PROBABILITY OF INFECTION WITH *TRYPANOSOMA CRUZI* OF THE VECTOR *TRIATOMA INFESTANS* FED ON INFECTED HUMANS AND DOGS IN NORTHWEST ARGENTINA

RICARDO E. GURTLER, MARIA C. CECERE, MONICA B. CASTANERA,
DELMI CANALE, MARTA A. LAURICELLA, ROBERTO CHUIT,
JOEL E. COHEN, AND ELSA L. SEGURA

*Laboratorio de Ecologia General, Departamento de Ciencias Biologicas, Universidad de Buenos
Aires, Buenos Aires, Argentina; Laboratory of Populations, Rockefeller University, New York,
New York; Servicio Nacional de Chagas, Cordoba, Argentina; Instituto Nacional de Chagas Dr.
Mario Fatala Chaben, Buenos Aires, Argentina; Direccion de Epidemiologia, Ministerio de Salud
y Accion Social de la Nacion, Buenos Aires, Argentina*

Abstract. The probability that an uninfected *Triatoma infestans* would become infected with *Trypanosoma cruzi* after a single feed on people or dogs seropositive for *T. cruzi* was estimated in Amama, a rural village in northwest Argentina where transmission had resurged four years earlier. The prevalence of seropositivity for *T. cruzi* was 34.2% among 225 people tested, and 65.1% among 83 dogs tested. Parasitemia was detected by xenodiagnosis in 29.3% of 41 seropositive persons and in 85.3% of 34 seropositive dogs. Parasitemia decreased with age more sharply in seropositive people than in seropositive dogs. Seropositive humans infected 2.6% (95% confidence interval = 1.6%–3.6%) of 963 third or fourth instar nymphs fed once on them, whereas dogs infected 48.7% (44.7%–52.7%) of 610 nymphs. The probability of bug infection increased significantly with instar and was positively related to molting success. The infectivity to bugs of seropositive dogs was 12 times higher than that of seropositive children, and 100 times higher than that of seropositive adults. The weighted probability of infection of an uninfected bug fed randomly on any dog (0.3082) was about 50 times higher than that of bugs fed on any human (0.0062). Such differences in relative infectivity, combined with the relative host-feeding preference of domiciliary *Triatoma infestans* for dogs, reinforces the important role of domestic dogs as a risk factor for the domestic transmission of *T. cruzi*.

The duration of infectivity to vector of *Trypanosoma cruzi* in infected humans is lifelong in the absence of effective chemotherapy.[1] After the acute phase subsides, parasitemia decreases with age to levels below detection by direct methods.[2] During the inapparent or chronic stage of infection, parasitemia is usually detected by xenodiagnosis with laboratory-reared, uninfected triatomine bugs or by blood culture.[3]

Extensive studies have shown that approximately 30–50% of humans seropositive for *T. cruzi* have parasitemia detectable by xenodiagnosis at any one moment.[1-5] Most xenodiagnosis trials examined the bugs' feces in pools. In the relatively few studies that undertook the individual examination of the bugs used, the percentage of infected *Triatoma infestans,* after a single blood meal on people seropositive for *T. cruzi,* ranged from 2–3%[5, 6] to 26%.[7]

In contrast, in seropositive dogs naturally infected with *T. cruzi,* 55–60% of the bugs became infected after a single blood meal, and parasitemia detected by xenodiagnosis did not depend on the age of the dog.[8, 9] As a first approximation, we estimated that seropositive dogs had an infectivity to bugs 10 times higher than children seropositive for *T. cruzi* from the same area.[10]

The probability that an uninfected vector acquires the infection after a single blood meal on infected hosts is important for understanding the transmission dynamics of Chagas disease. Furthermore, it is an important parameter in mathematical models of vector-borne diseases.[11] For *T. cruzi,* however, no field study has been specifically designed to estimate this probability. Although there is a large and scattered body of empirical evidence, many of the data were collected for other purposes through biased sampling designs. As part of a larger project aimed at building an em-

pirically based mathematical model of *T. cruzi* transmission, the infection probability of the vector *Triatoma infestans* after a single feed on people or dogs seropositive for *T. cruzi* from a defined rural population of northwest Argentina was measured. We also made an extensive literature review for the selection and synthesis of quantitative data in published studies on the instar-specific proportion of infected *Triatoma infestans* that fed on seropositive humans.

