Epidemiology of Chagas Disease in Guatemala: Infection Rate of *Triatoma dimidiata, Triatoma nitida* and *Rhodnius prolixus* (Hemiptera, Reduviidae) with *Trypanosoma cruzi* and *Trypanosoma rangeli* (Kinetoplastida, Trypanosomatidae)

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A five-year domiciliary collection in the 22 departments of Guatemala showed that out of 4,128 triatomines collected, 1,675 were Triatoma dimidiata *(Latreille, 1811), 2,344 were* Rhodnius prolixus *Stal 1859, and only 109 were* T. nitida *Usinger 1939. The Chagas disease parasite,* Trypanosoma cruzi*, was found in all three species. Their natural infection rates were similar in the first two species (20.6%; 19.1%) and slightly lower in* T. nitida *(13.8%). However there was no significant difference in the infection rates in the three species (p = 0.131).* T. dimidiata *males have higher infection rates than females (p = 0.030), whereas for* R. prolixus *there is no difference in infection rates between males and females (p = 0.114).*

The sex ratios for all three species were significantly skewed. More males than females were found inside houses for T. dimidiata *(p < 0.0001) and* T. nitida *(p = 0.011); a different pattern was seen for* R. prolixus *(p = 0.037) where more females were found. Sex ratio is proposed as an index to show the mobility of* T. dimidiata *in different populations.*

T. dimidiata *is widely distributed in the country, and is also the main vector in at least ten departments, but* R. prolixus *with higher vectorial capacity is an important vector in at least two departments.*

Key words: *Trypanosoma* infection rate - *Triatoma dimidiata* - *Triatoma nitida* - *Rhodnius prolixus* - vectors' sex ratio - Guatemala

Trypanosoma cruzi and *Trypanosomana rangeli* are parasitic protozoans of insect vectors of the Triatominae subfamily and vertebrates, specifically mammals. *T. cruzi* is the causative agent of Chagas disease, while *T. rangeli* is non pathogenic to humans and other vertebrate hosts. In the vector, *T. cruzi* develops in the intestinal tract, while *T. rangeli* has the ability to invade and develop in the hemolymph and salivary glands (Grisard et al. 1999). In Guatemala, the first reports of the presence of *T. cruzi* and *T. rangeli* in humans were in 1932 and 1934, respectively (Reichnow 1933, Blanco 1943, De León 1949).

Reports of *T. cruzi* and *T. rangeli* infection in triatomine vectors of Central America, suggest that rates of infection differ among species and regions. For example, in Panama, Sousa and Johnson (1973) reported that 68.8% of 740 *Rhodnius pallescens* collected were found infected with either *T. cruzi* or *T. rangeli*. However, from 94 *Triatoma dimidiata* examined, 17.7% contained *T. cruzi* but *T. rangeli* was not seen in the salivary glands of the species. Acosta et al. (1991) found only *T. cruzi* in all the *T. dimidiata* isolates collected in Honduras, using several techniques.

Reports of the infection rates of *T. rangeli* in the vectors from South America also give some idea of differences between regions. Marinkelle (1968), in Colombia, reported five out of 29 *T. dimidiata capitata* infected with *T. rangeli* in the salivary glands.

In addition to variable rates of parasite infection among vectors, different rates of human infection are related with different vector species or populations, suggesting that vectors may differ in their ability to transmit *T. cruzi*. For example, in Guatemala, *T. dimidiata* appears to be associated with lower levels of human seroprevalence compared to *R. prolixus*. In one village of the department of Zacapa where *R. prolixus* was the principal vector, the seroprevalence amongst 373 people tested was 38.8%. In another village in the department of Santa Rosa, where the only vector found was *T. dimidiata*, a much lower seroprevelance rate was found: 8.9% of the 428 people tested were seropositive for *T. cruzi* (Paz-Bailey et al. 2002). This trend was also noted in Honduras in a region where 35% of houses were infested with *R. prolixus*, seroprevalence in the population was 40%, while in another area, where only *T. dimidiata* was present, the prevalence of human infection was only 15% (Ponce et al. 1995). These results suggest that *R. prolixus* is a more efficient vector than *T. dimidiata*.

