diagnostics were computed with a SAS/IML macro for GEEs described by Hammill and Preisser (Hammill and Preisser, 2006).

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CHAPTER 4. HOUSEHOLD RISK FACTORS FOR *TRYPANOSOMA CRUZI* SEROPOSITIVITY IN TWO GEOGRAPHIC REGIONS OF ECUADOR

Abstract

Few studies of the relationship between environmental factors and *Trypanosoma cruzi* transmission have been conducted in Ecuador. We conducted a cross-sectional study of household risk factors for *T. cruzi* seropositivity in two distinct geographical regions of Ecuador. Exposure information was collected via household surveys, and all subjects were tested for serologic evidence of infection with *T. cruzi*. 3,286 subjects from 997 households were included in the study. In the coastal region, factors associated with being seropositive were living in a house with a palm roof (odds ratio, OR=2.63 [95% confidence interval, 1.61, 4.27]), wood walls (OR=5.75 [2.04, 16.18]) or cane walls (OR=2.81 [1.31, 6.04]), and the presence of firewood in the peridomicile area (OR=2.48 [1.54, 4.01]). Accumulation of trash outside the home was associated with a reduced risk of seropositivity (OR=0.25 [0.12, 0.51]). In the Andean region, living in a house with adobe walls was the only factor predictive of *T. cruzi* seropositivity. In conclusion, risk factors for *T. cruzi* transmission in Ecuador varied by geographic region, likely due to differing behavior of the triatomine vector species in each region. These findings illustrate that an understanding of the transmission dynamics of *T. cruzi* in a particular area are necessary for the development of effective Chagas disease control strategies in those areas.

Introduction

An estimated 120,000 to 200,000 people in Ecuador are infected with *Trypanosoma cruzi*, the causative agent of Chagas disease, and 25% of the entire population of Ecuador is estimated to be at risk for infection (Aguilar et al, 1999). However, few studies have been conducted to examine the relationship between environmental factors and risk of *T. cruzi* transmission in Ecuador. An understanding of these risk factors is necessary for the development of appropriate intervention strategies in endemic areas of Ecuador. Previous studies from other parts of Latin America have identified housing construction materials and characteristics of the peridomicile area such as accumulation of firewood outside the house and the presence of palm trees as factors associated with both infection with *T. cruzi* and household infestation with the triatomine vectors of *T. cruzi* (Mott et al, 1978; De Andrade et al, 1995; Greer et al, 1999; Rizzo et al, 2003; Starr et al, 1991; Zeledon and Vargas, 1984).

Risk factors for *T. cruzi* transmission in a particular area depend on regional living habits and the behavior of local vector species. The Southern Cone Initiative has achieved success in interrupting transmission of *T. cruzi* in Chile, Uruguay, and parts of Brazil and Argentina through systematic spraying with residual insecticides aimed at eliminating domiciliary populations of *Triatoma infestans*, the insect vector of *T. cruzi* in this region. Unlike *T. infestans*, which is almost strictly domiciliated, the main insect vectors of disease in Ecuador are reported to be *Triatoma dimidiata*, *Triatoma carrioni*, and *Rhodnius ecuadoriensis*, which have been found in domestic, peridomestic, and sylvatic habitats (Abad-Franch, 2001; WHO, 2000). Interventions targeted at controlling domestic vector populations such as *T. infestans* may not be as effective against other triatomine species.

Ecuador is divided into several unique geographic regions, each with different insect vectors of *T. cruzi*. Risk factors for *T. cruzi* infection may vary in differing parts of the country. A recent study from the Amazon region of Ecuador found that *T. cruzi* seropositivity in this area was

associated with living in a house with a thatch roof or open wall construction (Grijalva et al, 2003). The aim of the current study is to evaluate the relationship between housing characteristics and *T. cruzi* seropositivity in two other geographically distinct regions of Ecuador, the coastal lowlands and the Andean highlands.

Methods

Study population

The study population consists of all residents of 14 selected communities in rural areas of the Manabi, Guayas, and Loja provinces of Ecuador. Manabi and Guayas are both coastal regions with a tropical climate while Loja has a temperate climate and is located in the Andean highlands of southern Ecuador. In order to be included in the study, the head of the household to which the subject belonged must have consented to participate in a household interview, and the subject must have attended a study-sponsored medical clinic established in collaboration with the Ecuadorian Ministry of Health where a blood sample was drawn for serologic testing for *T. cruzi*. All subjects with serologic evidence of infection with *T. cruzi* were considered cases, and all subjects without serologic evidence of infection with *T. cruzi* served as controls. Subjects were excluded from the analyses in the present study if they did not provide a blood sample or if the blood sample provided was not suitable for serologic testing. All study procedures were approved by the Institutional Review Board of Ohio University and the Ethical Committee of Catholic University of Ecuador. Approval for secondary data analysis was granted by the Institutional Review Board of The University of North Carolina School of Public Health.

Serological testing

For all three study sites, initial screening was performed by enzyme-linked immunosorbent assay (ELISA) using detergent extracted *T. cruzi* epimastigote antigens as previously described (Grijalva et al, 1995). The optical density (OD) values of positive and negative controls were

analyzed and used to define the limits for seropositivity and seronegativity of the assay. OD values within 2.5 standard deviations (SDs) of the OD average for the positive controls were considered seropositive and all OD values within 2.5 SDs of the OD average for negative controls were considered negative. Sera previously confirmed as anti-*T. cruzi* seropositive served as positive controls. Samples that did not fulfill the criteria for positive or negative were classified as borderline. Positive and borderline samples were assayed at least two more times. Samples that were positive at least three times were considered positive. Samples with repeated borderline results were considered negative. All tests were based on a single blood sample.

For samples collected in the Manabi province in 2001, all samples positive by the initial ELISA were confirmed by immunofluoresence at the Centers for Disease Control and Prevention (Atlanta, GA). All samples collected in the Loja province in 2002 that were positive by the initial ELISA and approximately 50% of the negative samples were subsequently tested with two commercially available ELISA kits (Chagatest ELISA recombinante V 3.0, Wiener Labs, Rosario, Argentina; Chagas Serum Antibody Detection Assay Microwell ELISA, IVD Research Inc, Carlsbad, CA) and a commercial indirect hemagglutination test (Chagatest HAI, Wiener Labs, Rosario, Argentina). All samples collected in Guayas province were also tested using Chagatest ELISA recombinante V 3.0 and Chagatest HAI (Wiener Labs). These tests were performed according to the manufacturer's instructions. Samples from these provinces that were positive by the recombinant ELISA or by at least two of the other tests were considered positive.

Analysis of risk factors

Risk factors of interest were the type of material used in the construction of the roof, walls, and floor of the house, the presence of palm trees near the house, and the accumulation of firewood, lumber, rocks and bricks, trash, and organic matter (defined as palm tree leaves, agricultural products or agricultural refuse) in the peridomicile area. The peridomicile was defined

as the area within 20 meters of the house. Exposure information was ascertained by trained study personnel who visited each household. Each variable was evaluated as a separate main exposure, and for each exposure a unique logistic regression model was constructed containing the appropriate confounding and interaction terms.

Statistical analyses. Odds ratios (ORs) and corresponding 95% confidence intervals (CIs) for the associations between *T. cruzi* seropositivity and each exposure were calculated with logistic regression. Generalized estimating equations (GEEs) were used to account for the correlation of individuals within households. GEEs were implemented with the GENMOD procedure in SAS. For instances in which zero cases occurred in one or more exposure categories and thus an OR could not be calculated, differences between exposed and unexposed categories were compared with the Fisher's exact test. A p-value of ≤ 0.05 was considered indicative of a statistical difference between the two groups. All analyses were performed using SAS version 8.2 (Cary, NC).

Assessment of interaction. Since the species of triatomine vectors and their behavior vary by geographic region, we speculated that risk of seropositivity associated with each exposure might differ by geographic region. To test for such an effect modification, an interaction term between exposure and region was included in each model. Region was dichotomized as "coastal" for the Manabi and Guayas provinces and "highlands" for the province of Loja. Manabi and Guayas are contiguous provinces with the same insect vectors of *T. cruzi* reported from both locations, while Loja is geographically distinct with different triatomine vector species. The significance of the interaction term was tested with the Wald test. An alpha level of 0.10 was considered statistically significant for the presence of interaction.

Results for the evaluation of interaction are shown in Appendix 2, Table A2.1. The interaction term between region (coastal vs. highlands) and each respective exposure was statistically significant in the GEE logistic regression models for firewood and trash. Though not

statistically significant in the model for rocks, the odds ratio for the association between rocks and seropositivity was on the opposite side of the null for the different geographic regions. Therefore, an interaction term between exposure and region was included in the models for all peridomicile exposures. Interaction could not be assessed in the models for roof type, wall type, and palm trees because zero cases occurred in the referent categories of these variables in the highland region. Therefore, the logistic regression analyses of these exposures were restricted to subjects in the coastal region. An interaction term could not be included in the model for floor type due to collinearity between flooring materials and geographic region. Separate models with floor type as the main exposure were constructed for subjects in the coastal and highland regions.

Assessment of confounding. Variables to be considered as potential confounders for each exposure were initially assessed through the use of a directed acyclic graph (DAG) based on the theory of causal diagrams proposed by Greenland, Pearl, and Robbins (Greenland et al, 1999; Hernan et al, 2002). The DAG illustrating the effect of household exposures on infection with *T. cruzi* is shown in Figure 4.1. The potential set of confounders identified for each exposure of interest according to the rules of casual diagrams is listed in Table 4.1. Each variable in the potential adjustment set for each exposure was further evaluated as a confounder according to the two conventional criteria for confounding: 1) the variable was associated with the exposure of interest and 2) the variable was associated with *T. cruzi* seropositivity independent of the exposure of interest (Rothman and Greenland, 1998). Assessment of confounding factors for each exposure according to these criteria is shown in Appendix 2, Tables A2.2-A2.3.

All of the potential confounders for each exposure identified from the DAG and listed in Table 4.1 fulfilled the two additional criteria for confounding with the exception that the presence of palm trees was not associated with *T. cruzi* seropositivity among subjects in households without organic matter in the peridomicile; therefore, the OR for the association between seropositivity and organic matter was not adjusted for palm trees in the final analysis. Construction material of walls

was so highly correlated with both type of roof and type of floor (Appendix 2, Table A2.2) that collinearity was deemed to be present between these factors and wall type. Therefore, the variables for roof type and floor type were not included in the model for which wall type was considered the main exposure. Likewise, the variable for wall type was not included in the models for roof type or floor type. According to the rules of the DAG, palm trees no longer confound the wall type-seropositivity relationship once roof type is removed from the model; thus, the variable representing palm trees was removed from the model for wall type was well.

Although the variables for floor type and palm trees fulfilled all criteria to be considered as confounders in the association between roof type and seropositivity in the coastal region, the OR comparing palm roofs to metal roofs (the only roofing materials found in this region) was unchanged when these variables were removed from the multivariate model. Consequently, the estimate presented for the association between roof type and seropositivity is unadjusted for these variables. However, removal of the roof type and palm tree variables from the multivariate model for floor type resulted in a change of >10% in the OR for at least one of the categories of floor type and so these variables remained in the final model for floor type. The final models upon which all estimates for each of the exposures are based are shown in Table 4.2.

Results

Subjects from 1029 (80%) of the 1293 households in the study areas presented to the medical clinics and were eligible for participation in the study. Twenty-seven percent (1,244) of the 4530 eligible subjects did not contribute a blood sample and were excluded from further analyses. Approximately 75% of those not contributing a blood sample were children less than 10 years of age (Appendix 3, Table A3.1). Therefore, the study population on which this analysis is based consists of 3286 subjects from 997 households. The houses of study participants were significantly more likely to have zinc roofs and wood board floors and significantly less likely to have dirt

floors, palm trees, and organic matter accumulated in the peridomicile than non-participating houses (Appendix 3, Table A3.2).

Housing characteristics by province are given in Table 4.3. The majority of houses in Loja were constructed of tile roofs (92.7%), adobe walls (77.8%), and dirt floors (81.3%). Tile roofs and adobe walls were completely absent in the Manabi and Guayas provinces. In the coastal provinces of Manabi and Guayas the main housing construction materials were zinc or asbestos roofs, cane walls, and wood board floors. In the Manabi province, palm roofs (30.8%) and cane floors (27.1%) were also common. Palm trees outside the home were more common in Manabi (57.7%) than in Guayas (16.5%) and Loja (6.5%).

Overall, 73 seropositive cases were detected in the coastal region and 32 cases were detected in the highland region, corresponding to a prevalence of *T. cruzi* seropositivity of 5.7% (59/1041), 1.0% (14/1343), and 3.6% (32/902) in the provinces of Manabi, Guayas, and Loja, respectively.

The frequency of cases in each exposure group and the corresponding odds ratios for the association between each household exposure and seropositivity are given in Table 4.4, stratified by region. In the coastal region, factors associated with being seropositive were living in a house with a palm roof (OR=2.63 [1.61, 4.27]), wood walls (OR=5.75 [2.04, 16.18]) or cane walls (OR=2.81 [1.31, 6.04]), and the presence of firewood in the peridomicile area (OR=2.48 [1.54, 4.01]). Accumulation of trash outside the home was associated with a reduced risk of seroposivity (OR=0.25 [0.12, 0.51]). The estimates associated with palm roofs and cane walls are very similar (odds ratios of 2.63 and 2.81, respectively). Since these two factors are highly correlated and were not controlled for one another in a single model, it cannot be determined if both are independently associated with seropositivty or if the observed effect of one of these factors is due to confounding by the other. However, in an analysis restricted to subjects living in households with zinc or asbestos roofs, the relationship between cane walls and seropositivity for *T. cruzi* was similar to that seen in the overall analysis (OR=2.37 [1.07, 5.28]), suggesting that cane walls are related to

seropositivity independently of roof type. An analysis of roofing materials stratified by wall type was not possible because no houses with cement walls had a palm roof.