MATERIALS AND METHODS

Study area. Studies were undertaken in the rural village of Amama, Santiago del Estero, Argentina, which has been described elsewhere.[10] After being sprayed with deltamethrin for the first time in 1985, the village became reinfested by *Triatoma infestans* and evidence of renewed transmission of *T. cruzi* was obtained in 1988–1989.[12]

Study design. A house-to-house census of people, dogs, and cats was carried out in October 1991 and updated in March 1992. All houses were identified with a numbered metal plaque. For each person, we recorded the number of his or her current household, full name, place and date of birth (as stated in his or her identity card), age, sex, time of residence in the village and travel history for the last three years. House dwellers were asked for similar data regarding their dogs and cats. All inhabitants were categorized as to their status of residence into permanent stable residents, temporarily absent stable residents, and migrant workers (spending more than two months per year out of home). Census data were checked against previous records and those of the local primary health care agent. Heads of each family were explained the objectives and activities of the entire research project and signed a written informed consent.

The study was carried out in two steps: 1) a house-to-

house entomologic and serologic survey of humans and dogs was conducted in March 1992, and 2) xenodiagnosis of a sample of the seropositive humans and dogs was carried out from October to December 1992. This design avoided disturbing seronegative persons by the unnecessary application of xenodiagnosis. Because of the six-month lag between serologic screening and xenodiagnosis, our estimates can be regarded mostly as pertaining to individuals not in the acute stage of *T. cruzi* infection.

People and dogs tested by xenodiagnosis had been seropositive for *T. cruzi* by at least two different serologic tests (see below), except for eight dogs 3–12 months old that were detected as infected by xenodiagnosis. We believe that inclusion of these dogs did not produce a selection bias because young dogs usually have 100% concordant results between serology and xenodiagnosis.[8]

For the purpose of our study and within operational constraints, we planned to test by xenodiagnosis 40 seropositive people and 40 seropositive dogs. Because infected children are an especially important fraction of the human reservoir of infectious hosts, we attempted to test all seropositive children less than 15 years of age present during house-to-house visits made in October 1992, and sampled one of four older seropositive people (15 years of age and older) who attended a clinical and electrocardiographic examination conducted at the local health post in November 1992. All households with seropositive dogs detected in the baseline survey were visited in November–December and the available animals were tested by xenodiagnosis. Because five seropositive animals had died or were elsewhere and six dogs could not be handled by the available family members, additional nonsystematic xenodiagnoses were carried out to recruit more infected dogs as noted above.

Serology. Blood samples from humans were obtained by venipuncture and tested by the indirect hemagglutination test (IHA), the indirect immunofluorescent antibody test (IFAT), and an enzyme-linked immunosorbent assay (ELISA) at the Instituto Nacional de Chagas following standard procedures.[13] Titers of 1/32 or greater (for the IHA and IFAT) and an optical absorbance of 0.2 or greater (for the ELISA) were considered positive for *T. cruzi* infection. Although the serologic tests used cross-react with *T. rangeli*, *T. rangeli* has not been detected in Argentina in *Triatoma infestans* or in humans tested by xenodiagnosis in the past.[14] The main vectors of *T. rangeli* are *Rhodnius* species, but these are absent south of the Amazon basin.

Blood samples from dogs were similarly obtained and the sera were tested in the same laboratory by IHA and ELISA.[15] All IHA titers of 1/16 or greater and ELISA readings of 0.2 or greater were considered positive for *T. cruzi* infection. For human and dog sera, seropositive refers to samples reactive by at least two different serologic tests.

Xenodiagnosis. We did not use blood culture and the feeding of bugs through an artificial membrane because these techniques were not suitable under our field conditions and additional errors might arise during the extra steps required between blood extraction and feedings. Feeding of bugs directly on the study subjects under field conditions reproduced the natural process and was relevant to the objectives of our study.

All the nymphs used were F_1 progeny of wild-caught *Tria-*

toma infestans from various areas of Argentina. The F_1 progeny were used to mimic the susceptibility to *T. cruzi* of natural *Triatoma infestans* populations and to avoid the potential loss of susceptibility of bugs reared for generations in laboratories. Nymphs were reared on chickens at 27°C and a relative humidity of 80% at the insectarium of the National Chagas Control Service in Punilla (Cordoba) and starved for three weeks before use.

