

A PROSPECTIVE STUDY OF THE EFFECTS OF SUSTAINED VECTOR SURVEILLANCE FOLLOWING COMMUNITY-WIDE INSECTICIDE APPLICATION ON *TRYPANOSOMA CRUZI* INFECTION OF DOGS AND CATS IN RURAL NORTHWESTERN ARGENTINA

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Abstract. Domestic dogs were used as natural sentinels to assess prospectively the long-term impact of selective, community-based spraying with pyrethroid insecticides after community-wide spraying on transmission of *Trypanosoma cruzi* in rural villages under surveillance between 1992 and 2002. In 2000 and 2002 light infestations by *Triatoma infestans* were recorded, and 523 dogs and cats were examined serologically or by xenodiagnosis. The prevalence of *T. cruzi* infection in dogs decreased from 65% at baseline to 8.9% and 4.7% at 7.5 and 10 years after sustained vector surveillance, respectively. The average annual force of infection dropped 260-fold from 72.7 per 100 dog-years at baseline to <0.3% in 2002, as determined prospectively and retrospectively from the age-prevalence curve of native dogs born during surveillance. Multiple logistic regression analysis showed that prevalent cases in dogs in 2000 and 2002 were associated positively and significantly with the peak number of *T. infestans* caught in domestic areas at the dog’s compound during its lifetime. The sustained decline in *T. cruzi* infections in dogs and cats is the result of selective, community-based insecticide spraying that kept the abundance of infected *T. infestans* at marginal levels, fast host population turnover, and low immigration rates from areas with active transmission.

INTRODUCTION

Chagas disease, caused by the protozoan *Trypanosoma cruzi*, may be the most important parasitic disease in the Americas, with an estimated 0.67 million disability adjusted life years and 17 million people currently infected.¹ *T. cruzi* is mainly transmitted by triatomine bugs, and may be transmitted vertically or by blood transfusions. In the absence of an effective vaccine, prevention currently relies on residual spraying of houses with insecticides and on screening of blood donors. *Triatoma infestans*, the only domestic vector of *T. cruzi* in Argentina and other Southern Cone countries, is the target of a regional elimination program that interrupted vector-mediated transmission to humans in Chile, Uruguay, most of Brazil and parts of Argentina.¹ Progress made in the interruption of *T. cruzi* transmission has usually been demonstrated through human seroprevalence panel surveys,^{2,3} but ethical considerations and acceptability issues pose a significant restriction to regular screening surveys of the affected human populations.

The human transmission of *T. cruzi* is most intense in the domestic environment where it may involve triatomine bugs, humans, dogs, cats, and rodents.^{4–6} Dogs are important domestic reservoir hosts of *T. cruzi* in many endemic rural areas,^{5,6} especially in northern Argentina, where they serve as one of the main blood meal sources for domestic *T. infestans*.^{7–9} Both dogs and cats showed very high infectiousness to bugs and a 10-fold higher incidence of infection than local children in an area of active transmission.⁸ Based on these findings, dogs are natural sentinels of transmission of *T. cruzi* after insecticide spraying.^{7,10} Because of the difficulties in

handling and drawing blood samples from rural domestic cats, only small xenodiagnosis surveys of cats have been done in the Chaco region and very few have been conducted elsewhere.⁵ The precise role of cats in domestic transmission of *T. cruzi* or as a bridge reservoir host linking domestic and sylvatic cycles of transmission remains unclear.

In spite of clear relative progress, vector control programs throughout Latin America still face recurrent reinfestation as the key problem threatening elimination attempts, especially in sparsely populated rural areas where official control actions are sporadic. In northern Argentina, after a community-wide one-time application of deltamethrin, and in the absence of surveillance measures, reinfestation resulted in domestic infestations by *T. infestans* returning to baseline levels within 3–7 years post-spraying and in renewed transmission.^{8,11} In the same area, a subsequent community-wide deltamethrin spraying of compounds that also covered all peridomestic structures and was followed by vector surveillance strongly reduced the abundance of *T. infestans* (especially in domestic areas) and the prevalence of *T. cruzi* in bugs and in dogs over 5 years post-intervention.^{10,12,13} New cases of infected dogs were detected when only very low-density populations of *T. infestans* or of the secondary vector *Triatoma guasayana* were present, but these new cases could also be attributed to vertical transmission.¹⁰ Surveillance activities were transferred to the communities during 1996 and monitored regularly by the research team. The long-term effects of such community-based surveillance system on the occurrence of *T. cruzi* infection in *T. infestans*, domestic dogs and cats have not been assessed.

As part of a long-term prospective study aimed at modeling the transmission dynamics of *T. cruzi* in a well-defined rural area in northwestern Argentina, here we use domestic dogs as natural sentinels of transmission of *T. cruzi* during the surveillance phase to assess the impact of selective, community-based spraying with residual insecticides and to identify the

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potential emergence of new cases from hitherto unidentified sources. For this purpose we monitored regularly infestations and bug infection in all compounds jointly with measurements of demography, prevalence, and incidence of infection in dogs before, during, and after transferring the surveillance system to the communities. *T. cruzi* infection in cats was also diagnosed in 2002. The effects of selective insecticide spraying on infestation will be presented elsewhere. Based on previous results,¹⁰ we hypothesized that during the surveillance phase new cases of *T. cruzi* infections in dogs would occur through introduction from nearby infested villages, or acquired locally through vertical and vector-mediated transmission in households already harboring infected dogs and cats. If oral infection by ingestion of wild mammals infected with *T. cruzi* occurred at all, we hypothesized that hunter dogs and cats would be at greater risk of becoming infected.