In the highlands region, a formal OR could not be calculated for tile or palm roofs, adobe or wood walls, wood board floors, or palm trees because zero cases occurred in the referent categories of these exposure groups. However, the proportion of cases living in houses with adobe walls (27/744) was significantly higher than in households with cement walls (0/138, p=0.0154). The difference was not statistically significant for wood walls or for the comparison between tile roofs and metal roofs (p=0.1628). No characteristics of the peridomicile were strongly associated with *T. cruzi* seropositivity in the highlands region (Table 4.3). Weak positive associations were observed for rocks and bricks (OR=1.79 [0.76, 4.21]) and trash (OR=1.66 [0.69, 3.98]), though these estimates are imprecise due to the low number of cases in this region.

Discussion

We found that compared to cement walls, cane walls and adobe walls in the coastal and highland regions of Ecuador, respectively, were associated with an increased risk of *T. cruzi* seropositvity. Previous studies investigating wall construction and seropositivity failed to find an association (Gurtler et al, 1998; Greer et al, 1999), though a few studies have shown adobe walls to be associated with household infestation of triatomines (Starr et al, 1991). Adobe walls are prone to cracking and provide hiding places and breeding sites for triatomine insect vectors of *T. cruzi*. Wood or cane walls can contain open spaces and are also subject to cracking and perhaps facilitate triatomine infestation in a similar manner.

In the coastal provinces, individuals living in a house with a palm thatch roof were almost three times as likely to be seropositive for *T. cruzi* as those individuals living in houses with roofs composed of zinc or asbestos. Previous studies reported associations between thatched roofs and *T. cruzi* seropositivity in areas where *Rhodnius prolixus* and *Triatoma dimidiata* were the main

vectors of transmission (Greer et al, 1999; Rizzo et al, 2003). Sylvatic populations of *R. prolixus* are found in palm trees and are thought to colonize houses via passive transport in the palm leaves used in the construction of thatched roofs (WHO, 2000; D'Alessandro et al, 1984). Sylvatic populations of *Rhodnius ecuadoriensis* have been reported from the Manabi province (Abad-Franch et al, 2001) and could be introduced into thatched roof homes in this area by a similar mechanism. The observed association with palm roofs could also be an artifact resulting from the high correlation between palm roofs and cane walls.

The presence of firewood, junk piles, and agricultural products outside of the house have been reported to be associated with household infestation of triatomines (Zeledon and Vargas, 1984; Enger et al, 2004). Interestingly, we found in the coastal region that accumulation of trash in the peridomicile area was protective against seropositivity to *T. cruzi*. Though the estimates were unstable, the accumulation of organic matter, rocks, and lumber appeared to be protective as well. Only the presence of firewood outside the home was associated with an increased risk of seropositivity. A protective effect against *T. cruzi* infection by any of these items has not been reported in the literature. However, in a previous analysis by our group of a different population from the Manabi province, we found that the accumulation of organic matter outside the house was inversely associated with *T. cruzi* seropositivity (Grijalva, unpublished data). Perhaps the presence of these materials in the peridomicile provides sufficient refuge for the triatomines such that they have colonized these habitats rather than the intradomicile. An entomologic search conducted in the Manabi province three years after the data for the present study were collected seems to support this hypothesis, as the majority of captured triatomines were found outside of houses in piles of wood and brick, chicken nests, and guinea pig pens (Grijalva, unpublished data). The finding that firewood is associated with an increased risk of infection is also consistent with this hypothesis as firewood is brought into the home on a regular basis and could serve to passively transfer the triatomine vectors into the domicile.

The idea that the presence of triatomione habitats in the peridomicile decreases the likelihood of intradomicilliary colonization is not supported by a recent study from Mexico, which reports that junk piles in the yard are associated with intradomicilliary and peridomicilliary infestation by *Triatoma pallidipennis*, while agricultural products in the yard were associated with intradomiciilairy infestation but not peridomicilliary infestation (Enger et al, 2004).

An additional explanation for the protective effect of trash and other materials in the peridomicile is that these items might attract predators of the triatomine, such as rodents and other small mammals, thereby reducing the triatomine populations. Alternatively, these small mammals may serve as a food source for the triatomine insects, decreasing the need for the vectors to enter the houses in search of a blood meal and therefore serving as a buffer for intradomiciliary infestation.

Unlike in the coastal provinces, no peridomicile characteristics were strongly associated with *T. cruzi* seropositivity in the highlands region. The difference in risk factors between the regions is probably due to differences in the triatomine vectors. Although *R. ecuadoriensis* is found in both regions, this species is found in sylvatic and domestic habitats in the coastal region, while only synanthropic populations of this species have been reported in the Loja province (Grijalva et al, 2005, Abad-Franch et al, 2001). The other main vector of *T. cruzi* in the Loja province, *Triatoma carrioni*, is found only in the Andean regions. In contrast to the coastal provinces, in which insects were found mainly in peridomicile areas, entomological searches in the Loja province found triatomines almost exclusively in the intradomicile, with peridomicile populations found only in association with occupied chicken nests. None of the aforementioned peridomicilliary characteristics were associated with household infestation by the triatomine vectors in an entomologic survey of the Loja province (Grijalva et al, 2005). Thus, it follows that materials in the peridomicile would also not be associated with *T. cruzi* infection in humans.

In conclusion, we identified several household characteristics that were related to *T. cruzi* seropositivity. Materials used in the construction of houses were associated with seropositivity in both the coastal and highland regions, despite the fact that the specific construction materials used differed by region. Peridomicilliary risk factors varied by geographic region. These differences were likely due to differing behavior of the triatomine vector species in each region. Our findings illustrate that there is no one-size-fits-all control strategy for Chagas disease. An understanding of the way in which humans, vectors, and environmental factors interact to promote the transmission of *T. cruzi* in a particular area is necessary for the development of an effective strategy for eliminating *T. cruzi* transmission in that area. The mainstays of Chagas disease control programs, such as housing improvement and systematic household spraying with residual insecticide, may not be sufficient to achieve interruption of transmission in Ecuador. Interventions here should be supplemented with additional activities such as spraying of peridomicle areas and continued surveillance for re-colonization of domestic environments by peridomestic and sylvatic species of triatomine vectors.

Tables and Figures

Table 4.1. Potential adjustment variables for each exposure necessary to eliminate confounding in the exposure-seropositivity relationship*

Exposure	Adjustment set	Comments
Type of roof	Walls, floor, palm trees	Walls, floor associated with roof through unmeasured factors (i.e, SES)
Type of walls	Roof, floor, palm trees	By adjusting for roof, an association between walls and palm trees is created; therefore, palm trees must be included as adjustment variable
Type of floor	Roof, walls, palm trees	By adjusting for roof, an association between floor and palm trees is created; therefore, palm trees must be included as adjustment variable
Palm trees	None – univariate analysis sufficient	Roof and organic matter are intermediates on the palm trees-serology pathway
Organic matter	Palm trees	All other factors on pathway are descendants of organic matter
Firewood	None – univariate analysis sufficient	All other factors on pathway are descendants of firewood
Rocks	None – univariate analysis sufficient	All other factors on pathway are descendants of rocks
Trash	None – univariate analysis sufficient	All other factors on pathway are descendants of trash
Lumber	None – univariate analysis sufficient	All other factors on pathway are descendants of lumber

* Table refers to Figure 1.

Table 4.2. Final models used in the determination of association between *T. cruzi* seropositivity and each main exposure after assessment of interaction and confounding

Exposure	Full model[1]
Type of roof[2]	$\text{logit}(Y) = \alpha + \beta_1\text{roofpalm}$
Type of walls[2]	$\text{logit}(Y) = \alpha + \beta_1\text{wallwood} + \beta_2\text{wallcane}$
Type of floor[3]	
Coastal	$\text{logit}(Y) = \alpha + \beta_1\text{floorwood} + \beta_2\text{floorcane} + \beta_3\text{floordirt} + \beta_4\text{roofpalm} + \beta_5\text{palms}$
Highland	$\text{logit}(Y) = \alpha + \beta_1\text{floorwood} + \beta_2\text{floordirt}$
Palm trees[4]	$\text{logit}(Y) = \alpha + \beta_1\text{palms}$
Organic matter	$\text{logit}(Y) = \alpha + \beta_1\text{organic} + \beta_3\text{region} + \beta_4\text{organic*region}$
Firewood	$\text{logit}(Y) = \alpha + \beta_1\text{firewood} + \beta_2\text{region} + \beta_3\text{firewood*region}$
Rocks	$\text{logit}(Y) = \alpha + \beta_1\text{rocks} + \beta_2\text{region} + \beta_3\text{rocks*region}$
Trash	$\text{logit}(Y) = \alpha + \beta_1\text{trash} + \beta_2\text{region} + \beta_3\text{trash*region}$
Lumber	$\text{logit}(Y) = \alpha + \beta_1\text{lumber} + \beta_2\text{region} + \beta_3\text{lumber*region}$

[1] Where Y = 1 if seropositive, 0 if seronegative; α = intercept term; region = 1 if coastal region, 0 if highland region; all other variables take the value of 1 if exposed, 0 otherwise

[2] Models for coastal region only. Models could not be constructed for these variables in the highland region due to lack of cases with metal roofs or cement walls

[3] categories of floor exposure differ by region because cane floors were not present in the highland region. Adjustment variables for roof type and palm trees could not be included in the model for the highland region because zero cases occurred in the referent categories of these variables in this region.

Table 4.3. Housing characteristics by province; N = number of households

Exposure	Manabi N=278 N (%)	Loja N=304 N (%)	Guayas N=415 N (%)
Type of Roof			
Cement/Asbestos/Zinc	181 (66.06)	21 (6.98)	398 (96.36)
Tile	0 (0)	279 (92.69)	0 (0)
Palm	85 (30.80)	1 (0.33)	13 (3.15)
Other	1 (0.36)	0 (0)	2 (0.48)
Type of Walls			
Cement	60 (21.82)	48 (15.89)	155 (37.53)
Adobe	0 (0)	235 (77.81)	0 (0)
Wood	9 (3.27)	11 (3.64)	11 (2.66)
Cane	206 (74.91)	7 (2.32)	244 (59.07)
Other	0 (0)	1 (0.33)	3 (0.73)
Type of Floor			
Cement/Tile/Parquet	53 (19.13)	48 (16.00)	105 (25.42)
Wood boards	132 (47.65)	8 (2.67)	227 (54.96)
Cane	75 (27.08)	0 (0)	58 (14.04)
Dirt	17 (6.14)	244 (81.33)	23 (5.57)
Palm trees	153 (57.74)	18 (6.45)	68 (16.46)
Organic matter	64 (24.24)	75 (25.00)	68 (16.46)
Firewood	132 (50.00)	154 (51.33)	86 (20.77)
Rocks and Bricks	12 (4.55)	37 (12.33)	60 (14.49)
Trash	34 (12.88)	63 (21.00)	199 (48.07)
Lumber	24 (9.09)	21 (7.00)	61 (14.73)

Table 4.4. Frequency of cases and non-cases by exposure status and odds ratio (OR) and 95% confidence interval (CI) for the association between each exposure and seropositivity in each geographic region

	Coastal 73 cases / 2311 non-cases		**Highlands 32 cases / 870 non-cases**	
Exposure	**Number of cases / non-cases**	**OR (95% CI)**	**Number of cases / non-cases**	**OR (95% CI)**
Type of Roof				
Cement/Asbestos/Zinc	48 / 1911	1	0 / 67	--
Tile	0 / 0	--	32 / 797	--
Palm	25 / 382	2.63 (1.61, 4.27)	0 / 0	--
Type of Walls[1]				
Cement	10 / 717	1	0 / 138	--
Adobe	0 / 0	--	29 / 715	--
Wood	5 / 63	5.75 (2.04, 16.18)	1 / 11	--
Cane	58 / 1514	2.81 (1.31, 6.04)	0 / 0	--
Type of Floor[2]				
Cement/Tile/Parquet	11 / 536	1	3 / 138	1
Wood boards	40 / 1193	1.80 (0.85, 3.82)	0 / 24	--
Cane	15 / 441	1.30 (0.55, 3.06)	0 / 0	--
Dirt	7 / 131	2.17 (0.78, 6.03)	29 / 703	1.82 (0.55, 6.02)
Palm trees	29 / 730	1.44 (0.89, 2.33)	0 / 47	--
Organic matter[3]	13 / 441	0.87 (0.46, 1.65)	7 / 204	0.89 (0.33, 2.43)
Firewood[3]	42 / 39	2.48 (1.54, 4.01)	14 / 452	0.70 (0.32, 1.52)
Rocks and Bricks[3]	5 / 233	0.62 (0.23, 1.70)	6 / 98	1.79 (0.76, 4.21)
Trash[3]	8 / 762	0.25 (0.12, 0.51)	10 / 185	1.66 (0.69, 3.98)
Lumber[3]	6 / 266	0.63 (0.26, 1.54)	2 /60	0.85 (0.23, 3.07)

[1] Analysis in highlands region excludes 2 cases and 3 controls living in a house with no walls
[2] Separate models constructed for coastal and highland regions; Coastal model adjusted for roof type and palm trees
[3] Model contains interaction term between exposure and region