Xenodiagnoses were carried out with either 10 nymphs (small pups), 20 nymphs (older dogs and children less than 18 years old), or 40 nymphs per subject (people 18 years of age and older). All tests were made with third instars, except those carried out in October 1992 when fourth instars were used due to unavailability of third instars. We excluded the use of fifth instar nymphs because this would cause unacceptable discomfort to patients. The wooden boxes with the nymphs were applied to the belly of dogs and to the forearms or thighs of people for 25 min, and inspected after exposure to ensure that most of the nymphs had fully engorged. If they were not, nymphs were re-applied for another 10 min. Only one adult person showed erythema as a consequence of xenodiagnosis. The boxes were kept at 27°C and a relative humidity of 80% during the observation period. Fasting of bugs during the observation period does not decrease the likelihood of detecting *T. cruzi* in *Triatoma infestans*, although the intensity of infection slightly decreases from 30 days of fasting onwards.[3]

Fecal droplets were obtained by applying abdominal pressure to the bugs. The feces were mixed with physiological saline solution, covered with 22×22 mm² coverslips, and microscopically examined for *T. cruzi* infection at 400× at approximately 30 and 60 days after feeding.[3] Trypanosomes were judged to be *T. cruzi* on the basis of their morphology in stained preparations and zymodemes. Microscopists did not know whether the slides they examined came from bugs fed on dogs or humans. Bugs fed on dogs or children less than 18 years of age were individually examined on each occasion. Bugs from people more than 18 years of age were examined in pools of five bugs to save labor; when a pool gave a positive result, the bugs were immediately re-examined individually. The number of exuviae and dead bugs in each box was recorded on each examination to serve as indices of xenodiagnosis quality. The rationale behind this is that the more frequently the bugs consume blood, the higher their molting rate; a significant mortality rate during the initial 30-day period would suggest that the bugs were not healthy or were affected by other causes. The percentage of molting was calculated as the total number of molts produced during the 60-day examination period divided by the total number of live bugs at the first examination. The mortality rate of bugs was calculated as the total number of bugs dead at the first examination divided by the total number of bugs applied to each host. An overall proportion of infected nymphs was calculated among those bugs examined for infection at least once. Bugs dead before the first examination were not examined and were excluded from the calculations of infectivity (i.e., infectivity to the vector); this exclusion introduced no bias because infection with *T. cruzi* does not increase or decrease the chance of survival of *Triatoma infestans* bugs in good nutritional conditions.[16]

To check the sensitivity of our procedures, 35 dog-fed

Seropositive people*			Seropositive dogs*		
Age (years)	No. tested by XD	No. (%) with parasitemia	Age (years)	No. tested by XD	No. (%) with parasitemia
≤4	5	3 (60.0)	<1	13	12 (92.3)
5–9	10	4 (40.0)	1	5	5 (100)
10–14	13	3 (23.1)	2–3	8	7 (87.5)
≥15	13	2 (15.4)	≥4	8	5 (62.5)
Total	41	12 (29.3)	Total	34	29 (85.3)

* Seropositive for *T. cruzi* by at least two tests, except eight dogs < one year of age detected as infected by xenodiagnosis.

bugs that were still negative after two examinations were re-examined for *T. cruzi* infection by dissection of the final portion of the gut. These bugs were randomly drawn from boxes that had harbored infected bugs. In addition, a batch of unused bugs that traveled to the field together with those used was dissected and examined for *Blastocrithidia triatomae,* a monoxenous parasite of triatomines that could give false-positive results.[3] None of the 20 bugs examined had flagellates.

Data analysis. From the published studies of the instar-specific infection probability of *Triatoma infestans* after a single blood meal on humans seropositive for *T. cruzi,* we excluded from analysis those which selected subjects on the basis of a previous positive xenodiagnosis, which dealt with patients in the acute stage or which confounded the detection of *T. cruzi* and *T. rangeli* in the bugs.

To describe the bug infection probability in terms of the blood meal size of the instars used, we used estimates of blood meal size from a laboratory study.[3] If two or three instars were used in the same study, we considered the median blood meal size of those instars.

RESULTS

Of the seropositive people detected, we tested by xenodiagnosis 100% of children less than 10 years of age, 85% of those 10–14 years of age, and 27% of older people. Approximately 60% of the seropositive dogs previously detected were tested by xenodiagnosis. The overall percentage of *T. cruzi*-seropositive dogs with parasitemia (85.3%) was almost three times higher than that of seropositive people (29.3%) (Table 1). This difference is statistically highly significant ($\chi^2 = 23.54$, degrees of freedom [df] = 1, $P < 0.0001$). The prevalence of parasitemia among seropositive people (that is, the proportion of seropositive people with

positive xenodiagnosis) decreased with age more sharply, from 60% to 15%, than among seropositive dogs, from 92% to 62% (Table 1). The ranked ages of individuals with parasitemia were significantly different from those without parasitemia, among both people (Mann-Whitney test, $Z = 5.57$, $P < 0.001$) and dogs ($Z = 2.88$, $P < 0.001$). The percentage of seropositive individuals with parasitemia did not differ significantly by sex either among people (35% of 17 females versus 25% of 24 males) or dogs (100% of 11 females versus 78% of 23 males).