MATERIALS AND METHODS

Study area. Field studies were carried out in Amamá (27°12'33"S, 63°02'10"W) and the neighboring villages of Trinidad, Mercedes, Pampa Pozo, and Villa Matilde, in the Province of Santiago del Estero, Argentina. The communities are situated in a semi-arid hardwood thorny forest, and the history of infestation and control of *T. infestans* has been described.^{11,13} After the community-wide residual application of pyrethroid insecticides in 1992, regular triatomine surveillance was conducted by combining community participation and professional focal spraying in 1993–1995, and by householders themselves and community leaders between 1996 and 2002. Surveillance activities were described elsewhere.^{12,13} Starting in 1996, the capture of one *T. infestans* bug of any stage prompted treatment of all domestic and peridomestic areas of each compound with suspension concentrate deltamethrin (K-Othrina; Agrevo, San Isidro, Argentina) or cypermethrin (Sipertrin; Chemotecnica, Cañuelas, Argentina) diluted in water at 25 mg or 50 mg of active ingredient/meter², respectively. Local records of reported infestations and control actions were kept by community leaders. As part of a new intervention program, all houses were sprayed with pyrethroid insecticides by National Vector Control Program (NVCP) personnel in April of 2004.

Triatomine surveys. Two teams of 4 people each searched for bugs in 133 (96%) of inhabited compounds in March 2000 and in 129 (96%) compounds in October 2002. Skilled bug collectors from the NVCP searched for triatomine bugs in all bedrooms (1 person) and peridomestic areas (2 persons) from all compounds in which householders were present, using 0.2% tetramethrin (Icona, Buenos Aires, Argentina) for 30 minutes per compound.¹¹ Peridomestic structures included corrals for goats or sheep, cows or horses and pigs, chicken coops, trees where chicken roosted, storerooms, kitchens, and other possible refuges for triatomines within the area of human activity. Infestations were also monitored by domestic sensor boxes every 6 months and eventually by householders' bug collections.¹¹ All bugs were later identified to species and stage at the field laboratory and counted.¹² All live or moribund third to fifth instars and adults of *T. infestans* were individually examined for *T. cruzi* infection within 10 days of capture; *T. guasayana* and *Triatoma garciabesi* bugs were separately examined in pools of 3 insects from the same site. Bug feces were diluted with saline solution and microscopi-

cally examined at 220–400 × magnification. Minicircle-DNA based polymerase chain reaction was also applied to fecal lysates from *T. infestans* to detect *T. cruzi*.¹⁴ This PCR assay amplified a 330-bp fragment from the variable regions of minicircles of the kinetoplastid genome, and has 100% specificity.¹⁴

Domestic animal surveys. A house-to-house census of all dogs was undertaken in May 2000 and again in November 2002; the latter also included cats. The infection survey aimed for a complete census of the dog and cat populations. A questionnaire was completed for each animal on both occasions. Villagers were asked the name, age, sex, and place of birth of each pet they owned. The name of the pet's mother, and the pet's history of visiting or residing in villages outside the study area were also recorded. Additional data only requested in 2002 included the main function of the dog (hunting, herding goats, guard, and pet); where the animal usually slept at night; type of food, and for females, the number of litters and final destination of offspring. The questionnaire for cats, only conducted in 2002, additionally included main function (hunting rodents, snakes or birds, and pet), types of prey hunted and length and frequency of visits to the forest.

Dogs and cats aged ≥ 3 months were bled by venipuncture whereas younger animals (26 dogs and 6 cats) were examined only by xenodiagnosis using 20 laboratory-reared, third- or fourth-instar nymphs of *T. infestans* per animal.¹⁵ Six seropositive dogs and 6 with a borderline negative serologic result in 2000 were both bled by venipuncture and examined by xenodiagnosis in 2002. Cats were captured with a net and anesthetized using 2–5 mg/kg of tiletamine chlorohydrate and zolacepam chlorohydrate (Zelazol®, Fort Dodge Sanidad Animal, La Plata, Argentina) prior to venipuncture. Blood samples were processed and preserved as described elsewhere.¹⁰ All animal processing was conducted according to the Institutional Animal Care and Use Committee protocol No. 04223 at University of Illinois at Urbana-Champaign.

Serodiagnosis and xenodiagnosis. Each dog serum was tested for antibodies to *T. cruzi* by indirect hemagglutination assay (IHA; Polychaco, Buenos Aires, Argentina), indirect immunofluorescence test (IFAT), and enzyme-linked immunosorbent assay (ELISA) using standardized procedures.¹⁶ Each cat serum was tested for antibodies to *T. cruzi* by IHA (Polychaco, Buenos Aires, Argentina), IFAT (total anti-gamma LID, Laboratorio Inmunodiagnóstico, Buenos Aires, Argentina), and ELISA (goat anti-cat IgG HRP, Santa Cruz Biotechnology, Santa Cruz, California). Titers $\geq 1:16$ (IHA and IFAT) or an optical absorbance ≥ 0.2 (ELISA) were used as cut-off values, as determined from the distribution of serologic titers among 20 xenodiagnosis-positive and 53 xenodiagnosis-negative field dogs.¹⁶ Serum from a xenodiagnosis-positive cat seropositive for *T. cruzi* by IHA and IFAT was used as a positive control. Each serum was tested by ELISA in duplicate. Duplicates showing relative differences in optical absorbance greater than 0.2 and sera giving discordant results between serologic methods (i.e., those positive by only one method) were re-tested, with the second result considered as definitive. 'Seropositive' refers to samples reactive by at least 2 different serologic tests (among ELISA, IHA or IFAT) in any one survey. Seropositive animals were later examined by xenodiagnosis to confirm *T. cruzi* infection and isolate parasites as described previously.¹⁵ One seropositive

dog and one serologically discordant cat died before being xenodiagnosed.

