

DYNAMICS OF TRANSMISSION OF *TRYPANOSOMA CRUZI* IN A RURAL AREA OF ARGENTINA: II. HOUSEHOLD INFECTION PATTERNS AMONG CHILDREN AND DOGS RELATIVE TO THE DENSITY OF INFECTED *TRIATOMA INFESTANS*¹

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INTRODUCTION

The degree to which a home is infested with the triatomine insect vectors of Chagas' disease depends on both entomologic and anthropologic factors (1). Of the former, among the most critical is the particular vector species present, while of the latter a number of factors—including structural features of home construction (2, 3), the availability of human and other blood meal sources (4-6), use of insecticides by the inhabitants (7), and so forth—are known to be important.

It has been demonstrated that the domiciliary density of one *Trypanosoma cruzi* vector, *Panstrongylus megistus*, can have a direct influence on household children's seropositivity rates for *T. cruzi* antigens in cases where at least one *T. cruzi*-infected vector was encountered (2). Similarly, a preliminary report on work done in Brazil has found that the number of infected *P. megistus* caught per unit of effort was directly correlated to current infection

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patterns of family members (8), and a later report has indicated that this capture index could be used to help predict future human infections (9). More recently, an inverse correlation was found between the proportion of infected *P. megistus* captured in a study household and the age of the youngest seropositive resident (10).

Valuable information of this kind derived from cross-sectional studies is not yet available for the major Chagas' disease vector *Triatoma infestans*. This is unfortunate, for such information can provide important clues about transmission dynamics, assist in designing control programs, and help in estimating infestation densities associated with low-risk transmission levels.

One of the prime objectives of the study reported here was to collect data on the numbers of infected *T. infestans* in an area where *T. cruzi* was being actively transmitted and where no official insecticide campaign had been conducted, so as to investigate the intensity of *T. cruzi* transmission to household dogs and children. Also, since canine reservoirs have been found to play an important role in domestic *T. cruzi* transmission cycles in rural areas of Argentina (11, 12), and have recently been incriminated as amplifying hosts (13, 38), the investigation tried to gain further insight into the relationship between the presence of infected dogs in a household and *T. cruzi* infection among children.

MATERIALS AND METHODS

A cross-sectional survey was conducted at a locality named Amamá in the province of Santiago del Estero, Argentina, in December 1982. A general description of the study area and the survey design has been published previously (13).

A two-person collection team searched all areas of each house studied for triatomine bugs, using only forceps and a flashlight. Beds were searched separately. All the bugs captured were kept alive in labeled containers. Later, during a second stage of the collection effort, the house walls and roof were sprayed repeatedly with a synthetic pyrethrin solution (neopynamin, 0.2%), and insect captures were continued for a period averaging 1.5 hours per house (on the average, four man-hours were spent searching each house), after which it appeared in most cases that no more bugs could be caught. The captured bugs were subsequently shipped to a laboratory where fecal examinations for trypanosomes and blood meal studies (to be described in a separate article) were performed.

Blood samples were obtained by venipuncture from all members of each study household, and from all household dogs and cats.⁵ Human sera were mixed 1:1 with buffered glycerine and stored at room temperature. The procedures used to preserve dog and cat sera, as well as the complement fixation (CF), indirect hemagglutination (IHA), and direct agglutination (DA) tests per-

⁵ Only six cats were involved, and data about them have already been published (11), so no further discussion of them is presented here.

formed with these sera, have already been described (13).

Xenodiagnosis was performed on children up to 12 years old and on dogs and cats using 20 third or fourth instar *T. infestans* nymphs in two boxes. Examination of the bugs followed a previously described procedure (13).

Serologic studies on the collected human sera were performed by the Dr. Mario Fatała Chabén Institute of Chagas' Disease Diagnosis and Research in Buenos Aires, as described previously (14). The tests performed included indirect hemagglutination (IHA), direct agglutination (DA), indirect immunofluorescent antibody (IFA), and (for discordant results) enzyme-linked immunosorbent assay (ELISA) (15). Titers accepted as positive were 32 in the IHA test, 16 in the DA test, and 32 in the IFA test. An ELISA reading of 0.20 was also considered positive. Human subjects whose sera yielded positive results on two or more tests were considered seropositive. Human subjects were considered infected with *T. cruzi* if they were found seropositive or were found positive for *T. cruzi* by xenodiagnosis.