The aggregated percentages of bugs infected with *T. cruzi* in xenodiagnoses of seropositive dogs and seropositive people are shown in Table 2. On the average, approximately 50% of bugs fed on seropositive people and seropositive dogs molted to the next instar with a single feed during the stage. The mortality rate of bugs applied to both host species was similarly low (4%). The mean per capita number of bugs examined for infection was 17.9 (SD = 3.3) for dogs, 18.8 (SD = 1.2) for children, and 37.9 (SD = 2.3) for adults. On the average, seropositive dogs infected 48.7% of the *Triatoma infestans* whereas seropositive people infected only 2.6% of the bugs. Two (5.7%) of 35 dog-fed bugs that had been negative in two examinations were found infected with *T. cruzi* by dissection.

Infectivity to the vector (defined as the number of *T. cruzi*-positive bugs divided by the total number of bugs fed on seropositive hosts, excluding those bugs that did not survive to be examined for infection) decreased significantly with age both in seropositive humans (Spearman's r = −0.324, N = 41, P = 0.041) and in seropositive dogs (Spearman's r = −0.354, N = 34, P = 0.042) (Figure 1). For both hosts, the largest differences were between the young (0–14 years in humans and 0–1 years in dogs) and adults. The outlier value (10 of 19) observed among children 10–14 years of age was from a child showing other health disorders. This child had a positive xenodiagnosis in 1985 and was seropositive in 1989; thus, he was not in the acute stage of infection by 1992. The percentage of infected bugs did not differ significantly between women (2.4%) and men (2.7%). Female dogs infected more bugs (70%) than males (39.8%), but the median age of females was lower (0.4 years) than that of males (two years).

Before beginning specific chemotherapy, seropositive children who seroconverted between 1989 and 1992 had detectable parasitemias more frequently (62.5%, 5 of 8) than those who had been seropositive in 1989 (33.3%, 5 of 15; Fisher test, P = 0.221). Bugs fed on children who had seroconverted were infected (4.7%, 7 of 149) as frequently as bugs fed on children who had been seropositive in 1989

TABLE 2

Infectivity to *Triatoma infestans* third or fourth instar nymphs of humans and dogs seropositive to *T. cruzi* in Amama, northwest Argentina, March–November 1992

Host	No. of hosts tested	No. of bugs examined	% bugs		% infectivity*			
			Molts	Dead	Mean	95% CI†	Median	1st–3rd quartile
Dogs	34	610	52.3	4.3	48.7	44.7, 52.7	45.0	5.0–77.8
People	41	963	56.9	4.2	2.6‡	1.6, 3.6	0.0	0.0–5.0

* Infectivity = the percentage of infected bugs in each xenodiagnosis.
† CI = confidence interval.
‡ The mean infectivity of children less than 15 years of age was 4.2% (CI = 2.51, 5.86) and of adults was 0.48% (CI = −0.18, 1.15).
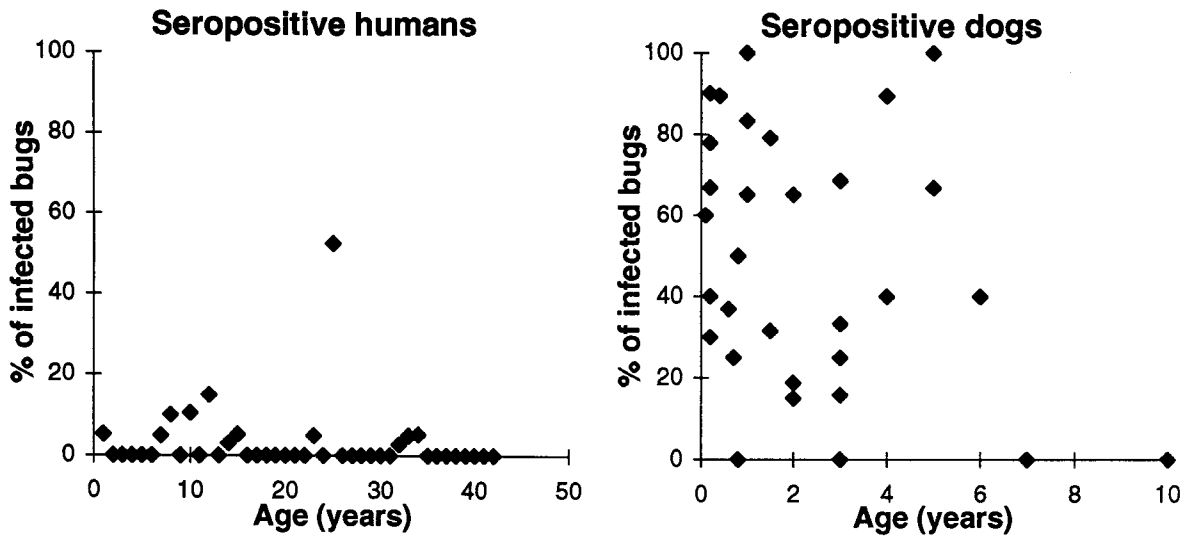
FIGURE 1. Age-specific percentages of third or fourth instar nymphs of *Triatoma infestans* infected with *Trypanosoma cruzi* after taking a single blood meal on humans or dogs seropositive for *T. cruzi* in Amama, northwest Argentina, March–November 1992.