'Infected' means animals having positive xenodiagnosis and/or being seropositive to *T. cruzi*. The results of serodiagnosis and xenodiagnosis were combined to calculate the composite prevalence of *T. cruzi* infection. Infected animals with lifetime residence (i.e., those that entered the household under 3 weeks of age) were considered autochthonous cases. Given that xenodiagnosis is 92–100% sensitive in pups < 1 year old,¹⁵ pups \leq 3 months old that were only tested by xenodiagnosis were assumed to be seronegative. Seronegative dogs and/or xenodiagnosis-negative pups on December 1996 or May 2000 (baseline surveys) showing seroconversion by 2 methods or a positive xenodiagnosis subsequently were regarded as incident cases of *T. cruzi*.

Data analysis. The ages of dogs as reported by their owners were checked against records from census conducted during 1992–2000, and corrected as required. As the recall for recent events is more accurate than for events in the distant past, the first record of a dog's age was assumed to be the most accurate. Demographic statistics were restricted to data collected in 2002 because the sample size was larger and the data more detailed than in 2000.

To evaluate the relationship between *T. cruzi* infection among dogs born after the community-wide insecticide spraying as determined in 2000 (number examined, $N = 202$) and in 2002 ($N = 245$) and potential risk factors, unadjusted odds ratios [OR] and 95% confidence intervals [CI] for univariate analyses were calculated by Woolf's method.¹⁷ The demographic variables considered were village of residence (categorized in 3 levels, Trinidad was grouped with Pampa Pozo and Mercedes with Villa Matilde because of their vicinity); age (in months, a surrogate of length of exposure); sex; unstable local residence (rural or urban immigrants and natives with history of travel; natives without history of travel outside of the study villages [i.e., dogs with permanent residence]); whether the dog's mother was seropositive for *T. cruzi* (2 levels); whether the dog cohabited with at least 1 dog infected with *T. cruzi* (2 levels), and hunting habit (2 levels). Reference levels were Trinidad-Pampa Pozo, the lowest age group, males, natives without history of travel, having a seronegative mother, not cohabiting with an infected dog, and no hunting habit, respectively. The entomological variables, derived from timed manual searches, sensor boxes, and householders' bug collections at each dog's compound approximately each 6 months from October 1993 to October 2000 and 2002, included the occurrence of *T. cruzi*-infected *T. infestans* bugs during the dog's lifetime (2 levels); both the cumulative (categorized in 3 levels) and peak number of *T. infestans* captured separately in 2 strata (domestic areas, and kitchens or store-rooms or ovens [i.e., nearby peridomestic sites]) during the dog's lifetime; and the occurrence of *T. guasayana* or *T. garciabesi* at domestic or peridomestic areas at the dog's compound between 2000 and 2002 (2 levels). Adjusted odds ratios and CI were estimated from maximum-likelihood logistic multiple regression analysis (Stata 9.0, College Station, TX). Regression analyses were clustered on dog house and provided robust standard errors. Only variables significant at the 10% level in univariate analyses were included in the multiple logistic regression analysis. Backward and forward stepwise procedures were used to obtain the most parsimonious model that retained independent variables at the 5% nominal sig-

nificance level. Interaction terms were then added to this model and tested for significance.

For computing prevalence and incidence of *T. cruzi*, 8 seronegative dogs and 5 seropositive dogs in 2002 that had the same seroreactivity status in 1994 or 1996 were assumed under such status in 2000, when they were not examined. In addition, 3 seropositive dogs in 1994–1996 were considered seropositive in 2000 when they were not examined. The instantaneous per capita rate of conversion from negative to positive (annual force of infection, λ) was estimated retrospectively from age-specific dog prevalence data using a catalytic model with recovery rate set to 0,¹⁸ reflecting the absence of serorecovery or specific chemotherapy. This model assumes that the incidence of infection is constant over time and independent of age; individual hosts are homogeneously exposed; no time lag occurs between infection and infectiousness, and the association between age and prevalence is observed at equilibrium. λ was estimated using nonlinear least-squares procedures (Matlab 6.3, The MathWorks, Natick, MA), and the catalytic model $p_a = 1 - \exp(-\lambda \cdot a)$, where p_a is the proportion of infected individuals within the age class whose midpoint is a . This model was used as a null hypothesis to test whether peaks in the observed age-prevalence curve deviated significantly from the 95% confidence interval for λ .

RESULTS

The prevalence of infestation by *T. infestans* in domestic sites ranged from 12–18%, 9–11% in kitchens and nearby peridomestic sites, to 22–27% in corrals in 2000 and 2002 (Figure 1A). Mean bug abundance increased with infestation prevalence, from 0.2–0.7 bugs per 0.5 person-hour per house in domestic sites or nearby peridomestic sites to 2.0–2.3 bugs per 0.5 person-hour in corrals. The prevalence of *T. infestans* infected with *T. cruzi* in domestic sites (4.5–8.8%) was less variable than in nearby peridomestic sites (0–13.7%) (Fig. 1B). Infected bugs were rare in corrals (0–0.7%). The pooled infection prevalence for peridomestic *T. infestans* was 2.5% in 2000 and 0.7% in 2002; for *T. garciabesi* it was 0% ($N = 85$) and 0% ($N = 24$); and for *T. guasayana* it was 0% ($N = 32$) and 0% ($N = 132$), respectively. *T. cruzi* DNA was detected in 91% of microscope-positive *T. infestans* and only in 3.4% of microscope-negative bugs, but not in other triatomines collected in 2002.¹⁴