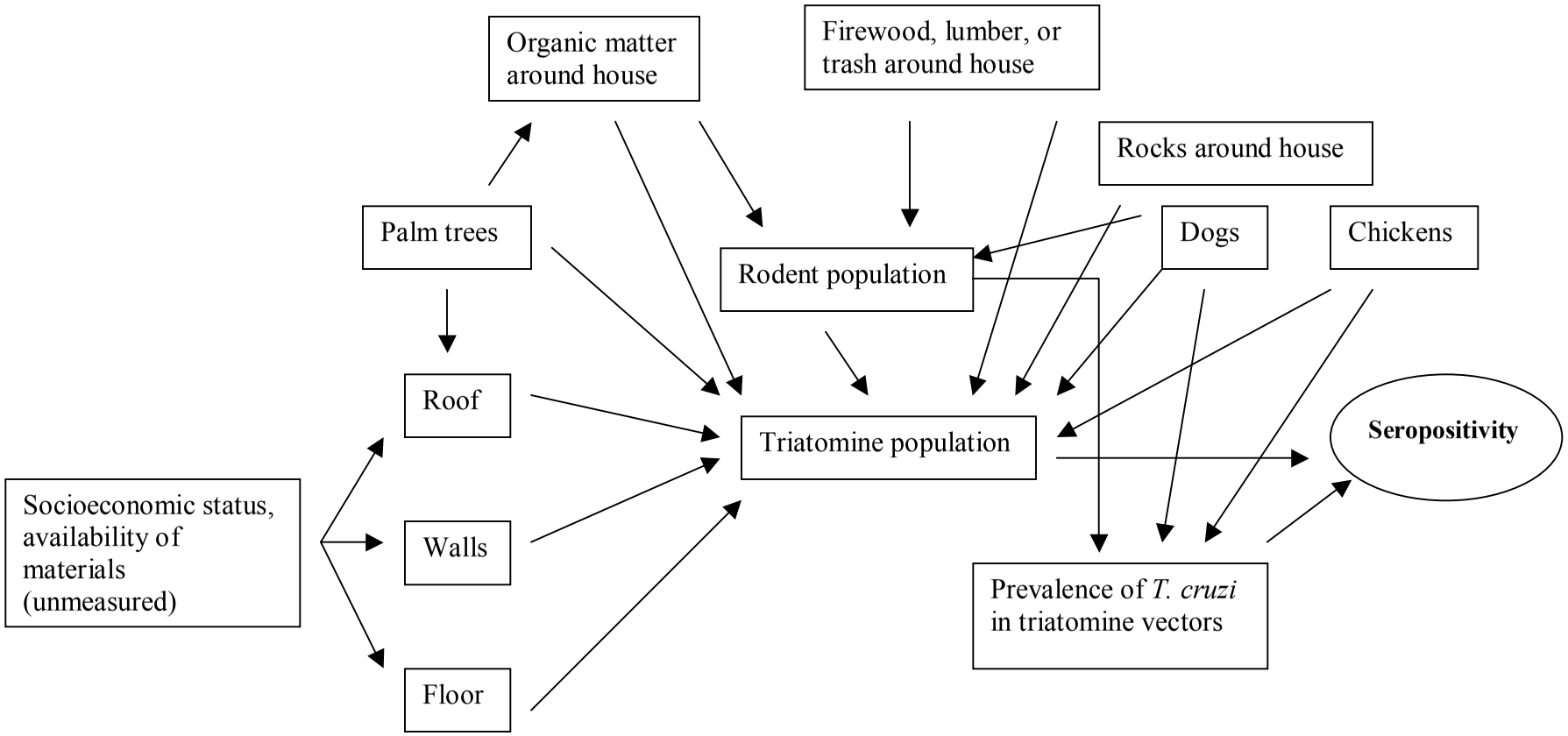

Figure 4.1. Directed acyclic graph illustrating the effect of household exposures on seropositivity with *Trypanosoma cruzi*

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CHAPTER 5. PREVALENCE OF *TRYPANOSOMA CRUZI* SEROPOSITIVITY IN RURAL ECUADOR AND CLUSTERING OF SEROPOSITIVITY WITHIN HOUSEHOLDS

Abstract

There are no recent population-based studies of the prevalence of *Trypanosoma cruzi* infection in Ecuador. We performed a cross-sectional study of *T. cruzi* seroprevalence in 14 communities in 3 provinces of Ecuador and assessed the extent to which human infection with *T. cruzi* clustered within households. 3,286 subjects from 997 households were tested for serologic evidence of infection with *T. cruzi*. The magnitude of the association of *T. cruzi* seropositive individuals within households was estimated in the form of pairwise odds ratios with the use of alternating logistic regressions. The prevalence of *T. cruzi* seropositivity was 5.7%, 1.0%, and 3.6% in rural communities of the Manabi, Guayas, and Loja provinces of Ecuador, respectively. Seroprevalence increased with increasing age in the provinces of Manabi and Guayas, while in Loja the highest prevalence of 7.1% was observed in children younger than 10 years. In the coastal provinces of Manabi and Guayas, clustering of seropositives within households was not observed after adjustment for known household risk factors for *T. cruzi* infection. However, in the Loja province, the risk of seropositivity was more than two times greater for an individual living in a household with another seropositive person. Our results indicate that transmission of *T. cruzi* is ongoing in Ecuador, though intensity of transmission and mechanisms of interaction between humans and the insect vectors of disease vary between geographic regions.

Introduction

The presence of human infection with *Trypanosoma cruzi*, the causative agent of Chagas disease, has been documented in Ecuador as early as 1927. During the 1940s and 1950s new disease foci were reported from the coastal provinces of Guayas, Manabi, and Los Rios, and the Andean provinces of Loja, Azuay, and Bolivar (Aguilar et al, 1999). However, there are no recent reports of population-based studies assessing the current status of *T. cruzi* transmission in these areas. We report the prevalence of *T. cruzi* seropositivity in population-based samples of communities in rural areas of the Manabi, Guayas, and Loja provinces of Ecuador.

Another objective of this study was to estimate the extent to which *T. cruzi* infections cluster within households. In a paper published in 1976, Mott et al state "Since transmission of Chagas' disease largely occurs within households, description and analysis of the characteristics of household clustering of seropositivity to *T. cruzi* are of particular interest." (Mott et al, 1976) Knowledge of the extent to which *T. cruzi* clusters within households can provide information about the extent to which an individual's risk of infection is affected by living in close proximity to another infected person, who can serve as a reservoir of *T. cruzi*. Additionally, the persistence of clustering within households after adjustment for other known household risk factors for *T. cruzi* infection may indicate that other as yet unknown factors are involved in the transmission of *T. cruzi* within households. Mott et al reported significant clustering of *T. cruzi* seropositivity within households but were unable to quantify the degree of clustering or control for the effect of other household factors (Mott et al, 1976). A later analysis by Gurtler et al found no effect of clustering after adjustment for other covariates using a random effects model (Gurtler et al, 1998). Though a random effects model can determine whether the parameter associated with clustering is statistically significant or not, the parameter itself does not have an easily understandable interpretation in terms of the magnitude of the clustering. We use the technique of alternating logistic regressions (ALR) introduced by Carey et al (Carey et al, 1993) to quantify the degree of

clustering of *T. cruzi* infections within households. ALR provides pairwise odds ratios (PORs) of association of the outcome, in this case *T. cruzi* seropositivity, within clusters while also taking into account the dependence of the outcome on individual and cluster-specific covariates. The pairwise odds ratios obtained from ALR are interpreted similar to conventional odds ratios, with a POR >1 indicating an association of seropositivity between individuals within a household. We also illustrate how the POR can be used in the calculation of design effects, which is useful for planning the sample size of future studies in which subjects are sampled in households or other related units. (Carey et al, 1993; Katz et al, 1993; Preisser et al, 2003)

Methods

Study population

The subjects included in this study were residents of 14 rural communities in the Manabi, Guayas, and Loja provinces of Ecuador. The study areas are denoted by circles in the map of Ecuador shown in Figure 1. Data were collected between June 2001 and August 2003. The study population has been previously described (Chapter 4). Briefly, study personnel visited all households in the selected communities as part of a study of household risk factors for *T. cruzi* transmission. All members of each household were invited to attend study-sponsored medical clinics. All subjects that presented to the medical clinic and donated a blood sample to be tested for serologic evidence of *T. cruzi* infection were included in the present study. All study procedures were approved by the Institutional Review Boards of Ohio University and Catholic University of Ecuador. Approval for secondary data analysis was granted by the Institutional Review Board of The University of North Carolina School of Public Health.

4,530 subjects presented to the medical clinics, representing 80% of the eligible households in the study communities. A further 1,244 subjects were excluded from the analysis because they did not consent to serologic testing, leaving a final sample of 3,286 subjects.

Serological testing

Blood was collected via venous puncture from all participants. For all three study sites, initial screening was performed by enzyme-linked immunosorbent assay (ELISA) using detergent extracted *T. cruzi* epimastigote antigens as previously described (Grijalva et al, 1995). The optical density (OD) values of positive and negative controls were analyzed and used to define the limits for seropositivity and seronegativity of the assay. OD values within 2.5 standard deviations (SDs) of the OD average for the positive controls were considered seropositive and all OD values within 2.5 SDs of the OD average for negative controls were considered negative. Sera previously confirmed as anti-*T. cruzi* seropositive served as positive controls. Samples that did not fulfill the criteria for positive or negative were classified as borderline. Positive and borderline samples were assayed at least two more times. Samples that were positive at least three times were considered positive. Samples with repeated borderline results were considered negative.

For samples collected in the Manabi province in 2001, all samples positive by the initial ELISA were confirmed by immunofluoresence at the Centers for Disease Control and Prevention (Atlanta, GA). All samples collected in the Loja province in 2002 that were positive by the initial ELISA and approximately 50% of the negative samples were subsequently tested with two commercially available ELISA kits (Chagatest ELISA recombinante V 3.0, Wiener Labs, Rosario, Argentina; Chagas Serum Antibody Detection Assay Microwell ELISA, IVD Research Inc, Carlsbad, CA) and a commercial indirect hemagglutination test (Chagatest HAI, Wiener Labs, Rosario, Argentina). All samples collected in Guayas province were also tested using Chagatest ELISA recombinante V 3.0 and Chagatest HAI, Wiener Labs. These tests were performed according to the manufacturer's instructions. Samples from these provinces that were positive by the recombinant ELISA or by at least two of the other tests were considered positive.

Analysis of Prevalence

Poisson regression was used to estimate age-specific prevalences and corresponding 95% confidence intervals for each province. The Poisson regression model included a linear and a squared variable for age, two indicator variables corresponding to the three provinces, and terms for linear and squared age by province interactions. Age was coded as an ordinal variable ranging from 0-7 representing 10-year age categories. Variables for age squared and squared age by province interactions were added to improve model fit. The number of subjects in each 10-year age category in each province was included in the model as an offset term. The variance estimates used in the calculation of confidence intervals were multiplied by the design effect to adjust for possible non-independence of *T. cruzi* infections within households. The design effect was calculated from the PORs obtained from the alternating logistic regressions described below.

Alternating Logistic Regressions

The magnitude of household clustering of *T. cruzi* seropositivity was estimated in the form of pairwise odds ratios (ORs) of the association of seropositivity within households. Pairwise odds ratios were estimated with the use of alternating logistic regressions (ALR), which fit a model for the within-household odds ratio while simultaneously adjusting for the effect of other covariates on the risk of seropositivity. (Katz et al, 1993) The pairwise OR between individual *j* and individual *k* within household *i* is defined as

$$\psi_{ijk} = \frac{\text{pr}\,(Y_{ij} = 1,\, Y_{ik} = 1)\ \text{pr}\,(Y_{ij} = 0,\, Y_{ik} = 0)}{\text{pr}\,(Y_{ij} = 1,\, Y_{ik} = 0)\ \text{pr}\,(Y_{ij} = 0,\, Y_{ik} = 1)}$$

where Y = 1 if subject is seropositive; otherwise Y = 0
and $j \neq k$. (Carey et al, 1993)

Thus, the POR is interpreted as the increased odds in favor of seropositivity for an individual from a household where another individual chosen at random from that household is seropositive

relative to the odds in favor of seropositivty if that randomly chosen individual is seronegative (Katz et al, 1993).

The ALR algorithm involves the simultaneous estimation of two logistic regression models: one for the within-household pairwise odds ratios, given by log (ψ_{ijk}) = α, and one for the probability of seropositivity, given by logit pr (Y = 1) = $\beta_0 + \beta_1 x_1 + \ldots + \beta_p x_p$ where $x_1 \ldots x_p$ is a set of p explanatory variables associated with the risk of seropositivity and the βs are the log odds ratios for the risk of seropositivity associated with the respective covariates. The algorithm iterates between two steps until convergence: (1) Given the current estimate of α, a generalized estimating equation logistic regression is performed to obtain an updated estimate of the βs, and (2) Given the current estimates of α and β, an offset logistic regression relating each outcome in a cluster to all other outcomes in that cluster is used to obtain an updated estimate of α. Thus, alternating logistic regressions are being applied, one to estimate α and to estimate the βs.

Separate ALR models were constructed for the coastal provinces, which include Manabi and Guayas, and the Loja province, which is located in the Andean highlands. These two regions are geographically distinct with different insect vectors of *T. cruzi*, and a previous analysis of these data showed that household risk factors for *T. cruzi* differed between the two regions (Chapter 4). In the previous analysis, type of materials used in the construction of roofs (palm or tile versus metal) and walls (cane or adobe versus cement) of houses were identified as risk factors for *T. cruzi* seropositivity. The presence of firewood and trash in the peridomicile area were additionally associated with seropositivity in the coastal provinces but not in the highlands. These factors were included as adjustment variables in the β model of the ALRs.

The design effect is the amount by which the variance of the prevalence estimated under the assumption of simple random sampling must be inflated to account for the clustering of disease. The design effect (D) caused by the correlation of *T. cruzi* seropositivity within households was

calculated from the PORs according to the following formula outlined by Katz and Zeger (Katz and Zeger 1994):

$$D = 1 + \{(p_{11}-p^2)/[p(1-p)]\}\{[(m-1/N]s^2+\bar{u}-1\}$$

Where
p = prevalence of infection
m = the number of households
N = the total sample size
$\bar{u}$ = the mean of the household sizes
s^2 = the variance of the household sizes
p_{11} = the probability that two subjects chosen at random both have disease

$$= \frac{1 - 2p(1-\alpha) - \{[1 - 2p(1-\alpha)]^2 - 4\alpha p^2(\alpha-1)\}^{1/2}}{2(\alpha-1)}$$

α = within-household pairwise odds ratio

The unadjusted PORs for each region were used in the calculation of the design effects.

ALR analyses were performed with the GENMOD procedure in SAS version 8.2 (Cary, NC). An exchangeable structure was specified for α, which assumes the POR is constant across all households (Stokes et al, 2000).

Results

Prevalence of T. cruzi *Seropositivity*

The prevalence of *T. cruzi* seropositivity was 5.67% (59/1041), 1.04% (14/1343), and 3.55% (32/902) in the studied communities of the Manabi, Guayas, and Loja provinces, respectively. Prevalence varied by community within each province (Table 5.1), ranging from 2.1% to 7.9% in Manabi, 0.7% to 2.0% in Guayas, and 1.2% to 7.2% in Loja.