(5.2%, 16 of 306; $\chi^2 = 0.06$, df = 1, $P = 0.808$), even when the anomalous value (10 of 19) was excluded (2.1%; $\chi^2 = 2.43$, df = 1, $P = 0.119$).

Probability of bug infection by instar. Based on the results in Table 1 and Figure 1, we grouped seropositive people who had similar infectivity to bugs into two broad age classes to examine the probability of bug infection according to bug instar and molting success (Table 3). Children seropositive for *T. cruzi* infected significantly more fourth instar nymphs (6%) than third instars (1%). Bugs that were fed on children or dogs and that molted to the next instar had significantly higher infection rates than those that did not molt. Seropositive adults infected only two bugs (0.5%).

Using data drawn from the literature, Figure 2 shows the relationship between the overall proportion of infected *Triatoma infestans* in xenodiagnoses of seropositive humans and the instars used or their average blood meal size. From two

previous reports,[8, 17] we calculated that 28 seropositive children infected approximately 4.1% (23 of 560) of *Triatoma infestans* third or fourth instar nymphs. From data by Hoff and others,[2] we calculated that 39 people seropositive for *T. cruzi* infected 13.6% (53 of 390) of *Triatoma infestans* fifth instars. In the same area, seropositive persons with parasitemia detected in two different surveys three years apart infected more (27.3%) *Triatoma infestans* fifth instar nymphs than those with parasitemia detected in one occasion (17.7%).[18] Overall, Figure 2 shows that the proportion of infected nymphs increases with increasing instars or their average blood meal size, despite differences in the ages of study subjects and type of setting.

Probability of bug infection. An average probability of bug infection after a single feed on any human or dog was estimated as the combined product of the proportion of seropositives to *T. cruzi*, the proportion of seropositive individuals with positive xenodiagnosis, and the proportion of infected bugs in individuals with positive xenodiagnosis. To allow for the fact that the prevalence of seropositivity increased with age while parasitemia in seropositives decreased, and that the sampling fraction also varied with age group, we calculated the infection probability of bugs stratified by human age group (Table 4). The weighted average was 0.0062, or 0.0042 if the anomalous value was excluded. The age group younger than five years of age had a lower weighted probability of bug infection (0.0006) than the other age groups, which ranged from 0.0011 to 0.0034.

Table 5 shows similar data for the dog population. The weighted infection probability of an uninfected bug fed randomly on any dog was 0.3082, about 50 times higher than that of bugs fed on any human (0.0062). Pups less than one year of age increased by more than half the potential contribution to transmission of all the older age groups combined.

TABLE 3

Infectivity to *Triatoma infestans* nymphs of children less than 15 years of age, adults (15 years of age or older), and dogs seropositive to *Trypanosoma cruzi* according to bug instar and molting success in Amama, northwest Argentina, March–November 1992

Host	Bug instar applied	Molting success	Proportion of infected bugs (%)	Total (%)
Children				
	III	No	0/64 (0)	
	III	Yes	2/136 (1.5)	2/200 (1.0)*†
	IV	No	5/183 (2.7)	
	IV	Yes	16/166 (9.6)	21/349 (6.0)*†
Adults				
	III	No	0/53 (0)	
	III	Yes	1/189 (0.5)	1/242 (0.4)
	IV	No	1/113 (0.9)	
	IV	Yes	0/59 (0)	1/172 (0.6)
Dogs				
	III	No	127/292 (43.5)‡	
	III	Yes	170/318 (53.5)‡	297/610 (48.7)

* Effect of instar: $\chi^2 = 7.97$, degrees of freedom (df) = 1, $P = 0.005$.
† Effect of molting success: Mantel-Haenszel $\chi^2 = 7.05$, df = 1, $0.001 < P < 0.01$.
‡ Effect of molting success: $\chi^2 = 6.05$, df = 1, $P = 0.014$.