A total of 218 (71%) of 309 dogs registered in 2000 and 257 (84%) of 306 dogs and 48 (86%) of 56 cats in 2002 were examined for infection. The total dog population in 2000 and 2002 was roughly of the same size (306–309 dogs), median age (2.6–2.7 years), and sex ratio (79–82% of males). In 2002, dogs were 5 times as abundant as cats, and nearly all of them were mongrels (Table 1). Sex ratios were significantly biased toward males among both dogs ($\chi^2 = 125.5$, degrees of freedom [df] = 1, $P < 0.001$) and cats ($\chi^2 = 7.4$, df = 1, $P < 0.01$). Of 119 households visited, 91% owned at least 1 dog and 37% at least 1 cat. The median age of the dog population was 3.0 years with an age distribution of 22% < 1 year, 29% 1–2 years, 27% 3–5 years, and 22% > 5 years of age. The median age of the cat population was 2.0 years with an age distribution of 18% < 1 year, 37% 1–2 years, 23% 3–5 years, and 22% > 5 years of age. Cats were born locally (82%) as frequently as dogs (73%) (Fisher's test, $P = 0.18$). Most dogs and cats were

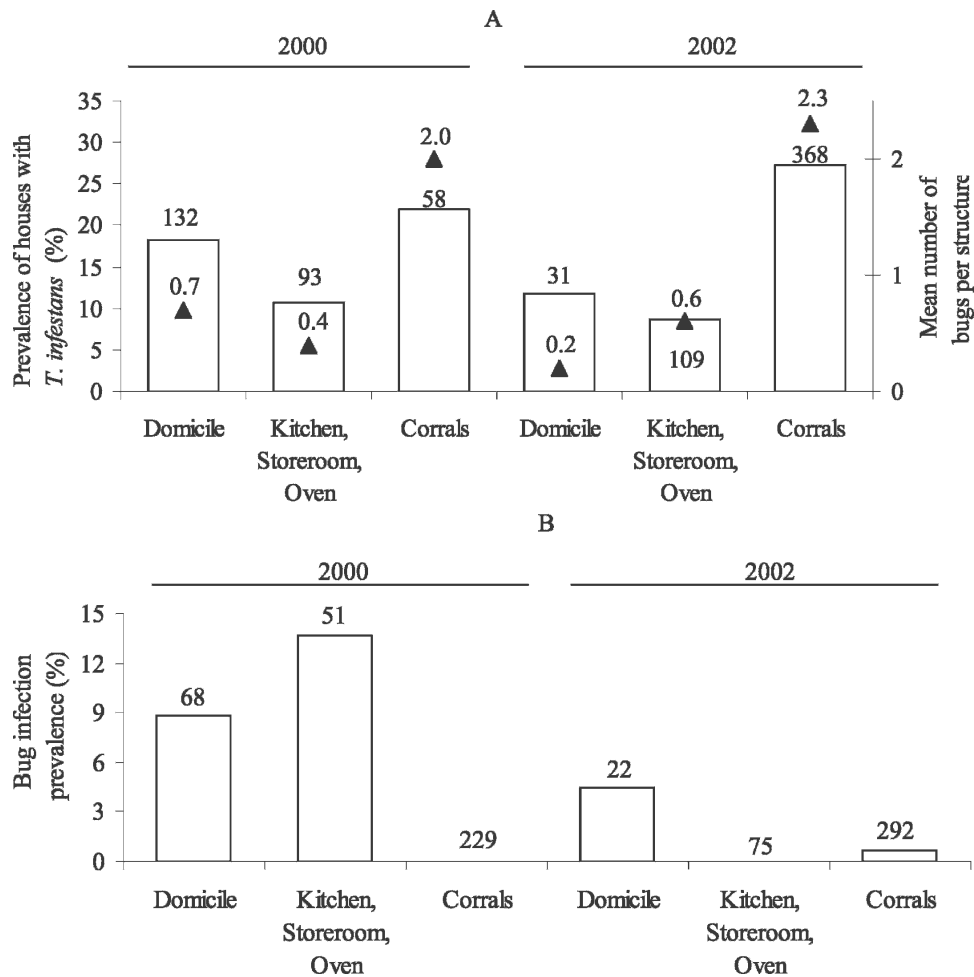


FIGURE 1. **A**, Prevalence (bars) and abundance (triangles) of infestation by *Triatoma infestans* and **B**, prevalence of *Trypanosoma cruzi* in bugs in domestic and peridomestic sites in Amamá and neighboring communities, Argentina, March 2000 and October 2002. Numbers on top of bars are bugs collected (**A**) or examined for infection (**B**).

unrestrained. One-third of dogs were used for hunting and 10% were reported to eat prey. More than half of the cats were reported to stray in the forest (56%) and to hunt rodents, birds, and snakes (54%).

The composite prevalence of *T. cruzi* infection in all dogs in 2000 (8.7%, $N = 218$) was significantly lower (Fisher's test, $P = 0.04$) than in 1996 (15.0%, $N = 237$), and was slightly higher than in 2002 (4.7%, $N = 257$) but not significantly so (Fisher's test, $P = 0.09$). The age-prevalence curve in all dogs in 2000 showed a minor peak at age 2 years (14%), a major peak at ages > 6 years (27–33%), and was 0–5% at other ages (Figure 2). The minor peak included 4 seropositive dogs, 3 of which had permanent residence and no previous serologic

result. Two of the latter were born to seropositive mothers and the remainder to a seronegative mother. In 2002, prevalence increased sharply from < 4% in dogs ≤ 3 years to 11% in dogs aged 4–5 years, to fall to < 3% in dogs 6–9 years old and then peaked at 31% in dogs ≥ 10 years (i.e., those present before the community-wide spraying campaign)(Fig. 2).