RESULTS

The density of *T. infestans* household infestations showed an approximately bimodal pattern, with five (25%) of the surveyed houses harboring less than 15 vectors and six (30%) harboring over 100 vectors per house (Table 1). Adults and fifth instar nymphs accounted for 80% to 100% of the total bug capture at each house.

Table 2 shows the percentages of children and dogs found infected with *T. cruzi* at homes with different densities of infected *T. infestans*.

TABLE 1. Infestation densities in study houses in Amamá, as indicated by the numbers of *T. infestans* collected during two-man searches lasting an average of two hours that were assisted by repeated spraying of the house walls and roof with 0.2% neopynamin.

Density (No. of bugs collected per house)	Households surveyed	
	No.	(%)
1-14	5	(25)
15-49	1	(5)
50-69	3	(15)
70-99	5	(25)
≥ 100	6	(30)
Total	20	(100)

Overall, the dog population showed a significantly higher percentage of infected individuals (83.9%) than did the child population (47.8%) (G test, $p < 0.001$) (16).⁶ A similar relationship was found at all levels of infestation, but statistically significant differences were found only when the number of infected bugs captured was 1-15 (Fisher test, $p = 0.034$) and more than 100 ($p < 0.001$) (16).

Household densities below 16 infected *T. infestans* were found associated with the lowest infection rates (15% in children, 53% in dogs). Conversely, when over 15 infected bugs were captured, the prevalence of *T. cruzi* infection among the study children increased to 54-70%, and among

⁶ As indicated in reference 16 (p. 590), the G test is a test of independence based on multinomial distribution. (The statistic G follows this distribution more closely than X.)

TABLE 2. Densities of *T. infestans* infected with *T. cruzi* and percentages of *T. cruzi* infected children and dogs found in houses with different densities of infected bug infestations. The densities are the numbers of infected *T. infestans* collected per house during two-man searches lasting an average of two hours that were assisted by repeated spraying of the house walls and roof with 0.2% neopynamin.

Infected vector density ranges per house	Range of infected vectors actually collected per house	No. of houses surveyed	Study children examined		Household dogs examined	
			No. examined	(% positive) ^a	No. examined	(% positive) ^a
0	0		20	(33.3)	7	(42.9)
1-15	2-11	2 } ^b 6	17	(11.8)	8	(62.5)
16-45	29-41	3 ^c	3	(66.7)	8	(87.5)
46-70	52-70	5	14	(64.3)	12	(91.7)
71-100	71-76	2 } ^b 6	10	(70.0)	4	(100)
>100	108-145	4	22	(54.5)	17	(100)
Total		20	69	(47.8)	56	(83.9)

^a Infections among the children (all under age 15) were detected by IHA, DA, IFA, and ELISA tests and by xenodiagnosis; infections among the dogs were detected by OF, IHA, and DA tests and by xenodiagnosis. See further details in text, page 282.

^b Includes one house with no children.

^c Includes one house with no children where 29 infected vectors were captured. At the other two houses the numbers of infected vectors captured were 40 and 41.

dogs the prevalence rose to 87-100%. Highly significant differences were found in the overall percentages of infected hosts (children + dogs) in these two groups of homes (G test, $p < 0.001$) as well as in the percentages of infected children (Fisher test, $p < 0.001$) and the percentages of infected dogs (Fisher test, $p < 0.002$). In the households with more than 15 infected *T. infestans*, referred to hereafter as "high-risk" households, the minimum number of infected *T. infestans* associated with the presence of a child (infected or not) was 40. Therefore, the lower limit of the range should be considered 40 bugs.