DISCUSSION

Our study shows that the infectivity to bugs of seropositive people (defined as the fraction of bugs that were fed on
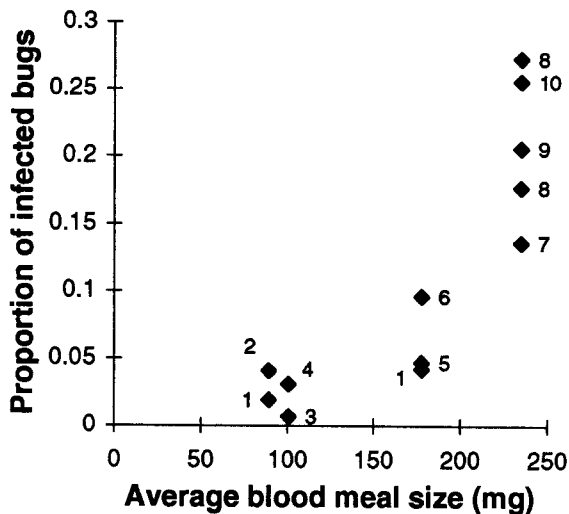
FIGURE 2. Proportion of *Triatoma infestans* nymphs infected with *Trypanosoma cruzi* in xenodiagnosis of people seropositive for *T. cruzi* according to average blood meal size (milligrams) of the instars used, indicating that the larger a blood meal, the higher the proportion of infected bugs, according to 10 studies. Data were selected from the literature. 1 = this study, mostly children less than 15 years of age; 2 = adults;[6] 3 = mostly adults;[5] 4 = children less than 13 years of age;[8,17] 5 = an entire family, all ages (third–fifth instars?);[19] 6 = adults (third–fifth instars);[20] 7 = an entire population, all ages;[2] 8 = an entire population, all ages;[18] 9 = adults;[7] 10 = adults.[21]

seropositive people and that were then found positive for *T. cruzi*) decreased with human age. The proportion of seropositive persons with detectable parasitemia also decreased with age and did not differ significantly by sex, which is in agreement with other field surveys including all age groups.[2,17,18] In contrast, in surveys that excluded children less than 10 years of age, the proportion of seropositive individuals with positive xenodiagnosis showed either 1) no significant age-related variation[4] or 2) a decrease at young ages followed by a less pronounced increase among the elderly,[1] possibly as a consequence of immunodepression associated with old age. Apart from this subtlety, parasitemia among seropositive people shows a clearly decreasing trend with age.

In contrast to seropositive people, in seropositive dogs, the age-specific prevalence of parasitemia or infectivity to bugs showed a slight and not significant trend in an earlier

study in Amama.[8,9] In the present data, the decrease was statistically significant. In a larger canine survey made in another rural area of Santiago del Estero, the proportion of seropositive dogs with a positive xenodiagnosis did not differ significantly among age groups, although the few dogs that were older than 10 years of age showed increasing infectivity to bugs.[15] For epidemiologic purposes, the infectivity to bugs of seropositive dogs can be considered to be marginally influenced by age.

In a group of 30 seropositive persons repeatedly tested by xenodiagnosis during 30 months, people chronically infected with *T. cruzi* showed a rather constant pattern of low, intermediate, or high infectivity to bugs over time.[3] Naturally infected dogs showed a similar phenomenon, partly attributed to their prior level of exposure to reinfections with *T. cruzi*[9] and nutritional status (unpublished data).

In xenodiagnosis trials of people seropositive for *T. cruzi*, variable percentages of infected *Triatoma infestans* nymphs were recorded depending on the selection criteria of the study subjects and the instar used (Figure 2). Infectivity estimates (means or medians calculated from actual observations) from studies that selected subjects on the basis of a previous positive xenodiagnosis[22–25] or that dealt with patients in the acute stage[26] invariably exceeded by several times the infectivity estimates for patients selected through serology. Estimates shown in Figure 2 are drawn from studies in which people were selected by the criterion of seropositivity. Age differences among data sets still remain as an uncontrolled source of variation.

Our estimates of infectivity with third or fourth instar nymphs (2.6%) were close to estimates from Brazil (1.9–3.1%),[5,6] in spite of differences in location, setting, and ages of the study subjects. The infection probability of bugs fed once on seropositive people increased from 1–3% in third instars to 13.6–27.3% in fifth instars. Infectivity estimates varied widely among subjects within each study.

There is a lack of data on the infectivity of seropositive humans to other instars. In two separate experiments with two different groups of patients,[27] 1.5–4% and approximately 3–14% of *Panstrongylus megistus* first instar nymphs became infected after a single feed on seropositive people with chronic infections, but an unstated number (many) of the patients appeared to be selected through a previous positive xenodiagnosis.