In 2002, the age-prevalence curve of native dogs was flat (range, 0–4%) in dogs ≤ 9 years of age and then rose sharply to 33% in the few dogs aged ≥ 10 years (Figure 3A). The observed infection prevalence registered for native dogs aged 4–5 years (4.3%) was significantly higher than that predicted by the catalytic model (1.5%), indicating a higher average force of infection acting during that period. The prevalence of

TABLE 1

Demographic parameters and prevalence of *Trypanosoma cruzi* infection in dogs and cats from Amamá and neighboring communities, Argentina, November 2002

Host	Mean no. per household (range)	Median age (first-third quartiles)	% males	% Native (n)*	% (n) positive by			Composite prevalence of infection (n)
					Sero-diagnosis only	Xeno-diagnosis only	Both	
Dogs	2.4 (0–8)	3.0 (1–5)	82	73 (306)	0.5 (202)	3.8 (26)	34.5 (29)	4.7 (257)
Cats	0.4 (0–3)	2.0 (1–5)	67	82 (56)	2.4 (41)	0 (6)	0 (1)	2.1 (48)

* n, number registered or examined.

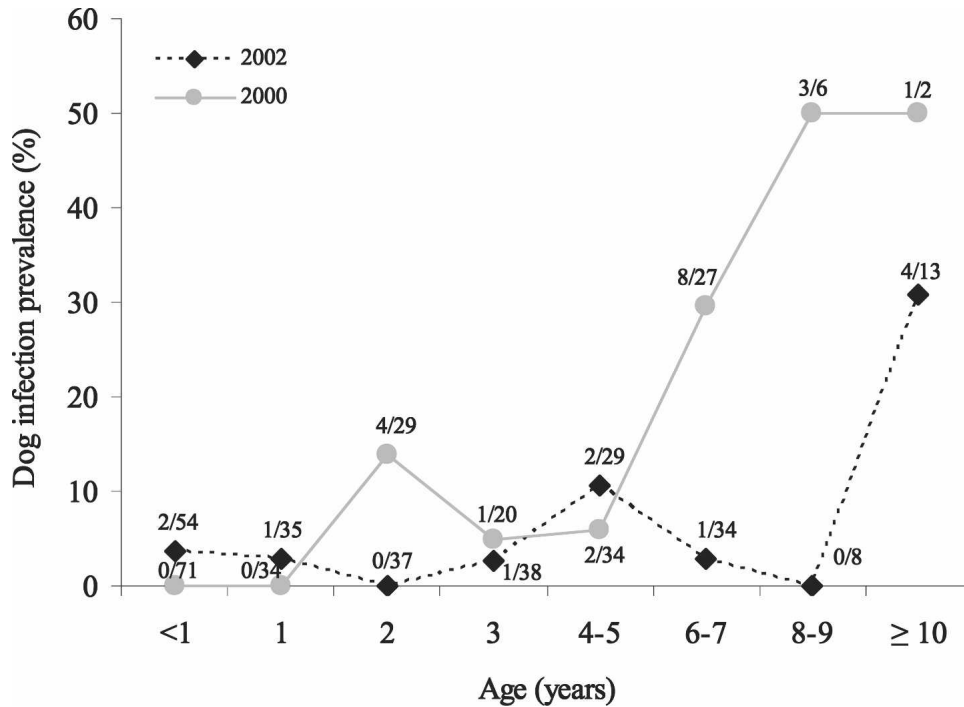


FIGURE 2. Age-specific prevalence of *Trypanosoma cruzi* infection in all dogs (native and immigrant) from Amamá and neighboring communities, Argentina, May 2000 and November 2002. Fractions are number of infected dogs to number of dogs examined for infection. Figure excludes 9 dogs of unknown age in 2002.

T. cruzi in immigrant dogs (7.9%, $N = 63$) was nearly twice as high as in native dogs (3.8%, $N = 185$) and peaked at 4–5 years of age (Fig. 3B), although the number of immigrant dogs studied was small. For dogs born after the spraying campaign (< 10 years old), the average annual force of infection was 0.28 per 100 dog-years for native dogs (CI = 0.00–0.72%), 1.41% for immigrant dogs, and 0.39% for all dogs.

Cats (2.1%) had similar prevalence of *T. cruzi* as dogs (4.7%) in 2002 (Fisher's test, $P = 0.70$) (Table 1). In cats from Trinidad and Mercedes, the infection prevalence in 2002 (6.7%) was significantly lower (Fisher's test, $P = 0.03$) than before community-wide insecticide spraying in 1992 (39.3%).¹⁹ The only cat seropositive for *T. cruzi* (ELISA 0.244 and IFAT 1:64) and xenodiagnosis-negative was a