To help assess the intensity of *T. cruzi* transmission to both the child and canine populations, the rates of *T. cruzi* infection among children (Table 3) and dogs (Table 4) in different age groups residing at "low-risk" and "high-risk" homes were calculated. Overall infection rates among the children showed a statistically significant linear trend increasing with age (Z test, $p = 0.032$) (17);⁷ similar but not statistically significant trends were found for both of the bug density ranges involved (≤ 15 infected *T. infestans* per house and ≥ 40 infected *T. infestans* per house). Also, a statistically significant difference was found in the percentages of infected children 0-4 years old residing in the low-risk and high-risk households ($p < 0.001$).

Dogs in the study households generally exhibited higher rates of infection than children at all levels of comparison (Table 4), though not all these differences were statistically significant because of the small numbers involved.

⁷ As indicated in reference 17 (pp. 307-309), the Z test is a lineal regression test, the statistic of which follows a normal distribution.

TABLE 3. Age-specific prevalences of *T. cruzi* infection found among study children below age 15, according to levels of household risk indicated by collection of infected *T. infestans*.

Household risk level	Children examined (by age group) ^a						All study children ^a	
	0-4 years		5-9 years		10-14 years			
	No. examined	(% positive)	No. examined	(% positive)	No. examined	(% positive)	No. examined	(% positive)
Low-risk (≤ 15 infected vectors collected)	9 ^b	(0)	7	(14.3)	4	(50.0)	20	(15.0)
High-risk (≥ 40 infected vectors collected)	15 ^c	(53.3)	20	(60.0)	14	(71.4)	49	(61.2)
Total	24	(33.3)	27	(48.1)	18	(66.7)	69	(47.8)

^a According to the Fisher test (two-tailed), the differences between the numbers infected in the low-risk and high-risk households were statistically significant for the 0-4 year age group ($p < 0.001$), not significant for the 5-9 year or 10-14 year group, and significant for the overall group ($p < 0.001$).

^b Figure includes four children less than one year old.

^c Figure includes four children less than one year old, of whom two (a pair of twins) were found to be infected.

TABLE 4. Age-specific prevalences of *T. cruzi* infection found among study household dogs according to levels of household risk indicated by collection of infected *T. infestans*.

Household risk level	Dogs examined (by age group) ^a						Total ^a	
	0-4 years		5-9 years		10-14 years			
	No. examined	(% positive)	No. examined	(% positive)	No. examined	(% positive)	No. examined	(% positive)
Low-risk (≤ 15 infected vectors collected)	13 ^b	(46.1)	2	(100)	0	—	15	(53.3)
High-risk (≥ 40 infected vectors collected)	29 ^c	(93.1)	9	(100)	3	(100)	41	(95.1)
Total	42	(78.6)	11	(100)	3	(100)	56	(83.9)

^a According to the Fisher test (two-tailed), the differences between the numbers infected in the low-risk and high-risk households were statistically significant for the 0-4 year age group ($p < 0.005$) and for the overall (0-14 year) group ($p < 0.002$).

^b Figure includes four dogs not over one year old, none of which were found to be infected.

^c Figure includes 14 dogs not over one year old, of which 12 were found to be infected.

Specifically, comparing the data in Tables 3 and 4, the Fisher test (two-tailed) showed a significant difference between the data on infected dogs and children 0–4 years old in both the low-risk households (6/13 dogs versus 0/9 children, $p=0.046$) and the high-risk households (27/29 dogs versus 8/15 children, $p<0.01$), and a marginally significant difference between the data on infected dogs and children 5–9 years old in the high-risk households ($p=0.059$). When considering the proportion of infected dogs in each household, four out of five low-risk households were found to have between 33% and 50% of their dogs infected, while 14 out of 15 high-risk households were found to have between 80% and 100% of their dogs infected.

Seven seronegative mothers (58%) were found among 12 mothers inhabiting high-risk houses. The ages of these women ranged from 36 to 75 years. In contrast, only one seropositive mother (who was 23 years old) was found among the six mothers from low-risk houses who were examined. This latter seropositive mother had a five-year-old infected son who was born in the home, which according to the moth-

er's account had been devoid of triatomine bugs for a long time.