The median infectivity of seropositive dogs to bugs ob-

TABLE 4

Age-specific proportions of people seropositive to *Trypanosoma cruzi*, of seropositive individuals with a positive xenodiagnosis, and of *T. cruzi*-infected third or fourth instar nymphs of *Triatoma infestans* fed once on seropositive individuals with a positive xenodiagnosis in Amama, northwest Argentina, March–November 1992

Age group (years)	No. of people censused	Proportion of age group in population	Proportion of seropositive individuals	Proportion of seropositive individuals with positive xenodiagnosis	Proportion of infected bugs*	Weighted probability of bug infection†
≤4	50	0.192	0.106	0.600	0.051	0.0006
5–9	36	0.138	0.257	0.400	0.078	0.0011
10–14	36	0.138	0.455	0.231	0.237‡	0.0034‡
≥15	138	0.531	0.436	0.154	0.028	0.0010
Total	260		0.342	0.293	0.094	0.0062§

* Bugs fed on seropositive individuals with a positive xenodiagnosis.
† The age-specific probability of bug infection is calculated as the combined product of these three proportions weighted by the proportion of individuals in each age class.
‡ Exclusion of an anomalous value of 0.53 for the age class 10–14 years of age gave a corrected value of 0.0015, and an overall weighted estimate for the probability of bug infection of 0.0042.
§ Weighted average.

TABLE 5

Age-specific proportions of dogs seropositive to *Trypanosoma cruzi*, of seropositive individuals with a positive xenodiagnosis, and of *T. cruzi*-infected third or fourth instar nymphs of *Triatoma infestans* fed once on seropositives with a positive xenodiagnosis in Amama, northwest Argentina, March–November 1992

Age group (years)	No. of dogs censused	Proportion of age group in population	Proportion of seropositive individuals	Proportion of seropositive individuals with positive xenodiagnosis	Proportion of infected bugs*	Weighted probability of bug infection†
<1	51	0.405	0.471	0.923	0.626	0.1100
1	20	0.159	0.643	1.000	0.719	0.0733
2–3	23	0.183	0.875	0.875	0.348	0.0487
≥4	29	0.230	0.789	0.625	0.670	0.0761
Total	126‡		0.651	0.853	0.580	0.3082§

* Bugs fed on seropositive individuals with a positive xenodiagnosis.
† Calculations are as in Table 4.
‡ Includes three adult dogs with unknown age, not tested serologically or by xenodiagnosis.
§ Weighted average.

served here (45%) was slightly lower than that found before (55–60%),[9] in spite of comparable procedures. In the present study, 5.7% of the bugs fed on infective dogs and that were diagnosed as negative had a detectable rectal infection with *T. cruzi* on dissection. The infectivity ratio of seropositive dogs to seropositive children (12x) was close to our prior rough estimate of a factor of approximately 10.[10] Measured with third instar nymphs, the infectivity of seropositive dogs was approximately 50 times higher than the infectivity of seropositive children. This is consistent with two previous field observations on the natural infection rate of domiciliary *Triatoma infestans* in our study area: 1) the younger the infected dog, the higher the infection rate of *Triatoma infestans* in houses with or without infected children,[10] and 2) the youngest instar infected with *T. cruzi* was younger in houses with than without an infected dog.[28] Because the infectivity of seropositive humans to fifth instar nymphs may average approximately 20% (Figure 2), the infectivity ratio of seropositive dogs to seropositive humans measured on fifth instar nymphs may differ significantly from the infectivity ratio of 50 in third instar nymphs.

The median age of the infected dogs was approximately three years, and most of them acquired their infection before reaching three years of age, as suggested by age-specific prevalence rates of seropositivity in Table 5. Therefore, on average, dogs had experienced the acute stage more recently than the infected human population. However, comparison of the infectivity of children who were incident cases between 1989 and 1992 with that of infected dogs three years of age or less in 1992 still yielded significant differences. The dogs had higher infectivity to bugs than the children, though the infections in both groups lasted less than three years.