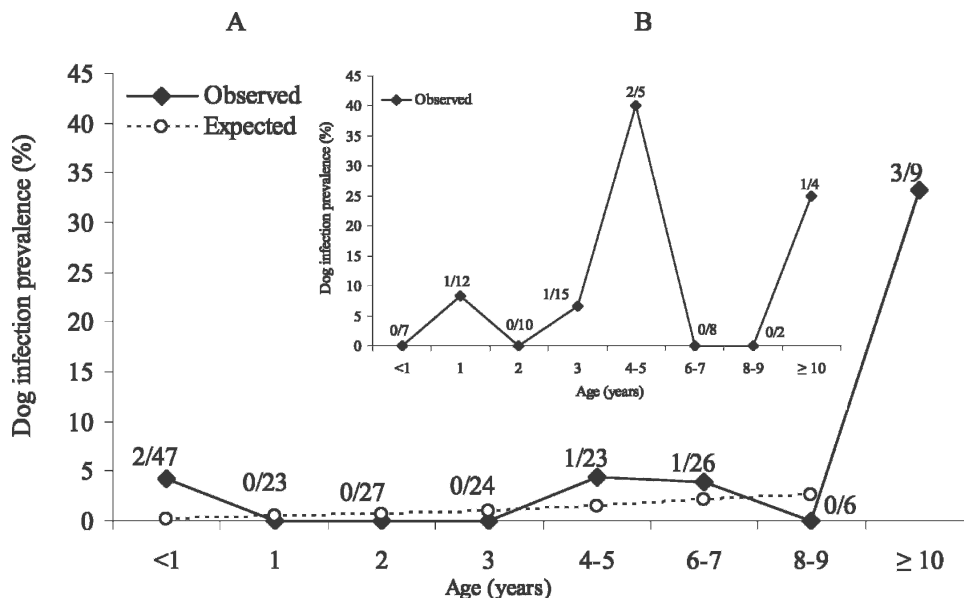


FIGURE 3. Observed (solid line) and expected (dashed line, according to a catalytic model) age-specific prevalence of *Trypanosoma cruzi* infection in 248 dogs from Amamá and neighboring communities in November 2002. **A**, Native dogs. **B**, Immigrant dogs. Fractions are number of infected dogs to number of dogs examined for infection. Figure excludes 9 dogs of unknown age.

TABLE 2

Comparison of serologic and xenodiagnosis results in dogs from Amamá and neighboring communities, Argentina, 2000–2002

Xenodiagnosis	No. tested	Seroreactivity to ELISHA, IFAT, IHA, respectively				
		+, +, +	+, +, -	+, -, +	-, +, +	-, -, -
Positive	16*	14	1	1	0	0
Negative	25	5†	1	0	0	19
Total	41	19	2	1	0	19

* Excludes one xenodiagnosis-positive 5-month-old pup that was not examined by serology.

† Includes one xenodiagnosis-negative dog in 2002 that was xenodiagnosis-positive in 2000.

5-year-old native cat with no history of traveling outside of the study area and that lived in a house with infected domestic *T. infestans* in 2002. One cat with discordant serodiagnosis (ELISA \geq 0.25 and IFAT or IHA \leq 1:8) died before it could be examined by xenodiagnosis.

Serologic titers of dogs showed a clear-cut distinction between non-reactive and reactive sera, with nearly perfect concordance between ELISA and IFAT in 2000–2002. A total of 409 sera with ELISA absorbance values $<$ 0.15 and IFAT titers \leq 1:8 were clearly negative, whereas 22 sera with ELISA \geq 0.25 and IFAT \geq 1:64 were clearly positive. In 2000, only 1 seropositive, xenodiagnosis-negative dog was ELISA $>$ 0.20, IFAT \geq 64, and IHA \leq 1:8. In 2002, only 1 seropositive, xenodiagnosis-positive dog was ELISA $>$ 0.40, IHA \geq 32, and IFAT \leq 1:8. All 8 seropositive dogs tested in December 2000 were xenodiagnosis-positive, and 10 of 11 seropositive dogs and cats tested in March or July 2003 were xenodiagnosis-positive. Concordance between xenodiagnosis and serodiagnosis in dogs was 85% (35/41) (Table 2). The

ELISA test detected all dogs with positive xenodiagnosis, while IFAT and IHA each missed 1 seropositive and xenodiagnosis-positive dog, though not the same one. One 5-year-old dog in 2002 was simultaneously positive by all serologic methods and xenodiagnosis-negative 3 times between 2000 and 2003.

The 12 infected dogs detected in 2002 were at 9 (7.6%) compounds, with 1 compound (A-109) harboring 4 infected dogs. Four infected dogs were immigrant, and 4 other infected dogs had been “censused” or found infected in 1992, when intense transmission was occurring. The remaining 4 infected dogs had permanent residence, and all of them were born at the A-109 house to 2 related mothers seropositive for *T. cruzi*. Two of the dogs were positive serologically and by xenodiagnosis in 1996 or 2000, and 2 were 5-month-old litter-mates born to a xenodiagnosis-positive mother. The A-109 compound was found heavily infested with infected *T. infestans* in both domestic and nearby peridomestic sites on 2000, and was immediately sprayed with insecticides. In 2002, when the pups were born, no bugs were collected at domestic or nearby peridomestic sites.

The relationship between *T. cruzi* infection in dogs born after the community-wide insecticide spraying and potential risk factors is shown in Table 3. In univariate analyses, *T. cruzi* infection was significantly higher for dogs with a mother seropositive for *T. cruzi*, and was associated positively and significantly with the number of *T. infestans* collected in domestic sites at the dog’s compound over its entire lifetime in both 2000 and 2002. The prevalence of infection increased significantly with age and with the occurrence of infected *T. infestans* at the dog’s compound in 2000, but not in 2002. In 2002 infection was significantly higher for dogs with unstable residence at the study villages. This variable combined data

TABLE 3

Prevalence of *Trypanosoma cruzi* infection and potential risk factors in dogs born after the community-wide insecticide spraying campaign in Amamá and neighboring villages, Argentina, May 2000 (n = 202) and November 2002 (n = 245)