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Triatominae sex ratio under laboratory conditions is usually 50% (1 male: 1 female). However, under specific climatic and environmental conditions, this sex ratio may vary. For example, some species such as *Panstrongylus geniculatus* were observed to produce significantly more males during the dry season (Naiff et al. 1998). Zeledón et al. (2001) reported more wild *T. dimidiata* males than females in several provinces of Costa Rica with an especially notable difference in sex ratio in the Guanacaste province.

In a previous paper (Tabaru et al. 1999), we reported the vector distribution in Guatemala and the departments with higher risk of human infection. This is a continuation of that study in which we report the protozoan infection rate of each species, and discuss the sex ratio as an index of mobility in different populations of *T. dimidiata.*

MATERIALS AND METHODS

Triatominae were collected between 1993 and 1998 in selected houses in the 22 departments of Guatemala. Every month vectors were collected in one department (22 consecutive months) during the rainy and dry seasons; in some departments several visits were needed in different months and years. Municipalities, villages and ultimately houses were selected based on previously determined risks for infestation including the type of construction such as mud bricks, containing walls with cracks and crevices, and poverty indices (Tabaru et al. 1999). Each house was inspected for 1 h. Ten to 12% of the houses in each of the selected villages was searched. The collected bugs were taken to the laboratory for later dissection (Tabaru et al. 1999).

In order to detect the presence of *T. cruzi,* the rectal gland, and sometimes the intestines, were dispersed in a drop of saline solution (phosphate buffer) and examined (under 400 X) for approximately 2 min. Specimens were dissected within few days after their death, stored at 4°C until the dissection was performed. *T. cruzi* was reported when found in the rectal glands in epimastigote or trypomastigote forms. Small epimastigote forms were also considered to be *T. cruzi*. To detect *T. rangeli*, only live bugs were analyzed because of the fragility of the salivary glands. A positive result for *T. rangeli* was recorded when parasites were seen in the salivary glands.

In the locality of San Miguel Huite, Zacapa, infection rates of *R. prolixus* with *T. cruzi* and *T. rangeli* were compared in three different houses by means of a Chi square test. The three houses were made of the same material and in the same general condition, such as: the number of inhabitants, measurement (square footage) and the presence of firewood inside the houses. Several visits to the same houses were made to collect bugs, and dissections were performed on living vectors.

A house located in Villa Canales, Guatemala, was found to be infested with *T. dimidiata* and *T. nitida*. In order to compare the infection rate of both species, the house was completely demolished and the pieces examined on white cloths to count every single bug. All the bugs collected after the complete demolition were transported to the laboratory and dissected to search for parasites (Monroy et al. 1998).

RESULTS

We first analyzed the infestation by the three vector species on each department of Guatemala (Table I). *T. dimidiata* was found to be widespread throughout the country (in 16 out of 22 departments), while the distribution of *R. prolixus* was punctual, being found in four departments, and was abundant in only two of them. *T. nitida* was found in five departments, in three of these, only one bug was collected. No vectors were found on six departments: Peten, Izabal, Sacatepequez, Huehuetenango, Solola, and Totonicapan.

Infection rates in the same species of bugs are different among departments. *T. dimidiata* is more likely to be infected with *T. cruzi* in the departments from the eastern part of the country (Santa Rosa, Chiquimula, Jutiapa), except for Jalapa and El Progreso, where no infection was observed. On the other hand, lower infection rates are common in the western departments (Quetzaltenango, San Marcos). In the central area, Chimaltenango showed no infection while Guatemala showed a high rate (Table I). Areas traditionally not considered endemic like Quiche and the Verapaces, showed significant infection rates. For *R. prolixus* the department of Chiquimula showed the highest rate of infection with *T. cruzi*.

Table II shows the infection rates with *T. cruzi* of the three species of vectors by growth stages. Only *R. prolixus* showed infection in the first larval stage and all species show some infection by the second stage. There is a considerable tendency of the percentage of infection to increase with age in the case of *R. prolixus* ($r = 0.771$, $p = 0.036$) and *T. dimidiata* ($r = 0.886$; $p = 0.009$). But this tendency is not seen in *T. nitida* ($r = 0.406$; $p = 0.212$). Infection rates of *R. prolixus* and *T. dimidiata* are very similar. The Chi square analysis shows no significant differences of infection rates with *T. cruzi* among the three species of vectors ($p = 0.131$).