The increase in bug infection probability with instar is explained by increasing blood meal size in successive instars. All instars of triatomine bugs are susceptible to *T. cruzi* but there is contradictory evidence related to whether they are equally susceptible.[29–31] Several studies showed that the proportion of bugs that acquired *T. cruzi* infection increased with the size of the infective blood meal and molting success[27, 29] and the number of trypomastigotes ingested.[32–34] However, paired testing of infected individuals by xenodiagnosis and other methods showed that some bugs did not acquire *T. cruzi* infection at times when direct or indirect detection methods yielded positive results.[26, 31, 35] Thus, parasites may be ingested by the bugs but infections may not

become established. Whether the ingested trypomastigotes may not survive the passage through the insect's midgut or their infectivity was already impaired by the host's immune response is unclear. Vector infection by *T. cruzi* is also sensitive to several other factors (symbionts, strains of *T. cruzi*) that are beyond the scope of this study.[36]

Because most of the bugs engorged close to their maximum capacity, our calculated estimates of bug infection probability may be regarded as the upper level achievable with a full blood meal. The blood meal size of triatomine bugs under field conditions is not known. Whether they take full or much smaller blood meals may be crucial to the actual infection probability of bugs. For instance, the blood meal size of fifth instar nymphs and adults of *Triatoma infestans* reared in experimental chicken houses was one-fifth to one-sixth of those reported for single feeds achieved in the laboratory.[37] Host irritation as a consequence of multiple bites may interrupt bug feeding; depending on the size of the blood meal achieved, interruptions would be expected to reduce the probability of bug infection.

The most developed model of Chagas disease transmission assumed that the infection probability of bugs after a single feed (0.5) was independent of host species, host age, and bug instar.[38] Our study, showing a marked dependence of the infection probability on those variables, may contribute toward a more realistic model of the domestic transmission of *T. cruzi*. As suggested in reference to malaria control,[39] estimates of the infection probability may serve as a yardstick for assessing the impact of a transmission-blocking vaccine.

The observed differences in relative infectivity of dogs and humans, combined with the relative host-feeding preference of domiciliary *Triatoma infestans* for dogs, reinforces the important role of domestic dogs as a risk factor for the domestic transmission of *T. cruzi*.[10] Other animal reservoirs of *T. cruzi* (e.g., rats, opossums, cats) may play roles similar to dogs in other domestic or peridomestic transmission cycles.[10]

ceptibilidade a infeccao por *Trypanosoma cruzi* dos estagios evolutivos de *Rhodnius neglectus. Rev Brasil Biol 33:* 43–52.

30. Perlowagora-Szumlewics A, Muller CA, de Carvalho Moreira CJ, 1987. Studies in search of a suitable experimental insect model for xenodiagnosis of hosts with Chagas disease. 3. On the interaction of vector species and parasite strain in the reaction of bugs to infection by *Trypanosoma cruzi. Rev Saude Publica 22:* 390–400.

31. Philips NR, Bertram DS, 1967. Laboratory studies on *Trypanosoma cruzi* infections in: *Rhodnius prolixus*-larvae and adults-, in: *Triatoma infestans, T. protracta,* and *T. maculata*-adults. *J Med Entomol 4:* 168–174.

32. Neal RA, Miles MA, 1977. The numbers of trypomastigotes of *Trypanosoma cruzi* required to infect *Rhodnius prolixus. Rev Inst Med Trop Sao Paulo 19:* 177–181.

33. Alvarenga NJ, Leite MAB, 1982. Injection of *Trypanosoma cruzi* into the gut of triatomine bugs: number required to infect the vector. *Trans R Soc Trop Med Hyg 76:* 708–709.

34. Moll-Merks C, Werner H, Doenges J, 1988. Suitability of in--vitro xenodiagnosis: development of *Trypanosoma cruzi* in *Triatoma infestans* depending on larval stage of bugs and number of trypomastigotes taken during in-vitro blood meal. *Zentralbl Bakt Hyg A 268:* 74–82.

35. Avila HA, Pereira JB, Thiemann O, Paiva E, Degrave W, Morel CM, Simpson L, 1993. Detection of *Trypanosoma cruzi* in blood specimens of chronic chagasic patients by polymerase chain reaction amplification of kinetoplast minicircle DNA: comparison with serology and xenodiagnosis. *J Clin Microbiol 31:* 2421–2426.

36. Zeledon R, 1987. Life cycle of *Trypanosoma cruzi* in the insect vector. Brenner RR, Stoka AM, eds. *Chagas Disease Vectors.* Volume II. Boca Raton, FL: CRC Press, 59–76.

37. Catala S, 1994. Bloodmeal size and nutritional status of *Triatoma infestans* under natural climatic conditions. *Med Vet Entomol 8:* 104–106.

38. Rabinovich JE, Himschoot P, 1990. A population-dynamics simulation model of the main vectors of Chagas' Disease transmission, *Rhodnius prolixus* and *Triatoma infestans. Ecol Model 52:* 249–266.

39. Graves PM, Burkot TR, Carter R, Cattani JA, Lagog M, Parker J, Brabin BJ, Gibson FD, Bradley DJ, Alpers MP, 1988. Measurement of malarial infectivity of human populations to mosquitoes in the Madang area, Papua New Guinea. *Parasitology 96:* 251–263.