Age-specific prevalence for each province is shown in Table 5.2 and Figure 5.2. In the Manabi province, prevalence increased with increasing age, ranging from 1.5% among children less than 10 years of age to a peak of 11.4% in persons aged 50-59 years. Prevalence also increased with age in the Guayas province, from 0.3% among 0-9 year olds to 2.4% in persons aged 70 and above. In the province of Loja, the highest prevalence of 7.1% occurred in children

less than 10 years of age. Prevalence decreased with age until age 30, after which it remained steady at approximately 2%.

Clustering of T. cruzi *Seropositivity Within Households*

In the coastal provinces of Manabi and Guayas, 73 seropositive subjects were identified from 693 households. Seven of these households had two cases living in the same house. The unadjusted pairwise odds ratio for seropositivity within households was 1.42 (0.76, 2.65). The design effect based on this estimate was 1.1. After adjustment for type of roof and the presence of firewood and trash in the peridomicile area, the POR was reduced to 0.97 (0.54, 1.74). A similar estimate of 0.96 (0.52, 1.77) was obtained in a model adjusted for wall type, firewood, and trash. Roof type and floor type were not included in the same model due to collinearity between the two variables.

In the Loja province, 32 cases were identified in 304 households, with five households having two cases each. The unadjusted POR was 2.72 (1.18, 6.29) with a corresponding design effect of 1.8. Although housing construction materials were associated with *T. cruzi* seropositivity in the Loja province as well, these variables could not be included as covariates in the ALR model because no seropositive subjects lived in households constructed of cement walls or metal roofs, the referent categories for these variables. All seropositives lived in houses with adobe walls and tile roofs, with the exception of two cases living in a house without walls. An analysis restricted only to subjects from households with tile roofs and adobe or no walls reduced the within household association of *T. cruzi* seropositivity only slightly (POR = 2.39 [1.05, 5.44]), indicating that factors other than shared exposure to substandard housing conditions are contributing to the clustering of infections within households. Although the presence of firewood and trash in the peridomicle were not identified as risk factors for seropositivity in the highlands region, a model containing these two variables was run in order to produce and estimate directly comparable to that

from the coastal region. The POR resulting from this model was 2.51 (1.08, 5.81), also similar to the unadjusted estimate.

Discussion

Our finding of 5.7% overall prevalence of *T. cruzi* seropositivity in the Manabi province is consistent with previous studies, which report seropositivity in various locations in Manabi ranging from 1% to 17% (Aguilar et al, 1999). The pattern of age-specific prevalence is lowest in the youngest ages and steadily increases with increasing age, a pattern that is indicative of a chronic infection that has been endemic in the population for a long period of time. Our results indicate that *T. cruzi* infection remains a significant problem in this region, and there is no evidence of a reduction in transmission over the past 50 years. The continued transmission of *T. cruzi* is also supported by a recent entomologic survey from the same areas of Manabi, which found 21% of households to be infested with triatomine insects capable of serving as vectors of *T. cruzi* (Grijalva, unpublished data).

Like Manabi, the province of Guayas is historically considered to be endemic for *T. cruzi*, with previous estimates of prevalence as high as 24% reported in 1959 (from Aguilar et al, 1999). We found an overall prevalence in Guayas of 1.0%, an estimate lower than those from previous studies. A trend for increased prevalence with increasing age was observed, suggesting the *T. cruzi* was once endemic in this area. However, the prevalence was very low among those in the youngest age categories (< 1% in all age groups below 30). These results are indicative of a reduction or possible recent interruption of *T. cruzi* transmission in this area. An entomologic survey of 476 households in Guayas conducted in conjunction with the present study found evidence of triatomine vectors in only one domicile (Grijalva, unpublished data).

The prevalence of *T. cruzi* infection in the Loja province has not been widely studied. A prevalence rate of 2% from a 1955 survey of schoolchildren is the only previous report from this

area that could be found in the medical literature (Aguilar et al, 1999). In the present study, we found a prevalence of 7.1% among children younger than 10 years, suggesting that active transmission of *T. cruzi* is occurring in this area. Prevalence did not increase with increasing age as would be expected if *T. cruzi* has been endemic in this area for many years. Conversely, prevalence actually decreased with age in the youngest age groups and then remained steady after age 30. This pattern suggests possible recent introduction of *T. cruzi* into this area. An entomologic survey in the same communities of Loja found a household infestation rate by triatomine insects of 35%. The study also found evidence to suggest that the triatomine vectors in this area can colonize human dwellings in a relatively short period of time (Grijalva et al, 2005). This further supports the notion that humans have recently been exposed to triatomine vectors that previously circulated *T. cruzi* in wild ecotopes and have presently become domiciliated.

Because different protocols for serologic testing were used in each province, the actual prevalence estimates are not directly comparable between provinces, though trends across age categories within provinces should be comparable. In the Manabi province, only those samples that were positive by the initial screening test were tested again, and only one additional test was performed on these samples. As such, this was the least sensitive and most specific testing scheme of the three sites, and the reported prevalence from Manabi likely underestimates the true prevalence. The testing scheme used in Guayas was the most sensitive and least specific, as every sample was tested with three separate tests. The true prevalence in the Guayas province was possibly even lower than that which we reported. Seventy-five percent of the 1,244 subjects excluded from the analysis due to lack of serology were children under 10 years of age. Had these subjects been included in the analysis, the resulting overall prevalence would likely be lower than the reported prevalence in Manabi and Guayas and higher than the reported prevalence in Loja.

In the coastal provinces, clustering of seropositives within households was not observed after adjustment for shared household risk factors for *T. cruzi* infection. However, in the Loja province,

the risk of seropositivity was more than two times greater if another randomly selected person from the same household was seropositive than if that randomly selected person was seronegative. The difference in household clustering might be explained by different behavior of the triatomine vectors in the two regions. In the entomologic search in Manabi, the majority of the triatomines were found in the peridomicile in piles of wood and brick and pens of domestic animals (Grijalva, unpublished data). If humans are being infected outside of the house or by vectors that only sporadically enter the house, an infected person within the house is not likely to serve as a reservoir of *T. cruzi* by which other triatomine insects can become infected. In Loja, triatomines were found mostly in the intradomicile, with peridomicilliary populations present only in occupied chicken nests (Grijalva et al, 2005). Here, humans are probably infected by vectors that have colonized their homes. Once infected, a person then serves as a reservoir capable of infecting other insects inside the house, who in turn infect other persons sleeping in close proximity to the index case. Also, since circulating levels of parasitemia decrease over time (Maguire et al, 1982), if *T. cruzi* transmission in Loja is indeed a recent phenomenon, infected persons in Loja likely have higher levels of parasitemia than persons in Manabi and Guayas who may have been infected for several decades. Thus, persons in Loja could be more infective to the triatomine vectors when bitten, and prevalence of *T. cruzi* infection in the vector population is more likely to be associated with human infections here than in the coastal regions, where rodents and domestic animals are probably more important sources of infection for the vectors. Another possible explanation for the observed clustering of seropositivity within households in Loja is that other unmeasured household characteristics are associated with *T. cruzi* transmission in this area.

In conclusion, we found evidence of *T. cruzi* infection in all three studied provinces of Ecuador. Prevalence patterns were suggestive of endemic infection in the Manabi province, reduction in transmission over time in the Guayas province, and recent introduction of transmission in the Loja province. *T. cruzi* infections clustered within households in the Loja province, while

clustering was not observed in the coastal provinces. Differences in clustering between the regions may be due to differing behavior of the triatomine vectors in each region, or may be an indication that other unknown household factors play a role in the transmission of *T. cruzi* in the highlands region.

Tables and Figures

Table 5.1. Prevalence of *T. cruzi* seropositivity by community, Ecuador, 2001-2003

Province and Community	Number of Cases / Total Sampled	Prevalence (%)
Manabi		
Cruz Alta	42 / 534	7.87
Pasaje	6 / 284	2.11
Pimpiguasi	11 / 223	4.93
Total	*59 / 1041*	*5.67*
Guayas		
La Alegria	2 / 289	0.69
Lomas de Colimes	2 / 220	0.91
Los Angeles	3 / 175	1.71
Macul	2 / 337	0.59
Puerto Rico	3 / 222	1.35
San Antonio	2 / 100	2.00
Total	*14 / 1343*	*1.04*
Loja		
Bramaderos	6 / 173	3.47
Jacapo	9 / 125	7.20
Naranjo Dulce	9 / 179	5.03
Pindo Alto	5 / 173	2.89
Playas	3 / 252	1.19
Total	*32 / 902*	*3.55*

Table 5.2. Estimated prevalence of *T. cruzi* seropositivity and 95% confidence intervals (CIs) by geographic region and age category, Ecuador, 2000-2002

Region and Age (years)	Number of cases / Total Sampled	Estimated Prevalence[1] (%) (95% CI)
Manabi		
0-9	3 / 184	1.49 (0.64, 3.44)
10-19	5 / 276	2.96 (1.82, 4.81)
20-29	13 / 159	5.11 (3.58, 7.29)
30-39	9 / 127	7.67 (5.32, 11.07)
40-49	10 / 103	10.01 (6.92, 14.48)
50-59	8 / 83	11.35 (7.96, 16.17)
60-69	6 / 58	11.18 (7.04, 17.75)
≥ 70	5 / 42	9.56 (4.32, 21.15)
Guayas		
0-9	1 / 265	0.33 (0.07, 1.68)
10-19	1 / 302	0.54 (0.20, 1.45)
20-29	1 / 214	0.84 (0.40, 1.77)
30-39	1 / 162	1.20 (0.56, 2.58)
40-49	3 / 149	1.59 (0.74, 3.44)
50-59	1 / 118	1.96 (0.96, 4.01)
60-69	3 / 69	2.24 (0.96, 5.20)
≥ 70	1 / 64	2.37 (0.59, 9.59)
Loja		
0-9	7 / 99	7.40 (3.21, 17.09)
10-19	10 / 243	4.20 (2.42, 7.29)
20-29	4 / 111	2.77 (1.36, 5.66)
30-39	3 / 99	2.13 (0.90, 5.09)
40-49	0 / 84	1.91 (0.78, 4.68)
50-59	2 / 91	2.00 (0.87, 4.61)
60-69	2 / 90	2.44 (0.98, 6.04)
≥ 70	3 / 77	3.45 (0.88, 13.56)

[1] Estimated from Poisson regression model

Figure 5.1. Map of Ecuador. Circles denote study locations.

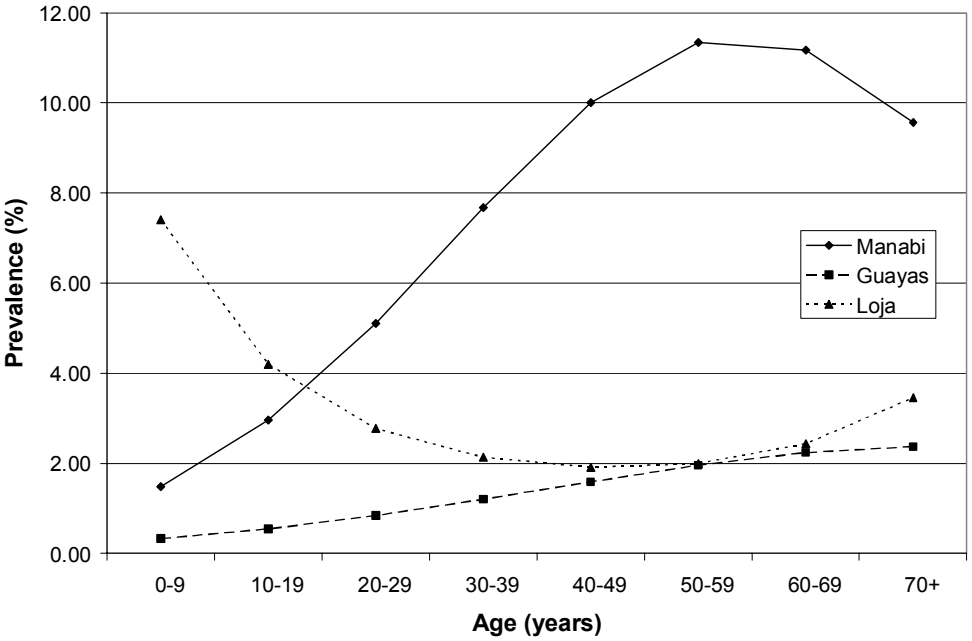

Figure 5.2. Prevalence of *T. cruzi* seropositivity by geographic region and age category, Ecuador, 2001-2003

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CHAPTER 6. CONCLUSIONS

Prevalence of *T. cruzi* in Ecuador

The prevalence of *Trypanosoma cruzi* seropositivity was 5.7%, 1.0%, and 3.6% in the studied communities in the provinces of Manabi, Guayas, and Loja, respectively. The finding of 5.7% overall prevalence of *T. cruzi* seropositivity in the Manabi province, with village prevalences ranging from 2% to 8%, is consistent with previous studies, which report seropositivity in various locations in Manabi ranging from 1% to 17% (Aguilar et al, 1999). The pattern of age-specific prevalence was lowest in the youngest ages and steadily increased with increasing age, a pattern indicative of a chronic infection that has been endemic in the population for a long period of time. These results indicate that *T. cruzi* infection remains a significant problem in this region, and there is no evidence of a reduction in transmission over the past 50 years. The continued transmission of *T. cruzi* is also supported by a recent entomologic survey from the same areas of Manabi, which found 21% of households to be infested with triatomine insects capable of serving as vectors of *T. cruzi* (Grijalva, unpublished data).

Like Manabi, the province of Guayas is historically considered to be endemic for *T. cruzi*, with previous estimates of prevalence as high as 24% reported in 1959 (from Aguilar et al, 1999). The prevalence in Guayas of 1.0% found in the present study is lower than expected based on previous reports. A trend for increased prevalence with increasing age was observed, suggesting the *T. cruzi* was once endemic in this area. However, the prevalence was very low among those in the youngest age categories (< 1% in all age groups below 30). These results are indicative of a reduction or possible recent interruption of *T. cruzi* transmission in this area. Interruption of

transmission is also supported by an entomologic survey of 476 households in Guayas conducted in conjunction with the present study in which evidence of triatomine vectors was found in only one domicile (Grijalva, unpublished data).