Factor	2000*			2002*		
	% Infected (n)	Unadjusted odds ratio (95% CI)	Adjusted odds ratio (95% CI)	% Infected (n)	Unadjusted odds ratio (95% CI)	Adjusted odds ratio (95% CI)
Age (in months)	- (202)	1.0 (1.01–1.07)§	1.0 (1.02–1.07)§	- (236)	0.98 (0.96–1.00)	1.0 (0.97–1.01)
Village of residence						
Trinidad-Pampa Pozo	2.0 (50)	1	-	1.7 (58)	1	-
Mercedes-Villa Matilde	2.2 (45)	1.1 (0.1–18.3)	-	5.8 (52)	3.5 (0.4–34.6)	-
Amamá	8.4 (107)	4.5 (0.6–36.5)	-	3.0 (135)	1.7 (0.2–15.9)	-
Unstable residence in the study villages†						
No	5.1 (176)	1	-	1.9 (210)	1	1
Yes	10.5 (19)	2.2 (0.4–10.9)	-	11.4 (35)	6.6 (1.6–27.9)§	12.5 (2.4–65.9)§
Dog’s mother seropositivity for <i>Trypanosoma cruzi</i>						
No	2.7 (73)	1	-	0.0 (80)	1	-
Yes	21.7 (23)	9.9 (1.8–55.0)§	-	26.7 (15)	62.2 (3.1–1232.9)§	-
No data	3.8 (106)	-	-	2.7 (150)	-	-
No. of <i>Triatoma infestans</i> caught in domestic areas during dog’s lifetime‡						
0	3.7 (135)	1	-	2.5 (160)	1	-
1–9	5.4 (56)	1.5 (0.3–6.4)	-	2.7 (74)	1.1 (0.2–6.1)	-
\geq 10	33.3 (9)	13.0 (2.5–67.6)§	1.1 (1.1–1.2)§	22.2 (9)	11.1 (1.7–71.5)§	1.1 (1.0–1.2)§
Infected <i>Triatoma infestans</i> caught in dog’s house in 2000 or 2002‡						
No	3.8 (185)	1	-	2.7 (226)	1	-
Yes	26.7 (15)	9.2 (2.3–36.4)§	-	11.8 (17)	4.9 (0.9–26.3)	-

* Excludes 29 dogs born before the community-wide insecticide spraying.

† Seven seronegative dogs without residence information were excluded.

‡ Two dogs without entomological data for their houses were excluded only for these variables.

§ Significant at the 0.05 level.

showing that rural immigrant dogs had 4-fold higher infection prevalence (10.7%, $N = 28$, odds ratio, OR = 4.2, 95% CI = 1.0–18.9) than urban immigrants or native dogs (2.7%, $N = 37$ and $N = 182$, respectively), whereas the few dogs with history of travel outside of the study villages (mainly extended visits to other rural areas for > 2 weeks) had 4.6-fold higher prevalence (14.3%, $N = 14$) than dogs with no travel history (3.1%, $N = 201$). Dog infection was not significantly associated with village of residence, sex, being a hunter dog, the number of *T. infestans* collected in kitchens, ovens, and storerooms at the dog's compound over its entire lifetime, and with the capture of *T. guasayana* or *T. garciabesi* at the dog's compound (data not shown). Multiple logistic regression analysis of data clustered by house confirmed the results from the univariate analysis. In 2000 and 2002, dog infection adjusted by age was associated positively and significantly with the peak number (31–42 bugs per 0.5 person-hour) of *T. infestans* caught in domestic areas at the dog's house over its entire lifetime (Table 3). In 2000, dog infection was also positively associated with age (Wald $\chi^2 = 40.7$, 2 df, $P < 0.0001$), whereas in 2002 dog infection was positively associated with unstable local residence (Wald $\chi^2 = 26.4$, 2 df, $P < 0.0001$). The status of seroreactivity of the dog's mother could not be evaluated in the multiple regression analyses because all 80 dogs with seronegative mothers were seronegative for *T. cruzi*.

Only 1 incident case was detected by seroconversion among 133 seronegative dogs, totaling 0.26 cases per 100 dog-years. Between 1996 and 2000, 86% of all seronegative dogs at baseline were exposed to *T. infestans* in domestic or nearby peridomestic sites at least once over their lifetime. One incident case was detected among 59 dogs that had been seronegative at baseline and were re-examined 3.5 years later. This incident case was a 3-year-old dog with permanent residence that lived in a house with *T. cruzi*-infected *T. infestans*. Among infested houses with candidate dogs, the total median catch of *T. infestans* in domiciles and nearby peridomestic sites decreased from 6 bugs (first and third quartiles, 3 and 17; range 1–77) in 1996–2000 to 3 bugs (2 and 8; range 1–56) in 2000–2002. Between 2000 and 2002 none of 74 dogs seroconverted for *T. cruzi*, even though 51% of them had been exposed to *T. infestans* in domestic or nearby peridomestic sites during the past 2.5 years. The only serologically discordant dog, xenodiagnosis-negative in 2000, was seronegative by all methods and by xenodiagnosis in 2002.

DISCUSSION

Our study demonstrates a large long-term decline in the prevalence and incidence of infection in dogs and cats resulting from community-wide insecticide application followed by sustained community-based vector surveillance. Interestingly, dog prevalence was reduced to similarly low levels in the 3 clusters of villages. Based on age-prevalence curves, the annual force of infection of *T. cruzi* in dogs dropped 260-fold, from 72.7% as of 1992⁸ to 0.26–0.28%, as determined prospectively between 1996–2000 or retrospectively in 2002 for native dogs born after the community-wide insecticide spraying. For comparison, in Amamá children, the average annual force of infection estimated prospectively was 4.3% when domestic reinfestation peaked in 1992.⁸