Infection rate in *T. nitida* females and the second larval stage of both sexes is higher, but in the third larval stage the rate of infection decreases. It was not possible to perform an statistical analysis to check the significance of these differences because of the small sample size.

The females in *R. prolixus* are more abundant than the males ($p = 0.037$), but in *T. nitida* ($p = 0.011$) and *T. dimidiata* ($p < 0.0001$) there are significantly more males. The males in *T. dimidiata* tend to show higher rates of *T. cruzi* infection than the females ($p = 0.030$).

Table III shows the results of the total infestation assessment in one house with both vectors *T. dimidiata* and *T. nitida*. *T. dimidiata* was more than six times as prevalent as *T. nitida*, however no significant differences ($p = 0.06$) were found between the *T. cruzi* infection rates of the two species. The third stage of *T. dimidiata* was the most prevalent, accounting 36% of the *T. dimidiata* bugs captured. The trend of increasing infection with increasing age of the bug was also seen.

The infection rate with *T. cruzi* and *T. rangeli* were compared in three different houses in the same village that only contained *R. prolixus* (Table IV). In one house only *T. cruzi* was found; in the other houses both parasites were present in *R. prolixus*. The non-pathogenic protozoan *T. rangeli* was only rarely found in the salivary

TABLE I

Natural infection with *Trypanosma cruzi* in three species of triatominae in different departments of Guatemala

Department	*Rhondius prolixus* (%) [a]	*Triatoma dimidiata* (%)	*Triatoma nitida* (%)	Total (%)
Chiquimula	282/779 [b] (36.2)	10/87 (11.5)	1/1 (100)	293/867 (34)
Santa Rosa		226/776 (29.1)		226/776 (29.1)
Retalhuleu		3/10 (30)		3/10 (30)
Guatemala		42/244 (29.1)	13/55 (23.6)	55/299 (18.4)
Zacapa	202/1534 (13.2)	0 /2 (0)		202/1536 (13.2)
Baja Verapaz		6/46 (13.0)		6/46 (13)
Alta Verapaz		3/120 (2.5)	0/1 (0)	3/121 (2.5)
San Marcos		1 /24 (4.2)		1/24 (4.2)
Quiche	0/10 (0)	3/34 (8.8)	1/1 (100)	4/45 (8.9)
Jutiapa		25/258 (9.7)		25/258 (9.7)
Escuintla		1/8 (13)		1/8 (13)
Progreso		0/21 (0)		0/21 (0)
Jalapa	0/21 (0)	0/25 (0)	0/51 (0)	0/97 (0)
Suchitepequez		0/12 (0)		0/12 (0)
Quetzaltenango		0/7 (0)		0/7 (0)
Chimaltenango		0/1 (0)		0/1 (0)
Total	484/2344 (20.6)	320/1675 (19.1)	15/109 (13.8)	819/4128 (19.8)

a: percentage of infected; *b*:positive bugs/bugs dissected

TABLE II

Infection rate of *Triatoma dimidiata, Triatoma nitida* and *Rhodnius prolixus* with *Trypanosoma cruzi (T. c.)*, by stage

Stage	*R. prolixus*			*T. dimidiata*			*T. nitida*		
	Total	*T. c.*[a]	% [b]	Total	*T. c.*	%	Total	*T. c.*	%
Male	454	105	23.1	402	93	23.1	17	3	17.6
Female	519	143	28	292	48	18.4	5	2	40.0
Fifth	330	60	18.2	233	55	23.6	14	4	28.6
Fourth	305	62	20.3	193	33	17.1	9	0	0
Third	393	87	22.1	409	78	19.1	36	2	5.6
Second	228	24	10.5	123	13	11.0	17	4	24.0
First	115	3	2.6	23	0	0	11	0	0
Total	2344	484	20.6	1675	320	19.1	109	15	13.8

a: number of bugs positive for *T. cruzi; b*: percentage of bugs positive for *T. cruzi*

TABLE III

Infection rate of *Triatoma dimidiata* and *Triatoma nitida* with *Trypanosoma cruzi (T. c.)*, in one house demolished in Villa Canales, Guatemala

	T. dimidiata			*T. nitida*		
Stage	Total	*T. c.*[a]	% [b]	Total	*T. c.*	%
Adult	33	20	61	29	12	41
Fifth	32	9	28	4	2	50
Fourth	34	8	24	1	0	0
Third	112	17	15	3	0	0
Second	81	12	15	6	2	33
First	16	0	0	5	0	0
Total	308	66	21	48	16	33

a: number of bugs positive for *T. cruzi; b*: percentage of bugs positive for *T. cruzi*

glands of *R. prolixus* (Table IV). No significant difference was found in the rates of flagellate infections for *R. prolixus* between the different houses in the same village ($p = 0.165$).