The prevalence of *T. cruzi* infection in the Loja province has not been widely studied. A prevalence rate of 2% from a 1955 survey of schoolchildren is the only previous report from this area that could be found in the medical literature (Aguilar et al, 1999). In the present study, a prevalence of 7.1% was observed among children younger than 10 years, suggesting that active transmission of *T. cruzi* is occurring in this area. Prevalence did not increase with increasing age as would be expected if *T. cruzi* has been endemic in this area for many years. Conversely, prevalence actually decreased with age in the youngest age groups and then remained steady after age 30. This pattern suggests possible recent introduction of *T. cruzi* into this area. An entomologic survey in the same communities of Loja found a household infestation rate by triatomine insects of 35%. The study also found evidence to suggest that the triatomine vectors in this area can colonize human dwellings in a relatively short period of time (Grijalva et al, 2005). This further supports the notion that humans have recently been exposed to triatomine vectors that previously circulated *T. cruzi* in wild ecotopes and have recently become domiciliated.

Household Risk Factors for *T. cruzi* Seropositivity

Construction materials used for house walls, specifically cane, adobe, and wood, were associated with an increased risk of *T. cruzi* seropositvity in both the coastal and highland regions of Ecuador. Previous studies linking wall construction to seropositivity have failed to find an association (Gurtler et al, 1998; Greer et al, 1999), though a few studies have shown adobe walls to be associated with household infestation of triatomines (Starr et al, 1999). Adobe walls are prone to cracking and provide hiding places and breeding sites for the triatomine insects. Wood or cane

walls can contain open spaces and are also subject to cracking and perhaps facilitate triatomine infestation in a similar manner.

In the coastal provinces, individuals living in a house with a palm thatch roof were almost three times as likely to be seropositive for *T. cruzi* as those individuals living in houses with roofs composed of zinc or asbestos. Previous studies have reported associations between thatched roofs and *T. cruzi* seropositivity in areas where *Rhodnius prolixus* and *Triatoma dimidiata* were the main vectors of transmission (Greer et al, 1999; Rizzo et al, 2003). Sylvatic populations of *R. prolixus* are found in palm trees and are thought to colonize houses via passive transport in the palm leaves used in the construction of thatched roofs (WHO, 2000; D'Alessandro et al, 1984). Sylvatic populations of *Rhodnius ecuadoriensis* have been reported from the Manabi province (Abad-Franch et al, 2001) and could be introduced into thatched roof homes in this area by a similar mechanism. The observed association with palm roofs could also be an artifact resulting from the high correlation between palm roofs and cane walls.

The presence of firewood, junk piles, and agricultural products outside of the house have been reported to be associated with household infestation of triatomines (Zeledon and Vargas, 1984; Enger et al, 2004). Interestingly, in this study accumulation of trash in the peridomicile area was protective against seropositivity to *T. cruzi* in the coastal region. Though the estimates were unstable, the accumulation of organic matter, rocks, and lumber appeared to be protective as well. Only the presence of firewood outside the home was associated with an increased risk of seropositivity. A protective effect against *T. cruzi* infection by any of these items has not been reported in the literature. However, in a previous analysis by our group of a different population from the Manabi province, we found that the accumulation of organic matter outside the house was inversely associated with *T. cruzi* seropositivity (Grijalva, unpublished data). Perhaps the presence of these materials in the peridomicile provides sufficient refuge for the triatomines such that they have colonized these habitats rather than the intradomicile. An entomologic search conducted in

the Manabi province three years after the data for the present study were collected seems to support this hypothesis, as the majority of captured triatomines were found in the peridomicile in piles of wood and brick, chicken nests, and guinea pig pens (Grijalva, unpublished data). The finding that firewood is associated with an increased risk of infection is also consistent with this hypothesis as firewood is brought into the home on a regular basis and could serve to passively transfer the triatomine vectors into the domicile.

An additional explanation for the protective effect of trash and other materials in the peridomicile is that these items might attract predators of the triatomine, such as rodents and other small mammals, thereby reducing the triatomine populations. Alternatively, these small mammals may serve as a food source for the triatomine insects, decreasing the need for the vectors to enter the houses in search of a blood meal, therefore serving as a buffer for intradomiciliary household infestation.

Unlike the coastal provinces, no peridomicile characteristics were strongly associated with *T. cruzi* seropositivity in the highlands region, though two factors, the presence of rocks and bricks and the presence of trash in the peridomicile showed weak positive associations. These results are contradictory to those in the coastal region, where both of these factors were inversely associated with seropositivity. The difference in risk factors between the regions is probably due to differences in the triatomine vectors. While *R. ecuadoriensis* is found in both regions, this species is found in sylvatic and domestic habitats in the coastal region, while only synanthropic populations of this species have been reported in the Loja province (Grijalva et al, 2005; Abad-Franch et al, 2001). The other main vector of *T. cruzi* in the Loja province, *T. carrioni*, is found only in the Andean region. In contrast to the coastal provinces, in which insects were found mainly in peridomicile areas, entomological searches in the Loja province found triatomines almost exclusively in the intradomicile, with peridomicile populations found only in chicken nests. None of the aforementioned peridomicilliary characteristics were associated with household infestation

of the triatomine vectors in the communities included in this study (Grijalva et al, 2005). Thus, it follows that materials in the peridomicile would not be associated with *T. cruzi* infection in humans.

Clustering of *T. cruzi* Infections Within Households

In the coastal provinces, clustering of seropositives within households was not observed after adjustment for shared household risk factors for *T. cruzi* infection. However, in the Loja province, the risk of seropositivity was more than two times greater if another randomly selected person from the same household was seropositive than if that randomly selected person was seronegative. The difference in household clustering might be explained by different behavior of the triatomine vectors in the two regions. In the entomologic search in Manabi, the majority of the triatomines were found in the peridomicile in piles of wood and brick and pens of domestic animals (Grijalva, unpublished data). If humans are being infected outside of the house or by vectors that only sporadically enter the house, an infected person within the house is not likely to serve as a reservoir of *T. cruzi* by which other triatomine insects can become infected. In Loja, triatomines were found almost exclusively in the intradomicile (Grijalva et al, 2005). Here, humans are probably infected by vectors that have colonized their homes. Once infected, a person then serves as a reservoir capable of infecting other insects inside the house, which in turn infects persons sleeping in close proximity to the index case. Also, since circulating levels of parasitemia decrease over time (Maguire et al, 1982), if *T. cruzi* transmission in Loja is indeed a recent phenomenon, infected persons in Loja likely have higher levels of parasitemia than persons in Manabi and Guayas who may have been infected for several decades. Thus, persons in Loja may be more infective to the triatomine vectors when bitten, and prevalence of *T. cruzi* infection in the vector population is more likely to be associated with human infections here than in the coastal regions, where rodents and domestic animals are probably more important sources of infection for the vectors. Another

possible explanation for the observed clustering of seropositivity within households in Loja is that other unmeasured household characteristics are associated with *T. cruzi* transmission in this area.

Strengths of Study

This study comprises a large sample of subjects distributed over a large geographical area, and is the first study to collect detailed data on risk factors for *T. cruzi* transmission in Ecuador. The study design has many strengths with respect to the assessment of risk factors for *T. cruzi* seropositvity. The model building strategy was based on a priori knowledge about the underlying biologic mechanisms of vector transmission of *T. cruzi*. This resulted in the selection of the most parsimonious model for each exposure, thus maximizing power and increasing the possibility that associations between exposures and *T. cruzi* seropositivity were found if they existed. This model building strategy also inspires more confidence that the exposure-seropositivity relationships discovered represent true associations and not spurious relationships created by chance as a result of analyzing many different variables. By evaluating interactions between exposures and geographic regions, the study design allowed for the possibility that the relationships between risk factors and seropositivity varied depending on the behavior of the triatomine vector species in each area.

Deletion diagnostics were run for the GEE models for each exposure to determine if the observed estimates of effect were due to particular households that had a disproportionately large influence on the estimated regression parameter. The results of the deletion diagnostic analyses for the factors that were shown in this study to be risk factors for *T. cruzi* seropositivity are shown in Appendix 4, Tables A4.1-A4.5. Each table gives the *DFBETA* or the Cook's distance for the five most influential households from the GEE model for that particular exposure and the resulting odds ratio with those observations deleted. Not surprisingly, the households with the largest influence on the estimates were those with more than one case or those with a large number of cases relative

to the total number of subjects in the household. However, the results of the deletion diagnostics show that none of the observed estimates were unduly influenced by any particular household. Removal of the five most influential households on the estimated associated with wood walls in the coastal region causes the odds ratio to become undefined because only five cases in this region lived in a house with wood walls. Though the odds ratio associated with the presence of trash in the peridomicile in the highlands region moved to the opposite side of the null after removal of the most influential households, this reinforces the previously made conclusion that that this estimate is unstable and the presence of trash in the peridomicile should not be considered a risk factor for seropositivity in the highlands region. Thus, removal of the most influential observations would not have changed the conclusions made about any of the risk factors for seropositivity.

The estimation of the clustering effect of *T. cruzi* infections within households with the use of alternating logistic regression is a useful element of this study. The presence of a clustering effect after adjustment for known household risk factors is an indication that additional household characteristics should be evaluated as risk factors for the transmission of *T. cruzi* or that living in close proximity to another infected person is itself an independent risk factor for transmission. If the latter is true, the presence of a seropositive person in a household could confound the relationship between *T. cruzi* infection and other household factors and should be controlled for in future studies of household risk factors, even if the study design does not necessitate the use of a cluster analysis. Mott et al first reported a formal statistical evaluation of household clustering of *T. cruzi* infections in 1976 (Mott et al, 1976), and this paper has been referenced many times since as evidence that clustering of *T. cruzi* infections within households occurs. Only one study since then has addressed the issue of household clustering of *T. cruzi* infections, and this analysis found no clustering effect after adjustments for other covariates (Gurtler et al, 1998b). The use of ALR illustrates a new technique that can provide both an interpretable estimate of the magnitude of within-household clustering and valid estimates of the effect of other covariates in the model. This

method could have applications in many other infectious diseases where the ability of disease to spread through contact between individuals in a shared environment is of interest. Though ALR was introduced in 1993 (Katz et al, 1993), there are few published uses in the literature regarding infectious diseases. The illustration of a practical use of this technique is an important contribution to the infectious disease literature.

Limitations of Study

The analysis of prevalence was limited by the sensitivity and specificity of the serologic testing scheme and by the high non-participation rate among younger subjects. Because different protocols for serologic testing were used in each province, the actual prevalence estimates are not directly comparable between provinces, though trends across age categories within provinces should be comparable. In the Manabi province, only those samples that were positive by the initial screening test were tested again, and only one additional test was performed on these samples. As such, this was the least sensitive and most specific testing scheme of the three sites, and the reported prevalence from Manabi likely underestimates the true prevalence. The testing scheme used in Guayas was the most sensitive and least specific, as every sample was tested with three separate tests. The true prevalence in the Guayas province was possibly even lower than that which is reported here. Seventy-five percent of the 1244 subjects excluded from the analysis due to lack of serology were children under 10 years of age. Had these subjects been included in the analysis, the resulting overall prevalence would likely be lower than the reported prevalence in Manabi and Guayas and higher than the reported prevalence in Loja.

Though the study included a large sample size, only 105 seropositive cases were detected among the 3,286 subjects. Only 32 cases occurred among subjects living in the highlands region, resulting in low power to detect associations between seropositivity and risk factors in this region. Also, as in any cross-sectional study, the risk factor analysis was limited by issues of temporality.

Since infection with T. *cruzi* is chronic, the time at which infection took place in relation to exposure to the various risk factors examined could not be determined. An analysis restricted to children, which would be more likely to include only those subjects recently inflected with T. *cruzi*, or an analysis restricted to subjects who reported living in their current house for their entire lives could have better answered this question. However, too few cases occurred among children younger than 10 years of age to permit such an analysis. Additionally, the question regarding length of residence in the current household was asked only of subjects living in the Guayas province, and the number of cases in this province was also inadequate to perform this analysis.

Both outcome and exposure measurements in this study were subject to misclassification. The low specificity in the serologic assays used to detect *T. cruzi* is well documented in the literature (WHO, 2000; Wendel and Gonzaga, 1993; Gomes et al, 1999), so some seronegative subjects were likely misclassified as being seropositive. Since the study was cross-sectional, information on household exposures was collected only at a single point in time. Infection with *T. cruzi* likely happened many years before the household exposures were observed in this study, so correct classification of exposure status relies on the assumption that current household characteristics are an adequate marker for the conditions in which a subject lived at the time he or she became infected. Thus, a proportion of subjects were likely to be misclassified regarding exposure status as well. Sensitivity analyses were conducted to estimate the potential magnitude of the bias of each exposure-seropositivity relationship due to these misclassifications. Tables A5.1-A5.18 in Appendix 5 show the ORs that would have resulted for each exposure under varying assumptions of sensitivity and specificity of the disease and exposure measurements. All analyses assume non-differential misclassification. In general, the observed ORs were robust to misclassification by exposure status and to imperfect sensitivity of disease ascertainment. However, the magnitude of the ORs changed greatly when the specificity of disease classification was assumed to be less than 100%. For most exposures, the odds ratios became undefined at

assumed specificities of less than 0.99 because applying these specificities to the observed distribution of the data resulted in zero cases among the exposed and/or unexposed subjects.

Another limitation of the study is that, although palm roofs and cane walls were both found to be associated with seropositivity in the coastal region, these two variables were highly correlated such that the effect of one could not be separated from the effect of the other. However, this issue is somewhat irrelevant in practice because any Chagas disease intervention aimed at improving housing conditions is likely to include upgrading both the walls and roofing materials of houses.