Relationships between *T. cruzi* infection among household children under age 10 and *T. cruzi* infection among household-associated dogs are shown in Table 5. Only dogs less than four years old were considered, because all of the older animals tested were infected. The degree of association between these child and canine infections was assessed using the method described by Mott et al. (18). That is, each dog under four years old in the study households was successively considered as a reference individual; its age and infection status (whether or not it was infected) were noted; and the numbers of infected and uninfected children under 10 in its household were determined. Using this method, the ratio of the prevalence of infection among "roommates" of infected reference animals and "roommates" of uninfected reference animals may be considered an indication of the relative risk in the two groups.

TABLE 5. Relationships between *T. cruzi* infection in study household dogs and children. The figures show the ages of dogs (≤ 1 year, 2–3 years) used as reference individuals, the percentages of children found to be infected when the reference individual was infected or uninfected, and the ratio of the percentage infected when the reference individual was infected to the percentage infected when the reference individual was not infected.

Age group of dogs serving as reference individuals ^a	Proportion of infected children according to status of reference individual (RI)						Prevalence ratio (% infected where RI infected over % infected where RI uninfected)
	RI infected			RI not infected			
	No. of children infected	All children in group	(% of children infected)	No. of children infected	All children in group	(% of children infected)	
≤ 1	19	33	(57.6)	1	19	(5.3)	10.9
2–3	32	57	(56.1)	1	4	(25.0)	2.2

^a All dogs under four years old in the study households were successively treated as reference individuals.

Pursuing this approach, the presence of an infected dog less than four years old in a household was found associated with infection rates among children under 10 that were two to 11 times higher than those encountered in households with no infected dogs under four years old. Also, it was found that the younger the infected dog, the higher the infection rate among the children. Thus, when an infected dog was less than two years old, the relative probability of a child less than 10 years old being infected was almost 11 times that found when the reference dog was not infected.

The collected data suggest that the status of *T. cruzi* infection among dogs up to age four in a household could be used to estimate the likelihood of infection among children under age 10 (Table 6) (19). Thus, in our case, 91% (21/23) of the *T. cruzi*-infected study children under 10 and 83% (10/12) of the homes inhabited by one or more infected study children under 10 could have been located by finding an infected dog under age four in the house.

DISCUSSION

The results of this study show that the households studied could be grouped into two broad risk categories with respect to *T. cruzi* infection of children, risk categories defined by the density of *T. infestans* vectors infected with the parasite. These findings are similar to those derived from a study on familial aggregation of seropositivity (20), but tend not to support the dose-response relationship of *T. cruzi* infection found by another survey (2).

More specifically, vector densities resulting in capture of less than 15 infected *T. infestans* appeared to be below threshold densities for successful transmission of *T. cruzi* to children—since only three children among 20 inhabiting such homes were found to be infected, and these infections were probably acquired before insecticide applications were begun at each house (see below). In contrast, over half the children under 10 were found to be infected in homes where 40 or more infected *T. infestans* were collected. Unfortunately, the small number of nuclear families studied and the lack of houses with children harboring between 15 and 39 infected vectors preclude closer examination of this threshold phenomenon, which should be investigated through broader surveys.

TABLE 6. *T. cruzi* infection rates in study children less than 10 years old in households with and without an infected dog less than four years old.

Infected dog < 4 years old	Children infected ^a			Houses positive ^b		
	No. infected	No. examined	(% infected)	No. positive	No. examined	(% positive)
Present	21	38	(55.3)	10	11	(90.9)
Absent	2	16	(12.5)	2	5	(40.0)

^a Sensitivity 91% (21/23); specificity 45% (14/31).

^b A house was considered positive if one child living in it was infected. Sensitivity 83% (10/12); specificity 75% (3/4).