DISCUSSION

T. cruzi vectors were found in all but six departments in Guatemala. Our method of sampling overestimates the entomological indices, because the houses were specifically selected by the higher probability of finding vectors. Our sample is not meant to be representative of the departments; it is only an estimate of presence, range of distribution and provides preliminary information about the species of vectors frequently found inside poor houses and their flagellate infection rates. At present, the National Control Program for Chagas vectors has found *T. dimidiata* in five out of the six departments we failed to find vectors during this study. Totonicapan is the only department where no vectors have been found inside houses.

Results of this study challenge the traditional idea that the endemic area of Chagas disease in Guatemala is only the Eastern part of the country. Departments as Quiche, Alta Verapaz, and Baja Verapaz must be considered in the present Chagas disease vector control program.

According to our results, *T. nitida* is not important as a vector of Chagas disease in Guatemala because it is scarce, produces low infestations and has punctual distribution. Its behavior may also limit its vectorial capacity. Galvão et al. (1995) demonstrated in laboratory colonies of *T. nitida* that the low number of defecations on the host and the delay to defecate affects its ability to transmit *T. cruzi*.

R. prolixus is important in at least two departments, and *T. dimidiata* is important in at least ten departments. The departments of Chiquimula and Santa Rosa urgently need control interventions; Chiquimula has three species of vectors, and Santa Rosa only has *T. dimidiata*. Both departments show very high rates of infestation and infection. These departments are on the border with Honduras and El Salvador, so effective control must include a coordinated effort.

The same species of vectors could show different infection rates in different localities. Although of the same species, different populations may differ in their susceptibility to or ability to transmit *T. cruzi*. In addition, available reservoirs or environmental factors may differ between locations and could affect infection rates.

Our results show that there is a considerable difference between *R. prolixus* infection in Chiquimula and Zacapa (Table I); this may be explained by climatic or other environmental conditions: Chiquimula is more humid, with more forest coverage and fauna than Zacapa, which is a deforested, hot and dry area. More extensive work needs to be done to understand the differences in natural infection rates that may be found in populations of the same vector but in different environments.

Peñalver (1959) in Guatemala reported, up to 34% of *T. rangeli* in *R. prolixus,* and 14% in the intestine of *T. dimidiata.* In our research, however, we report lower rates; the discrepancy may be due to our criteria that all small epimastigotes found in the rectal gland or intestines are considered to be *T. cruzi,* thus we may be underestimating the presence of *T. rangeli*.

It is not easy to differentiate *T. cruzi* from *T. rangeli* in the vector dissections (Vallejo 1988), especially the epimastigote forms (Monroy & Koga 1997). Dorn et al. (1999) reported the misdiagnosis of *T. rangeli*, comparing traditional dissection with PCR technique in *R. prolixus* from Guatemala.

A higher infection rate with *T. cruzi* in *T. nitida* and *T. dimidiata* was also reported by Peñalver (1959) and Blanco (1943), but this information comes from a single location. Galvão et al. (1996) reported a higher infection rate with *T. cruzi* in *T. nitida* captured by Leon in Guatemala. Cedillos et al. (2001) reported an overall infection rate for *T. dimidiata* in El Salvador, similar (17.8%) to the one reported here. For some departments, the infection rate they found is higher than what was found here (44.8%). The only intradomiciliary vector reported in the study from El Salvador is *T. dimidiata,* and seroprevalence in infested areas vary from 4.4% to 7.6%.

In the results reported here, the overall *T. cruzi* infection rates did not show statistically significant differences between *T. dimidiata, R. prolixus* and *T. nitida.*

The total number of bugs collected during the demolition of one house infested with *T. dimidiata* and *T. nitida* is considered small (Table III), compared with the ones reported for one house infested with *R. prolixus*. It is interesting that even when present at low densities *T. dimidiata* is capable of transmitting *T. cruzi* to humans. The dominance of *T. dimidiata* in houses shared with *T. nitida* is evident in the demolition study.