Final Conclusions

In conclusion, evidence of *T. cruzi* infection was found in all three studied provinces of Ecuador. Age-specific prevalence patterns were suggestive of endemic infection in the Manabi province, reduction in transmission over time in the Guayas province, and recent introduction or re-introduction of transmission in the Loja province. Several household characteristics were related to *T. cruzi* seropositivity. Materials used in the construction of houses were associated with seropositivity in both the coastal and highland regions, despite the fact that the specific construction materials used differed by region. Peridomicilliary risk factors varied by geographic region. These differences were likely due to differing behavior of the triatomine vector species in each region. Clustering of *T. cruzi* infections within households also differed by region. *T. cruzi* infections clustered within households in the Loja province, while clustering was not observed in the coastal provinces. Differences in clustering between the regions may also be due to differences in vector species between regions, or may be an indication that other unknown household factors play a role in the transmission of *T. cruzi* in the highland region.

These findings illustrate that there is no one-size-fits-all strategy for control of Chagas disease in Ecuador. An understanding of the way in which humans, vectors, and environmental factors interact to promote the transmission of *T. cruzi* in a particular area is necessary for the

development of an effective strategy for eliminating *T. cruzi* transmission in that area. To achieve interruption of transmission in Ecuador, traditional Chagas disease control programs that rely on housing improvement and household spraying with residual insecticides may need to be expanded to include such additional activities as spraying of peridomicile areas and continued surveillance for re-colonization of domestic environments by peridomestic and sylvatic species of triatomine vectors.

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APPENDICES

Appendix 1. Assessment of Goodness of Fit for Age-specific Prevalence Model

Table A1.1. Goodness of fit statistics for Poisson regression models of age-specific prevalence by province

Model	DF	Deviance	Comparison Model	Δ Deviance	Δ DF
Model 1	14	33.2444	--	--	--
Model 2	12	14.6972	1	18.5472	2
Model 3	10	7.2469	2	7.4503	2
Model 4	15	12.28	--	--	--
Model 5	14	11.4	4	0.88	1

DF = Degrees of freedom

Model 1: $\text{Log}(E[Y]) = \alpha + \beta_1\text{agecat1} + \beta_2\text{agecat2} + \ldots + \beta_7\text{agecat7} + \beta_8\text{loja} + \beta_9\text{guayas} + \log(N)$

Model 2: $\text{Log}(E[Y]) = \alpha + \beta_1\text{agecat1} + \beta_2\text{agecat2} + \ldots + \beta_7\text{agecat7} + \beta_8\text{loja} + \beta_9\text{guayas} + \beta_{10}\text{agecat\_loja} + \beta_{11}\text{agecat\_guayas} + \log(N)$

Model 3: $\text{Log}(E[Y]) = \alpha + \beta_1\text{agecat1} + \beta_2\text{agecat2} + \ldots + \beta_7\text{agecat7} + \beta_8\text{loja} + \beta_9\text{guayas} + \beta_{10}\text{agecat\_loja} + \beta_{11}\text{agecat\_Guayas} + \beta_{12}\text{agecat\_sq\_loja} + \beta_{13}\text{agecat\_sq\_guayas} + \log(N)$

Model 4: $\text{Log}(E[Y]) = \alpha + \beta_1\text{agecat} + \beta_2\text{agecat}^2 + \beta_3\text{loja} + \beta_4\text{guayas} + \beta_5\text{agecat*loja} + \beta_6\text{agecat*guayas} + \beta_7\text{agecat}^2\text{*loja} + \beta_8\text{agecat}^2\text{*guayas} + \log(N)$

Model 5: $\text{Log}(E[Y]) = \alpha + \beta_1\text{agecat} + \beta_2\text{agecat}^2 + \beta_3\text{agecat}^3 + \beta_4\text{loja} + \beta_5\text{guayas} + \beta_6\text{agecat*loja} + \beta_7\text{agecat*guayas} + \beta_8\text{agecat}^2\text{*loja} + \beta_9\text{agecat}^2\text{*guayas} + \log(N)$

Where Y = *T. cruzi* cases
N = Population size
α = intercept term

Loja = 1 if province of Loja; 0 if province of Manabi or Guayas
Guayas = 1 if province of Guayas; 0 of province of Manabi or Loja

Agecat = 0 if age 0-9
1 if age 10-19
2 if age 20-29
3 if age 30-39
4 if age 40-49
5 if age 50-59
6 if age 60-69
7 if age ≥ 70

Agecat1 = 1 if age10-19; 0 otherwise
Agecat2 = 1 if age 20-29; 0 otherwise
Agecat3 = 1 if age 30-39; 0 otherwise
Agecat4 = 1 if age 40-49; 0 otherwise
Agecat5 = 1 if age 50-59; 0 otherwise
Agecat6 = 1 if age 60-69; 0 otherwise
Agecat7 = 1 if age ≥ 70; 0 otherwise

Agecat_loja = agecat*loja
Agecat_guayas = agecat*guayas

Agecat_sq_loja = $agecat^2$*loja
Agecat_sq_guayas = $agecat^2$*guayas

Appendix 2. Assessment of Confounding and Effect Modification for Risk Factor Analysis Described in Chapter Three

Table A2.1. Assessment of effect modification. Odds ratio (OR) and 95% confidence interval (CI) for the association between each exposure and seropositivity in each geographic region and Wald test for interaction term between the exposure and geographic region

Exposure	Coastal OR (95% CI)	Highlands OR (95% CI)	p-value for Test of Interaction
Type of Roof*			
Cement/Asbestos/Zinc	1	1	
Tile	N/A	A	--
Palm	2.63 (1.61, 4.27)	N/A	--
Type of Walls*			
Cement	1	1	
Adobe	N/A	B	--
Wood	5.75 (2.04, 16.18)	B	--
Cane	2.81 (1.31, 6.04)	N/A	--
Type of Floor*			
Cement/Tile/Parquet	1	1	
Wood boards	1.67 (0.80, 3.51)	C	--
Cane	1.70 (0.73, 3.95)	C	--
Dirt	2.69 (1.04, 6.99)	1.82 (0.55, 6.02)	--
Palm trees*	1.44 (0.89, 2.33)	D	--
Organic matter	0.87 (0.46, 1.65)	0.89 (0.33, 2.43)	0.9661
Firewood	2.48 (1.54, 4.01)	0.70 (0.32, 1.52)	0.0066
Rocks	0.62 (0.23, 1.70)	1.79 (0.76, 4.21)	0.1163
Trash	0.25 (0.12, 0.51)	1.66 (0.69, 3.98)	0.0011
Lumber	0.63 (0.26, 1.54)	0.85 (0.23, 3.07)	0.7128

*Two separate models were run instead of one model with interaction term due to colinearity between region and exposure; therefore no formal test of interaction was conducted

N/A = Housing construction material not found in this region

A. OR undefined because all seropositives have tile roofs

B. OR undefined because all seropositives have adobe walls except two with no walls

C. Zero seropositives in Loja with wood or cane floors

D. OR undefined because 0 seropositives in Loja were exposed to palm trees

Table A2.2. Confounding criteria 1: Odds ratios* (OR) and 95% confidence intervals (CI) for the associations between exposures and potential confounders stratified by region

Exposure-confounder relationship	Coastal OR (95% CI)	Highlands OR (95% CI)	Overall OR (95% CI)
Organic matter-palm trees	2.5 (1.7, 3.6)	3.3 (1.3, 8.8)	2.1 (1.5, 3.0)
Roof type-wall type	14.9 (5.4, 41.0)	35.4 (11.2, 112.2)	6.4 (4.3, 9.4)
Roof type-floor type	4.3 (2.0, 9.0)	9.3 (3.6, 23.6)	2.9 (2.0, 4.2)
Wall type-floor type	30.3 (18.5, 49.8)	14.1 (6.9, 29.2)	23.5 (15.9, 34.7)
Roof type-palm trees	1.9 (1.2, 2.9)	0.2 (0.1, 0.8)	0.5 (0.3, 0.6)
Wall type-palm trees	1.3 (0.9, 1.8)	0.1 (0.05, 0.4)	0.8 (0.6, 1.2)
Floor type-palm trees	1.1 (0.8, 1.7)	0.1 (0.05, 0.4)	0.8 (0.6, 1.1)

*Odds of exposure for one variable relative to odds of exposure for the second variable
For organic matter and palm trees exposed = yes; for roof, walls, and floor exposed = open

Table A2.3. Confounding criteria 2: Associations between seropositivity and potential confounders for each exposure, coastal region[1]

Exposure	Population included in analysis	Potential confounder	Coastal OR (95% CI)
Roof type	Subjects in households with closed roofs (48 cases)	**Wall type**	
		Cement	1
		Wood	4.73 (1.55, 14.42)
		Cane	2.19 (0.99, 4.87)
		Floor type	
		Cement or tile	1
		Boards	1.36 (0.64, 2.93)
		Cane	0.71 (0.22, 2.30)
		Dirt	1.95 (0.54, 7.04)
		Palm trees	
		Yes vs no	2.11 (1.16, 3.82)
Wall type	Subjects in households with closed walls (10 cases)	**Roof type**	
		Zinc or asbestos	1
		Palm	N/A[2]
		Floor type	
		Cement or tile	1
		Boards	N/A[2]
		Cane	N/A[2]
		Dirt	1.57 (0.19, 13.31)
		Palm trees	
		Yes vs no	6.75 (1.33, 34.36)
Floor type	Subjects in households with closed floors (11 cases)	**Roof type**	
		Cement or tile	1
		Palm	N/A[2]
		Wall type	
		Cement	1
		Wood	13.95 (6.47, 30.06)
		Cane	0.79 (0.11, 5.73)
		Palm trees	
		Yes vs no	6.79 (1.42, 32.61)
Organic matter	Subjects in households with no organic matter (60 cases)	**Palm trees**	
		Yes vs no	1.37 (0.80, 2.34)

[1] A similar analysis could not be performed in the highland region due to zero cases in either the exposed or unexposed categories of all variables in the selected populations of interest

[2] Zero cases with exposure

Appendix 3. Demographic Characteristics of Study Population

Table A3.1. Comparison of demographic and housing characteristics between study participants and subjects that were excluded from study due to lack of serology

Variable	Participants N=3286 N (%)	Non-participants N=1244 N (%)	p-value[1]
Gender			
Male	1473 (44.92)	431 (44.11)	0.6559
Female	1806 (55.08)	546 (55.89)	
Age			
0-9	548 (16.76)	725 (74.74)	<0.0001
10-19	821 (25.11)	87 (8.97)	
20-29	484 (14.81)	37 (3.81)	
30-39	388 (11.87)	31 (3.20)	
40-49	336 (10.28)	34 (3.51)	
50-59	292 (8.93)	19 (1.96)	
60-69	217 (6.64)	20 (2.06)	
≥ 70	183 (5.60)	17 (1.75)	
Mean (std)	30.20 (21.29)	11.07 (17.36)	<0.0001
Median	24	4	
Province			
Manabi	1041 (31.68)	352 (28.30)	0.0006
Loja	902 (27.45)	543 (43.65)	
Guayas	1343 (40.87)	349 (28.05)	
Type of Roof			
Cement/Asbestos/Zinc	2020 (62.93)	598 (48.86)	<0.0001
Tile	829 (25.41)	484 (39.54)	
Palm	407 (12.48)	140 (11.44)	
Other	6 (0.18)	2 (0.16)	
Type of Walls			
Cement	857 (26.24)	286 (23.42)	<0.0001
Adobe	744 (22.78)	432 (35.38)	
Wood	80 (2.45)	30 (2.46)	
Cane	1572 (48.13)	468 (38.33)	
Other	13 (0.40)	5 (0.41)	

Table A3.1. Comparison of demographic and housing characteristics between study participants and subjects that were excluded from study due to lack of serology (cont.)

Variable	Participants N=3286 N (%)	Non-participants N=1244 N (%)	p-value[1]
Type of Floor			
Cement/Tile/Parquet	688 (21.03)	243 (19.92)	<0.0001
Wood boards	1257 (38.43)	363 (29.75)	
Cane	456 (13.94)	158 (12.95)	
Dirt	870 (26.60)	454 (37.21)	
Other	0	2 (0.16)	
Palm trees	806 (25.48)	318 (27.39)	0.2049
Organic matter	665 (20.63)	359 (30.32)	<0.0001
Firewood	1307 (40.51)	498 (42.06)	0.3548
Rocks	342 (10.60)	122 (10.30)	0.7755
Trash	965 (29.91)	363 (30.66)	0.6325
Lumber	334 (10.35)	131 (11.06)	0.4958

[1] p-value comparing mean ages was result of t-test; all other p-values were obtained from chi squared tests

Table A3.2. Comparison of houses in which residents participated in study and houses in which residents did not participate in study

Exposure	Participating houses N=997 n (%)	Non-participating houses N=296 n (%)	p-value
Province			
Manabi	278 (27.88)	123 (41.55)	<0.001
Loja	304 (30.49)	111 (37.50)	
Guayas	415 (41.62)	62 (20.95)	
Type of Roof			
Cement/Asbestos/Zinc	600 (60.73)	144 (51.25)	0.0219
Tile	280 (28.34)	92 (32.74)	
Palm	105 (10.63)	45 (16.01)	
Other	3 (0.30)	0 (0)	
Type of Walls			
Cement	263 (26.57)	73 (26.16)	0.3278
Adobe	247 (24.95)	85 (30.47)	
Wood	26 (2.63)	3 (1.08)	
Cane	450 (45.45)	118 (42.29)	
Other	4 (0.40)	0 (0)	
Type of Floor			
Cement/Tile/Parquet	206 (20.82)	58 (21.01)	0.0017
Wood boards	367 (37.07)	75 (27.17)	
Cane	133 (13.43)	45 (16.30)	
Dirt	284 (28.69)	96 (34.78)	
Other	0 (0)	2 (0.72)	
Palm trees	239 (24.97)	97 (38.65)	<0.001
Organic matter	207 (21.19)	97 (38.65)	<0.001
Firewood	372 (38.04)	88 (35.06)	0.3848
Rocks	109 (11.15)	19 (7.57)	0.0982
Trash	296 (30.27)	64 (25.50)	0.1389
Lumber	106 (10.84)	21 (8.37)	0.2513

Appendix 4. Deletion Diagnostics for the GEE Models of Roof Type, Wall Type, Firewood, and Trash

Table A4.1. Deletion diagnostics for estimate of palm roof in coastal region. Observations with the most influence on the estimate according to the value of *DFBETA*, and the change in odds ratio with the removal of the influential observations

House ID	# people in house	# cases in house	*DFBETA*	OR (95% CI) with observation and preceding observations removed
043	7	2	0.087451	2.38 (1.41, 4.04)
082	7	2	0.054923	2.21 (1.29, 3.78)
118	2	1	0.052988	2.06 (1.21, 3.53)
060	2	1	0.052988	1.92 (1.12, 3.29)
049	3	1	0.048524	1.85 (1.07, 3.19)

Odds ratio (95% CI) based on complete dataset = 2.64 (1.55, 4.50).