Also, considering that no vector control campaign had been carried out in the heavily infested study area, it appears noteworthy that some residents could keep the *T. infestans* population below "dangerous" levels by burning benzene hexachloride tablets and maintaining good hygienic conditions (7). This encouraging finding suggests that interruption of *T. cruzi* transmission to humans may be attainable with cheaper procedures than those currently being used. As previously indicated (2), control programs should give appropriate attention to these quantitative aspects of transmission.

One of the major obstacles to analyzing the data obtained is posed by the uncalibrated sampling method employed, whose lack of accuracy and bias toward large instars (fifth instar nymphs and adults) has been noted previously (9, 21). To minimize the lack of accuracy, an extensive effort was made to collect vectors, at the end of which it appeared that in most cases no more bugs could be captured. However, since *T. infestans* population studies conducted by means of house demolition have recorded large instar densities ranging from 100 in Argentina (22) to 90–500 (23) and even 2,300 (24) in Brazil, it appears likely that the numbers of infected bugs found in our study represent underestimates of the actual bug populations.

Also, the underlying assumption that infected vector numbers evaluated at the time of the survey represent the exposure level experienced by the cohort in the past has to be closely examined, for various reasons. Among them: (a) *T. infestans* populations tend to vary little under constant conditions, but they are subject to periodic fluctuations (25–28); (b) bug populations in the study area also seemed to depend on the frequency of domiciliary burning of

BHC tablets by householders (7), burning that was sometimes delayed by supply problems or lack of funds; (c) bug infection rates can vary according to the number and species of infected hosts available (10, 29, unpublished research); (d) thatched roofs in the study area were partially changed periodically (every one to seven years) as a consequence of natural deterioration, and shrubs were periodically burned (although the impact of these measures is unquantified, infestations are likely to persist and build up over time to previous levels.)

Despite these variables, the overall *T. cruzi* transmission pattern in the study area may be considered stable, since the human study population settled there long ago, and no dramatic ecologic or social changes or vector control campaigns have occurred. However, a few exceptions to this stable pattern were detected by retrospective interviews with residents of the study households. Among other things, the two older infected children (10–14 years old) from "low-risk" households lived in residences where conditions had changed drastically over the past 10 years because acute onset of disease in a child had moved the heads of both families to adopt bug-control measures. Hence, these two infections could have been acquired before the control measures began. Also, the one infected child under 10 residing in a low-risk household was born to a seropositive mother and lived in a house where the mother said triatomine bugs had been absent a long time and where insecticide applications were made frequently.

This boy's infection could thus constitute a congenital or breast-milk-transmitted case.

Another study finding—that uninfected individuals (most notably adult women) had resided for several years in highly infested houses—is similar to findings reported elsewhere in the literature (9, 23). This finding suggests the existence of undetermined factors, related to either sex or age, that act against acquisition of the infection. In this regard it should be noted that sex-specific asymmetries have been detected by previous experimental studies (30), and it has been reported that resistance to *T. cruzi* is greater among females than males (31).

The present survey not only reinforces previous evidence for highly efficient *T. cruzi* transmission to household-associated dogs (13), but it clearly shows that dogs in the study households had a higher probability of being infected than did children inhabiting the same house. To cite a specific example, in one low-risk household there were two infected dogs and two uninfected children in residence, both pairs being of similar ages and both having resided in the house for similar lengths of time. Complex circumstances involving relatively greater dog-vector contact, higher canine susceptibility to the parasite, and alternative avenues of transmission (licking of contaminated fur, congenital transmission, breast-milk transmission, and ingestion of infected bugs or rodents) have been said to produce such infection patterns among canine reservoirs (13).

Our data also document a strong association between the presence of infected dogs and infection among children residing in the same household. This suggests that dogs could be used in control programs for two purposes—as indicators of risk levels and

potentially infected children in specified households; and as natural sentinels to detect the introduction of *T. cruzi* into the domestic transmission cycle (especially in the surveillance phase, since the parasite is conveyed to canine reservoirs faster than to children).