Infestation by *T. nitida* alone is rare, competition can

TABLE IV

Infection of *Rhodnius prolixus* with *Trypanosoma cruzi (T. c.)* and *Trypanosoma rangeli (T. r.)* from three houses of San Miguel Huite, Zacapa

		House 1 N = 146			House 2 N = 108			House 3 N = 21	
Stage	*T. c.*	*T. r.*	Neg	*T. c.*	*T. r.*	Neg	*T. c.*	*T. r.*	Neg
Adults	5	1	22	28	0	54	3	1	7
Fifth	7	2	16	2	0	3	0	1	6
Fourth	4	2	15	2	0	7	0	0	1
Third	5	7	42	3	0	5	0	0	1
Second	2	4	18	0	0	2	0	0	0
First	0	0	1	0	0	2	0	0	0
Total	23	16	114	35	0	73	3	2	16
Observation		21% with *T. c* 6% with *T. r.* 5% mixed			32%. *T. c*.			14% with *T. c* 10% with *T. r.* 0% mixed	

N: total of bugs dissected; RG: rectal gland positive for *T. cruzi*; salivary gland positive for *T. rangeli*; Neg: negative rectal and salivary glands be observed since *T. nitida* is frequently found with *T. dimidiata* or with *R. prolixus* in the same house, but in lower numbers. It was observed that usually, only one or two houses in a village were found to be infested with *T. nitida*. This vector has little epidemiological importance for Chagas disease in Guatemala.

It is interesting to note the differences found in the sex ratios of the three species we found. Our work reports only domestic populations, but there are also populations of *T. nitida* and *T. dimidiata* that are peridomestic and sylvatic. Our idea is that there is a relationship with male abundance and mobility in both species since in *T. dimidiata*, males have more ability to fly than females (Tabaru et al. 1995). Dispersive flight of some triatominae species seems to be related with polyandry (one female mating with several males) (Forattini et al. 1983). In some species such as *Triatoma guasayana* the females have more dispersive flight and are more abundant than males (Wisnivesky-Colli et al. 1993).

Zeledón et al. (2001) reported more males than females in sylvan populations in *Panstrongylus geniculatus*, *Panstrongylus rufotuberculatus*, *Triatoma dispar*, *Eratyrus cuspidatus,* and *T. dimidiata.*

In the case of *R. prolixus* only domestic colonies have been reported in Guatemala, the sex ratio in these cases were more females than males. Female triatomines need more blood meals than males, because of the egg development. *R. prolixus* in Central America has a stable domestic environment that offers diverse hiding places with enough food throughout the year. Due to this stability, populations of these triatomines may reach higher densities and more females than males are easily found. In our work the *T. cruzi* infection rate is also very similar in females and males.

We propose that sex ratio could be used as an index of mobility for *T. dimidiata* populations. In our work we showed overall sex ratio, but there are differences in male abundances in different regions of the country (data not shown). If we take the data published by Zeledón et al. (2001), *T. dimidiata* populations from Costa Rica differ in the males abundance ratio, if our idea is correct the Limon *T. dimidiata* populations may be more mobile (male/female ratio = 13/2 = 6.5) compared with the populations from Guanacaste (176/38 = 4.6) or Puntarenas (13/9 = 1.4). In the three provinces, males are more abundant than females but from our field experience the sex ratio gives us an idea of the mobility of the males and the mobility of the populations.

The sex ratio index could also be used in those species with an abundance of males as *E. cuspidatus* or *P. geniculatus*, or in the species in which the females are more abundant as *T. dispar*, *P. megistus*, *T. guasayana*. However, further additional investigation in needed before establishing the sex ratio as an epidemiological index.

For *T. dimidiata* the epidemiological role of the male should be carefully analyzed since males are more abundant and more often infected with *T. cruzi*. In highly mobile species, the males that move from one ecotope to another have more chances of getting different sources of food and the infection rate could also increase. Males of *T. dimidiata* have to be considered carefully in relation to their epidemiological role.

A careful evaluation of the control methods to be used with *T. dimidiata* is needed. The different populations may show differences in mobility and sex ratio may help in control decisions. All of this is essential for planning effective control programs and a clear understanding of the epidemiology of Chagas disease in Guatemala.

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