Table A4.2. Deletion diagnostics for estimate of cane walls in coastal region. Observations with the most influence on the estimate according to the value of *DFBETA*, and the change in odds ratio with the removal of the influential observations

House ID	# people in house	# cases in house	*DFBETA*	OR (95% CI) with observation and preceding observations removed
082	7	2	0.029408	2.72 (1.27, 5.85)
041	7	2	0.029408	2.63 (1.22, 5.65)
153	7	2	0.029408	2.53 (1.18, 5.44)
043	7	2	0.029408	2.43 (1.13, 5.23)
012	13	2	0.022028	2.35 (1.09, 5.06)

Odds ratio (95% CI) based on complete dataset = 2.81 (1.31, 6.04).

Table A4.3. Deletion diagnostics for estimate of wood walls in coastal region. Observations with the most influence on the estimate according to the value of *DFBETA*, and the change in odds ratio with the removal of the influential observations

House ID	# people in house	# cases in house	*DFBETA*	OR (95% CI) with observation and preceding observations removed
042	4	1	0.13490	4.81 (1.58, 14.63)
158	4	1	0.13490	3.77 (1.10, 12.93)
092	4	1	0.13490	2.62 (0.62, 11.09)
210	5	1	0.11857	1.39 (0.19, 10.05)
PR-003	6	1	0.10285	NA

Odds ratio (95% CI) based on complete dataset = 5.75 (2.04, 16.18).

Table A4.4. Deletion diagnostics for estimate of firewood. Observations with the most influence on the estimate according to the value of Cook's distance, and the change in odds ratio with the removal of the influential observations

House ID	# people in house	# cases in house	Cook's distance	OR (95% CI) with observation and preceding observations removed
Coastal Region				
153	7	2	0.023841	2.64 (1.63, 4.27)
045	4	2	0.019713	2.52 (1.55, 4.07)
012	13	2	0.016582	2.67 (1.64, 4.35)
082	7	2	0.014707	2.56 (1.57, 4.17)
043	7	2	0.014707	2.44 (1.50, 3.99)
Highland Region				
1146	4	2	0.062783	0.60 (0.23, 1.34)
1641	5	2	0.058497	0.50 (0.22, 1.15)
1408	5	2	0.058497	0.41 (0.18, 0.94)
1110	4	2	0.046343	0.46 (0.20, 1.06)
1061	6	2	0.039080	0.52 (0.22, 1.21)

Odds ratios (95% CI) based on complete dataset:
Coastal – 2.48 (1.54, 4.01)
Highlands – 0.70 (0.32, 1.52)

Table A4.5. Deletion diagnostics for estimate of trash. Observations with the most influence on the estimate according to the value of Cook's distance, and the change in odds ratio with the removal of the influential observations

House ID	# people in house	# cases in house	Cook's distance	OR (95% CI) with observation and preceding observations removed
Coastal Region				
157	1	1	0.032006	0.21 (0.10, 0.47)
MA-011	2	1	0.030606	0.18 (0.08, 0.42)
PR-060	2	1	0.030606	0.15 (0.06, 0.38)
LA-056	3	1	0.029269	0.12 (0.04, 0.33)
AL-053	4	1	0.027991	0.09 (0.03, 0.29)
Highland Region				
1110	4	2	0.085858	1.34 (0.53, 3.39)
1641	5	2	0.079831	1.02 (0.38, 2.76)
1061	6	2	0.074109	0.70 (0.24, 2.01)
1146	4	2	0.040194	0.77 (0.27, 2.21)
1408	5	2	0.038002	0.85 (0.29, 2.44)

Odds ratios (95% CI) based on complete dataset:
Coastal – 0.25 (0.12, 0.51)
Highlands – 1.66 (0.69, 3.98)

Appendix 5. Sensitivity Analyses of Potential Bias Due to Misclassification of Disease and Exposure Status

In the following tables, the rows represent a range of values for the sensitivity and specificity of the serologic testing scheme used to detect T. cruzi seropositivity, and the columns represent a range of values for the sensitivity and specificity of the exposure measurement. The cells in the table represent the value of the "true" odds ratio at the given assumptions of sensitivity and specificity. The number in bold type in the upper left-hand corner of each table is the odds ratio observed from the study data. The final column of each table is the sensitivity and specificity determined from the validation study of the exposure measurements.

Table A5.1. Sensitivity of the odds ratio for the association between seropositivity and palm roof versus metal roof to misclassification of outcome and exposure, coastal region

		Exposure Measurement						
	Sensitivity	**1**	**0.99**	**0.95**	**0.90**	**0.85**	**0.80**	**0.75**
	Specificity	**1**	**0.99**	**0.95**	**0.95**	**0.95**	**0.95**	**0.989**
Disease Measurement								
Sensitivity	**Specificity**							
1	**1**		**2.61**	2.70	3.23	3.30	3.38	3.47	3.05
1	**0.99**		3.69	3.85	4.78	4.95	5.15	5.41	4.76
1	**0.98**		9.56	10.20	13.89	15.67	18.38	22.97	25.9
1	**0.975**		NA	NA	NA	NA	NA	NA	NA
0.98	**0.99**		3.69	3.85	4.79	4.95	5.16	5.42	4.76
0.98	**0.98**		9.57	10.20	13.90	15.69	18.4	23	25.92
0.98	**0.975**		NA	NA	NA	NA	NA	NA	NA
0.95	**0.99**		3.69	3.86	4.80	4.96	5.17	5.43	4.77
0.95	**0.98**		9.58	10.22	13.93	15.72	18.43	22.04	25.96
0.95	**0.975**		NA	NA	NA	NA	NA	NA	NA

NA = Odds ratio undefined

Table A5.2. Sensitivity of the odds ratio for the association between seropositivity and cane walls versus cement walls to misclassification of outcome and exposure, coastal region

		Exposure Measurement						
	Sensitivity	**1**	**0.99**	**0.95**	**0.90**	**0.85**	**0.80**	**0.887**
	Specificity	**1**	**0.99**	**0.95**	**0.95**	**0.95**	**0.95**	**0.846**
Disease Measurement								
Sensitivity	**Specificity**							
1	**1**	**2.75**	2.86	3.57	6.01	NA	NA	NA
1	**0.99**	7.33	8.54	36.04	NA	NA	NA	NA
1	**0.98**	NA	NA	NA	NA	NA	NA	NA
1	**0.975**	NA	NA	NA	NA	NA	NA	NA
0.98	**0.99**	7.34	8.55	36.06	NA	NA	NA	NA
0.98	**0.98**	NA	NA	NA	NA	NA	NA	NA
0.98	**0.975**	NA	NA	NA	NA	NA	NA	NA
0.95	**0.99**	7.34	8.56	36.09	NA	NA	NA	NA
0.95	**0.98**	NA	NA	NA	NA	NA	NA	NA
0.95	**0.975**	NA	NA	NA	NA	NA	NA	NA

NA = Odds ratio undefined

Table A5.3. Sensitivity of the odds ratio for the association between seropositivity and wood walls versus cement walls to misclassification of outcome and exposure, coastal region

		Exposure Measurement						
	Sensitivity	**1**	**0.99**	**0.95**	**0.90**	**0.85**	**0.80**	**0.887**
	Specificity	**1**	**0.99**	**0.95**	**0.95**	**0.95**	**0.95**	**0.846**
Disease Measurement								
Sensitivity	**Specificity**							
1	**1**	**5.69**	6.33	12.98	13.31	13.71	14.19	NA
1	**0.99**	18.00	20.53	47.14	52.17	59.30	70.26	NA
1	**0.98**	NA	NA	NA	NA	NA	NA	NA
1	**0.975**	NA	NA	NA	NA	NA	NA	NA
0.98	**0.99**	18.03	20.56	47.30	52.34	59.50	70.49	NA
0.98	**0.98**	NA	NA	NA	NA	NA	NA	NA
0.98	**0.975**	NA	NA	NA	NA	NA	NA	NA
0.95	**0.99**	18.07	20.61	47.55	52.62	59.82	70.87	NA
0.95	**0.98**	NA	NA	NA	NA	NA	NA	NA
0.95	**0.975**	NA	NA	NA	NA	NA	NA	NA

NA = Odds ratio undefined

Table A5.4. Sensitivity of the odds ratio for the association between seropositivity and wood board floors versus cement floors to misclassification of outcome and exposure, coastal region

Disease Measurement		Exposure Measurement							
		Sensitivity	**1**	**0.99**	**0.95**	**0.90**	**0.85**	**0.80**	**0.887**
		Specificity	**1**	**0.99**	**0.95**	**0.95**	**0.95**	**0.95**	**0.846**
Sensitivity	**Specificity**								
1	**1**		**1.63**	1.66	1.80	2.08	2.79	8.05	NA
1	**0.99**		2.25	2.32	2.73	3.86	11.82	NA	NA
1	**0.98**		NA	NA	NA	NA	NA	NA	NA
1	**0.975**		NA	NA	NA	NA	NA	NA	NA
0.98	**0.99**		2.25	2.32	2.73	3.86	11.83	NA	NA
0.98	**0.98**		NA	NA	NA	NA	NA	NA	NA
0.98	**0.975**		NA	NA	NA	NA	NA	NA	NA
0.95	**0.99**		2.25	2.32	2.73	3.87	11.84	NA	NA
0.95	**0.98**		NA	NA	NA	NA	NA	NA	NA
0.95	**0.975**		NA	NA	NA	NA	NA	NA	NA

NA = Odds ratio undefined

Table A5.5. Sensitivity of the odds ratio for the association between seropositivity and cane floors versus cement floors to misclassification of outcome and exposure, coastal region

Disease Measurement		Exposure Measurement							
		Sensitivity	**1**	**0.99**	**0.95**	**0.90**	**0.85**	**0.80**	**0.887**
		Specificity	**1**	**0.99**	**0.95**	**0.95**	**0.95**	**0.95**	**0.846**
Sensitivity	**Specificity**								
1	**1**		**1.66**	1.67	1.75	1.82	1.92	2.05	2.22
1	**0.99**		2.29	2.34	2.53	2.74	3.05	3.58	4.53
1	**0.98**		NA	NA	NA	NA	NA	NA	NA
1	**0.975**		NA	NA	NA	NA	NA	NA	NA
0.98	**0.99**		2.30	2.34	2.53	2.74	3.06	3.59	4.53
0.98	**0.98**		NA	NA	NA	NA	NA	NA	NA
0.98	**0.975**		NA	NA	NA	NA	NA	NA	NA
0.95	**0.99**		2.30	2.34	2.53	2.74	3.06	3.59	4.53
0.95	**0.98**		NA	NA	NA	NA	NA	NA	NA
0.95	**0.975**		NA	NA	NA	NA	NA	NA	NA

NA = Odds ratio undefined

Table A5.6. Sensitivity of the odds ratio for the association between seropositivity and dirt floors versus cement floors to misclassification of outcome and exposure, coastal region

Disease Measurement		Exposure Measurement							
		Sensitivity	**1**	**0.99**	**0.95**	**0.90**	**0.85**	**0.80**	**0.887**
		Specificity	**1**	**0.99**	**0.95**	**0.95**	**0.95**	**0.95**	**0.846**
Sensitivity	**Specificity**								
1	**1**		**2.60**	2.68	3.11	3.19	3.28	3.40	3.12
1	**0.99**		4.16	4.33	5.24	5.51	5.86	6.32	5.99
1	**0.98**		NA	NA	NA	NA	NA	NA	NA
1	**0.975**		NA	NA	NA	NA	NA	NA	NA
0.98	**0.99**		4.16	4.33	5.25	5.52	5.86	6.33	5.99
0.98	**0.98**		NA	NA	NA	NA	NA	NA	NA
0.98	**0.975**		NA	NA	NA	NA	NA	NA	NA
0.95	**0.99**		4.17	4.34	5.25	5.52	5.87	6.34	6.00
0.95	**0.98**		NA	NA	NA	NA	NA	NA	NA
0.95	**0.975**		NA	NA	NA	NA	NA	NA	NA

NA = Odds ratio undefined

Table A5.7. Sensitivity of the odds ratio for the association between seropositivity and dirt floors versus cement floors to misclassification of outcome and exposure, highland region

		Exposure Measurement						
	Sensitivity	**1**	**0.99**	**0.95**	**0.90**	**0.85**	**0.80**	**0.887**
	Specificity	**1**	**0.99**	**0.95**	**0.95**	**0.95**	**0.95**	**0.846**
Disease Measurement								
Sensitivity	**Specificity**							
1	**1**		**1.90**	2.00	2.84	NA	NA	NA	NA
1	**0.99**		2.68	2.95	6.98	NA	NA	NA	NA
1	**0.98**		NA	NA	NA	NA	NA	NA	NA
1	**0.975**		NA	NA	NA	NA	NA	NA	NA
0.98	**0.99**		2.68	2.95	6.99	NA	NA	NA	NA
0.98	**0.98**		NA	NA	NA	NA	NA	NA	NA
0.98	**0.975**		NA	NA	NA	NA	NA	NA	NA
0.95	**0.99**		2.68	2.95	6.99	NA	NA	NA	NA
0.95	**0.98**		NA	NA	NA	NA	NA	NA	NA
0.95	**0.975**		NA	NA	NA	NA	NA	NA	NA

NA = Odds ratio undefined

Table A5.8. Sensitivity of the odds ratio for the association between seropositivity and palm trees to misclassification of outcome and exposure, coastal region