Use of dogs as sentinels for *T. cruzi* has been suggested previously by different authors in Venezuela (32) and Brazil (33), where associations have been found between low prevalences of infection in dog populations and lack of domiciliary infestation by local vector species in areas under epidemiologic surveillance. It should be noted, however, that complementary data were lacking on the ways in which dogs were previously involved in active domestic transmission cycles in each of the study areas covered.

Also, in order to serve as surveillance tools, reservoirs should acquire the parasite readily and, ideally, should be more likely to contract the infection than members of the exposed human population. This may not be the case in certain areas, where the infection is transmitted by *Rhodnius prolixus* or *Panstrongylus megistus* (11, 34) and where canine prevalence rates are low. In these cases, large variances make it inadvisable to rely on small percentage decreases to indicate significant changes in transmission conditions; other parameters, such as infestation levels or the density of infected vectors, should be chosen. In all instances, however, baseline data on dog and child infection rates should be obtained locally before

starting to rely upon canine reservoirs as efficient disease sentinels. Finally, the operational feasibility of using dogs as disease sentinels obviously depends on the dog-owning habits of the local study population (in rural Argentina people typically have three or four dogs per household) (11).

The correlation found in this survey and in a previous survey from Brazil (35) between the presence of *T. cruzi*-infected dogs (or cats in the Brazil study) and high infection rates among children from the same household suggests that the presence of these animals constitutes a risk factor to the human population. Although a causal association would be hard to prove unless removal or reduction of the risk factor were followed by a decline in the disease rate of the exposed human population (36), the fact that the dogs in our study showed an age-independent persistence of parasitemia (13, 38) favors the hypothesis that there is an indirect vector-mediated causal link between infections in the animals and infection of human residents. A similar conclusion can be derived from the Brazilian survey, in which synergistic action between the presence of domestic infestation and infected pets was found to be reflected in the seropositivity rates of children studied.

Elimination of infected domestic animals, besides being desirable per se, should provide new and valuable information about those reservoirs' epidemiologic roles, especially in *P. megistus*-infested areas where a debate about the importance of dogs in this regard is

under way (6). Of course, sociologic considerations regarding dog-human relationships, as well as functions and values assigned to dogs and other pets that may influence public acceptance of disease control measures focusing on them, should be investigated locally before designing or executing any campaign to control canine or other household reservoirs (37).

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SUMMARY

A cross-sectional survey of 20 households was conducted at Amamá (Santiago del Estero Province, Argentina), an area where active *Trypanosoma cruzi* transmission was occurring and where no official insecticide applications against Chagas' disease vectors had been made. The survey sought to collect data on the numbers of *Triatoma infestans* vectors present, the intensity of *T. cruzi*

transmission to dogs and children, and relationships between the presence of infected household dogs and *T. cruzi* infection among children. Bugs were collected by two-man teams that sprayed the walls and roofs of the study houses with a synthetic pyrethrin solution. Blood samples, obtained from all members of each study household and from household pets, were tested serologically for *T. cruzi*; in addition, study subjects were tested for *T. cruzi* by xenodiagnosis.

The bug collection effort found relatively few *T. infestans* (less than 15 per house) in about a quarter of the study households, and relatively high densities (over 50 bugs) in all but one of the rest. Similarly, less than 16 *T. cruzi*-infected vectors were found in six study homes, while most of the rest yielded at least 40. Statistically significant differences were found between the *T. cruzi* infection rates of both children and dogs in "low-risk" and "high-risk" settings. Examination of the collected information also suggests that vector captures of less than 15 infected *T. infestans* appear to be below threshold densities for successful transmission of *T. cruzi* to children; that relatively cheaper control procedures than those currently employed may successfully interrupt *T. cruzi* transmission; that dogs in the study households were more likely to acquire the infection than children inhabiting the same house; that the presence of infected household dogs may increase the risk of *T. cruzi* transmission to human residents; that the status of *T. cruzi* infection among dogs can provide a basis for assessing the likelihood of *T. cruzi* infection of young children; and that dogs may serve as natural sentinels in the surveillance phase to detect introduction of *T. cruzi* into the domestic transmission cycle.

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