Disease Measurement		Exposure Measurement							
		Sensitivity	**1**	**0.99**	**0.95**	**0.90**	**0.85**	**0.80**	**0.80**
		Specificity	**1**	**0.99**	**0.95**	**0.95**	**0.95**	**0.95**	**0.929**
Sensitivity	**Specificity**								
1	**1**		**1.45**	1.46	1.52	1.54	1.57	1.6	1.64
1	**0.99**		1.71	1.73	1.83	1.88	1.93	1.99	2.06
1	**0.98**		2.76	2.83	3.12	3.31	3.56	3.92	4.21
1	**0.975**		8.02	8.46	10.85	14.63	24.98	NA	NA
0.98	**0.99**		1.71	1.73	1.84	1.88	1.93	1.99	2.06
0.98	**0.98**		2.76	2.83	3.13	3.31	3.56	3.92	4.21
0.98	**0.975**		8.02	8.46	10.85	14.63	24.99	NA	NA
0.95	**0.99**		1.72	1.74	1.84	1.88	1.93	2.00	2.06
0.95	**0.98**		2.77	2.83	3.13	3.31	3.56	3.92	4.21
0.95	**0.975**		8.03	8.46	10.86	14.64	25	NA	NA

NA = Odds ratio undefined

Table A5.9. Sensitivity of the odds ratio for the association between seropositivity and organic matter to misclassification of outcome and exposure, coastal region

Disease Measurement		Exposure Measurement						
		Sensitivity	**1**	**0.99**	**0.95**	**0.90**	**0.85**	**0.80**	**0.80**
		Specificity	**1**	**0.99**	**0.95**	**0.95**	**0.95**	**0.95**	**0.929**
Sensitivity	**Specificity**								
1	**1**		**0.89**	0.89	0.86	0.86	0.86	0.86	0.59
1	**0.99**		0.84	0.84	0.80	0.80	0.80	0.79	0.42
1	**0.98**		0.72	0.70	0.64	0.63	0.63	0.63	NA
1	**0.975**		0.52	0.50	0.38	0.39	38.00	0.37	NA
0.98	**0.99**		0.84	0.84	0.80	0.80	0.80	0.79	0.42
0.98	**0.98**		0.72	0.70	0.64	0.63	0.63	0.63	NA
0.98	**0.975**		0.52	0.50	0.38	0.38	0.38	0.37	NA
0.95	**0.99**		0.84	0.84	0.80	0.80	0.80	0.79	0.42
0.95	**0.98**		0.72	0.70	0.64	0.63	0.63	0.63	NA
0.95	**0.975**		0.52	0.50	0.38	0.38	0.38	0.37	NA

NA = Odds ratio undefined

Table A5.10. Sensitivity of the odds ratio for the association between seropositivity and organic matter to misclassification of outcome and exposure, highland region

Disease Measurement		Exposure Measurement						
	Sensitivity	**1**	**0.99**	**0.95**	**0.90**	**0.85**	**0.80**	**0.80**
	Specificity	**1**	**0.99**	**0.95**	**0.95**	**0.95**	**0.95**	**0.929**
Sensitivity	**Specificity**							
1	**1**	**0.90**	0.90	0.88	0.88	0.88	0.88	0.76
1	**0.99**	0.87	0.86	0.84	0.84	0.83	0.83	0.68
1	**0.98**	0.79	0.78	0.75	0.74	0.74	0.74	0.49
1	**0.975**	0.70	0.69	0.64	0.63	0.63	0.63	NA
0.98	**0.99**	0.87	0.86	0.84	0.84	0.83	0.83	0.68
0.98	**0.98**	0.79	0.78	0.75	0.74	0.74	0.73	0.49
0.98	**0.975**	0.70	0.69	0.64	0.63	0.63	0.63	NA
0.95	**0.99**	0.87	0.86	0.84	0.84	0.84	0.83	0.68
0.95	**0.98**	0.79	0.78	0.75	0.74	0.74	0.73	0.49
0.95	**0.975**	0.70	0.69	0.64	0.63	0.63	0.63	NA

NA = Odds ratio undefined

Table A5.11. Sensitivity of the odds ratio for the association between seropositivity and firewood to misclassification of outcome and exposure, coastal region

Disease Measurement		Exposure Measurement							
		Sensitivity	**1**	**0.99**	**0.95**	**0.90**	**0.85**	**0.80**	**0.80**
		Specificity	**1**	**0.99**	**0.95**	**0.95**	**0.95**	**0.95**	**0.929**
Sensitivity	**Specificity**								
1	**1**		**2.48**	2.52	2.75	2.91	3.13	3.44	3.54
1	**0.99**		3.81	3.93	4.49	5.03	5.88	7.43	7.71
1	**0.98**		38.99	50.33	NA	NA	NA	NA	NA
1	**0.975**		NA	NA	NA	NA	NA	NA	NA
0.98	**0.99**		3.82	3.93	4.49	5.03	5.89	7.43	7.72
0.98	**0.98**		39.01	50.37	NA	NA	NA	NA	NA
0.98	**0.975**		NA	NA	NA	NA	NA	NA	NA
0.95	**0.99**		3.82	3.94	4.5	5.04	5.9	7.44	7.73
0.95	**0.98**		39.06	50.42	NA	NA	NA	NA	NA
0.95	**0.975**		NA	NA	NA	NA	NA	NA	NA

NA = Odds ratio undefined

Table A5.12. Sensitivity of the odds ratio for the association between seropositivity and firewood to misclassification of outcome and exposure, highland region

Disease Measurement		Exposure Measurement						
	Sensitivity	**1**	**0.99**	**0.95**	**0.90**	**0.85**	**0.80**	**0.80**
	Specificity	**1**	**0.99**	**0.95**	**0.95**	**0.95**	**0.95**	**0.929**
Sensitivity	**Specificity**							
1	**1**	**0.71**	0.7	0.68	0.66	0.64	0.62	0.61
1	**0.99**	0.62	0.61	0.58	0.57	0.55	0.52	0.51
1	**0.98**	0.45	0.44	0.41	0.39	0.37	0.35	0.34
1	**0.975**	0.29	0.28	0.25	0.23	0.22	0.2	0.19
0.98	**0.99**	0.62	0.61	0.58	0.57	0.55	0.52	0.51
0.98	**0.98**	0.45	0.44	0.41	0.39	0.37	0.35	0.34
0.98	**0.975**	0.29	0.28	0.25	0.23	0.22	0.2	0.19
0.95	**0.99**	0.62	0.61	0.58	0.57	0.55	0.52	0.51
0.95	**0.98**	0.45	0.44	0.41	0.39	0.37	0.35	0.34
0.95	**0.975**	0.29	0.28	0.25	0.23	0.22	0.2	0.19

NA = Odds ratio undefined

Table A5.13. Sensitivity of the odds ratio for the association between seropositivity and rocks to misclassification of outcome and exposure, coastal region

Disease Measurement		Exposure Measurement						
	Sensitivity	**1**	**0.99**	**0.95**	**0.90**	**0.85**	**0.80**	**0.80**
	Specificity	**1**	**0.99**	**0.95**	**0.95**	**0.95**	**0.95**	**0.929**
Sensitivity	**Specificity**							
1	**1**	**0.64**	0.60	0.33	0.33	0.33	0.33	0.18
1	**0.99**	0.48	0.43	NA	NA	NA	NA	NA
1	**0.98**	NA	NA	NA	NA	NA	NA	NA
1	**0.975**	NA	NA	NA	NA	NA	NA	NA
0.98	**0.99**	0.48	0.43	NA	NA	NA	NA	NA
0.98	**0.98**	NA	NA	NA	NA	NA	NA	NA
0.98	**0.975**	NA	NA	NA	NA	NA	NA	NA
0.95	**0.99**	0.48	0.43	NA	NA	NA	NA	NA
0.95	**0.98**	NA	NA	NA	NA	NA	NA	NA
0.95	**0.975**	NA	NA	NA	NA	NA	NA	NA

NA = Odds ratio undefined

Table A5.14. Sensitivity of the odds ratio for the association between seropositivity and rocks to misclassification of outcome and exposure, highland region

Disease Measurement		Exposure Measurement						
	Sensitivity	**1**	**0.99**	**0.95**	**0.90**	**0.85**	**0.80**	**0.80**
	Specificity	**1**	**0.99**	**0.95**	**0.95**	**0.95**	**0.95**	**0.929**
Sensitivity	**Specificity**							
1	**1**	**1.80**	1.87	2.37	2.38	2.40	2.42	2.89
1	**0.99**	2.14	2.24	2.95	2.98	3.00	3.04	3.82
1	**0.98**	3.00	3.17	4.45	4.52	4.60	4.70	6.70
1	**0.975**	4.24	4.54	6.64	6.82	7.02	7.28	12.80
0.98	**0.99**	2.14	2.24	2.95	2.98	3.01	3.04	3.83
0.98	**0.98**	3.00	3.18	4.45	4.52	4.60	4.70	6.70
0.98	**0.975**	4.24	4.54	6.65	6.82	7.03	7.28	12.81
0.95	**0.99**	2.14	2.24	2.96	2.98	3.01	3.05	3.83
0.95	**0.98**	3.00	3.18	4.46	4.53	4.61	4.71	6.72
0.95	**0.975**	4.25	4.54	6.66	6.83	7.04	7.30	12.84

NA = Odds ratio undefined

Table A5.15. Sensitivity of the odds ratio for the association between seropositivity and trash to misclassification of outcome and exposure, coastal region

Disease Measurement		Exposure Measurement						
	Sensitivity	**1**	**0.99**	**0.95**	**0.90**	**0.85**	**0.80**	**0.893**
	Specificity	**1**	**0.99**	**0.95**	**0.95**	**0.95**	**0.95**	**0.708**
Sensitivity	**Specificity**							
1	**1**	**0.24**	0.23	0.15	0.15	0.14	0.14	NA
1	**0.99**	0.01	NA	NA	NA	NA	NA	NA
1	**0.98**	NA	NA	NA	NA	NA	NA	NA
1	**0.975**	NA	NA	NA	NA	NA	NA	NA
0.98	**0.99**	0.01	NA	NA	NA	NA	NA	NA
0.98	**0.98**	NA	NA	NA	NA	NA	NA	NA
0.98	**0.975**	NA	NA	NA	NA	NA	NA	NA
0.95	**0.99**	0.01	NA	NA	NA	NA	NA	NA
0.95	**0.98**	NA	NA	NA	NA	NA	NA	NA
0.95	**0.975**	NA	NA	NA	NA	NA	NA	NA

NA = Odds ratio undefined

Table A5.16. Sensitivity of the odds ratio for the association between seropositivity and trash to misclassification of outcome and exposure, highland region

Disease Measurement		Exposure Measurement							
		Sensitivity	**1**	**0.99**	**0.95**	**0.90**	**0.85**	**0.80**	**0.893**
		Specificity	**1**	**0.99**	**0.95**	**0.95**	**0.95**	**0.95**	**0.708**
Sensitivity	**Specificity**								
1	**1**		**1.66**	1.69	1.84	1.86	1.88	1.91	NA
1	**0.99**		1.96	2	2.22	2.26	2.3	2.36	NA
1	**0.98**		2.78	2.87	3.29	3.4	3.53	3.69	NA
1	**0.975**		4.14	4.3	5.13	5.43	5.82	6.36	NA
0.98	**0.99**		1.96	2.01	2.22	2.26	2.31	2.36	NA
0.98	**0.98**		2.78	2.87	3.3	3.4	3.53	3.69	NA
0.98	**0.975**		4.15	4.3	5.13	5.43	5.83	6.37	NA
0.95	**0.99**		1.96	2.01	2.23	2.26	2.31	2.36	NA
0.95	**0.98**		2.79	2.87	3.3	3.4	3.53	3.7	NA
0.95	**0.975**		4.15	4.31	5.14	5.44	5.83	6.37	NA

NA = Odds ratio undefined

Table A5.17. Sensitivity of the odds ratio for the association between seropositivity and lumber to misclassification of outcome and exposure, coastal region

Disease Measurement		Exposure Measurement						
	Sensitivity	**1**	**0.99**	**0.95**	**0.90**	**0.85**	**0.80**	**0.893**
	Specificity	**1**	**0.99**	**0.95**	**0.95**	**0.95**	**0.95**	**0.708**
Sensitivity	**Specificity**							
1	**1**	**0.67**	0.64	0.46	0.45	0.45	0.45	0.46
1	**0.99**	0.53	0.49	0.22	0.22	0.22	0.22	0.26
1	**0.98**	0.16	NA	NA	NA	NA	NA	NA
1	**0.975**	NA	NA	NA	NA	NA	NA	NA
0.98	**0.99**	0.53	0.49	0.22	0.22	0.22	0.22	0.26
0.98	**0.98**	0.16	NA	NA	NA	NA	NA	NA
0.98	**0.975**	NA	NA	NA	NA	NA	NA	NA
0.95	**0.99**	0.53	0.49	0.22	0.22	0.22	0.22	0.26
0.95	**0.98**	0.16	NA	NA	NA	NA	NA	NA
0.95	**0.975**	NA	NA	NA	NA	NA	NA	NA

NA = Odds ratio undefined

Table A5.18. Sensitivity of the odds ratio for the association between seropositivity and lumber to misclassification of outcome and exposure, highlands region

Disease Measurement		Exposure Measurement						
	Sensitivity	**1**	**0.99**	**0.95**	**0.90**	**0.85**	**0.80**	**0.893**
	Specificity	**1**	**0.99**	**0.95**	**0.95**	**0.95**	**0.95**	**0.708**
Sensitivity	**Specificity**							
1	**1**	**0.89**	0.87	0.63	0.63	0.63	0.63	0.73
1	**0.99**	0.85	0.83	NA	NA	NA	NA	0.64
1	**0.98**	0.76	0.72	NA	NA	NA	NA	0.42
1	**0.975**	0.65	NA	NA	NA	NA	NA	NA
0.98	**0.99**	0.85	0.83	NA	NA	NA	NA	0.64
0.98	**0.98**	0.76	0.72	NA	NA	NA	NA	0.42
0.98	**0.975**	0.65	NA	NA	NA	NA	NA	NA
0.95	**0.99**	0.85	0.83	NA	NA	NA	NA	0.63
0.95	**0.98**	0.76	0.72	NA	NA	NA	NA	0.42
0.95	**0.975**	0.65	NA	NA	NA	NA	NA	NA

NA = Odds ratio undefined

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