The prevalence of *T. cruzi* in dogs showed a steady decline

from 65% in 1992 to 15% 4 years after community-wide residual insecticide spraying when no incident case in dogs was detected,^{10,15} and the abundance of infected *T. infestans* was very low.^{12,13} During the extended follow-up, the dog population exhibited minor fluctuations in size, sex, or age structure, but pup immigration rates were highly variable among years (Castañera MB, unpublished data). Mostly during 1996, the surveillance system was transferred to the community and householders undertook vector control actions by themselves, usually concentrating their efforts on domestic premises. When domestic infestations peaked in late 1997, though at rather low levels,²⁰ they were extinguished by intensified insecticide spraying within a year; nevertheless they left a signature in the age-prevalence curve recorded in 2000, when 4 autochthonous cases with no travel history were detected. These included at least 1 incident dog and 1 prevalent case compatible with vector-mediated transmission, rather than with vertical transmission. The observed infection prevalence registered in native dogs aged 4–5 years in 2002 deviated significantly from expectations, indicating a higher average force of infection acting during that period. Between 1998 and 2002 infestations mostly persisted at relatively low levels in peridomestic sites,²¹ and the finding of infected bugs was rare. The absence of seroconversions among dogs between 2000 and 2002 demonstrates interruption of vector-mediated transmission of *T. cruzi* in the presence of light infestations, mostly at peridomestic sites. We conclude that: (i) in the presence of persistent peridomestic infestations, relaxation of vector surveillance actions led to domestic recolonization and renewed focal transmission of *T. cruzi*, and (ii) the sustained decline in prevalence and the low incidence of infection in dogs during 1996–2002 was the joint result of selective, community-based vector control actions that kept the abundance of infected *T. infestans* at marginal levels, fast dog population turnover (averaging 25% per year), and low immigration rates from neighboring villages with active transmission.

The role of domestic and peridomestic structures in *T. cruzi* transmission during the surveillance phase was not homogeneous. Transmission has usually been, and still is, most intense in domestic areas colonized by *T. infestans*. Even though *T. guasayana* was found to be significantly associated with *T. cruzi* infection in dogs over 1994–1996,¹⁰ current data do not show it is a putative secondary vector in the present epidemiologic context. Infected *T. infestans* may also occur in kitchens and storerooms, known resting places for dogs and cats, whereas they are consistently rare in peridomestic structures housing goats, sheep, pigs, and birds. The recurrent strong association between the presence of an infected dog or cat and the finding of infected *T. infestans* in their house is explained by frequent host-vector contact in domestic areas and high host infectiousness to bugs.^{8,15} In the current risk factor analysis, infection in dogs was significantly associated with the peak or cumulative number of *T. infestans* collected in domestic sites at the dog's compound during its entire lifetime. More importantly, the cumulative relative densities of *T. cruzi*-infected *T. infestans* in domestic or nearby peridomestic sites from houses with autochthonous cases compatible with vector-mediated transmission were clearly marginal (≤ 1 infected bug per 0.5 person-hour) with respect to pre-spraying levels in 1992. Total bug abundance in these houses ranged from 0–13 domestic bugs per 0.5 person-hour, but nevertheless led to new infections in at least 2 dogs and 1 cat. Taking

advantage of our detailed entomological database, here we show for the first time a significant association between dog or cat infection and past measurements of *T. infestans* abundance at each house compound over the relevant exposure period for each individual dog or cat. Current results in conjunction with a prospective study of the local child population suggest that the threshold abundance of *T. infestans* below which transmission of *T. cruzi* is unlikely was very low, if any such threshold exists at all, and fraught with several sources of inaccuracy.⁸ Interruption of the domestic transmission of *T. cruzi* in highly endemic areas requires zero tolerance of light triatomine infestations.

Household clustering of *T. cruzi* infection, also detected in humans,²² was reflected in only 1 compound harboring 4 of the 12 infected dogs detected. This cluster may have resulted from: (i) vector-mediated transmission, enhanced by the co-occurrence of infected dogs or cats at the same house, which may act as sources of *T. cruzi*; (ii) vertical transmission, if infected female dogs cohabited with their offspring; and (iii) dog to dog horizontal transmission, though this was observed only in dogs experimentally infected with large infecting doses.²³ Both vector-mediated and vertical transmission (detected in field dogs by Mazza S, unpublished data) apparently occurred in the current study. The occurrence of two *T. cruzi*-infected litter-mates born to an infected mother was most likely related to vertical transmission, because no bugs were collected from domestic or nearby peridomestic sites at the pups' compounds during their lifetime. The few bugs collected from corrals were not infected with *T. cruzi*. Only these 2 pups qualify as autochthonous cases during 2000–2002, and provide clear evidence that *T. cruzi* transmission was still occurring after 10 years of regular control activities. Molecular characterization of *T. cruzi* isolates obtained from the mother and the pups may provide concluding evidence on whether both cases originated from vertical transmission.

In native dogs, the prevalence of *T. cruzi* was markedly higher only in dogs ≥ 10 years old, consistent with the very high infestations observed before the community-wide insecticide spraying. In contrast, domestic dogs reportedly coming from nearby rural villages were at a significantly higher risk of infection than native dogs or urban immigrant dogs presumably less or not exposed to *T. cruzi*-infected *T. infestans*, as observed in the early surveillance phase.¹⁰ Triatomine control activities in nearby rural villages mainly consisted of pulsed insecticide spraying promoted by vector control services and conducted sporadically by householders themselves since 1994, with no external supervision (Spillmann C, unpublished data). These communities experienced higher domestic infestation and less frequent insecticide spraying than those within the Amamá area, and local human acute cases of Chagas disease were sometimes notified by health authorities.

Given their hunting habits, domestic dogs and cats may also acquire *T. cruzi* infection through the oral route.^{5,24} Local cats and dogs typically stray in the forest and were reported or observed to kill and eat rodents and opossums, respectively. In the Amamá area, the prevalence of *T. cruzi* in opossums (*Didelphis albiventris*) fell from 32–36% in 1984–1991²⁵ to 8% in 2002–2004. During the latter period, the only other species found infected with *T. cruzi* among some 500 wild mammals and rodents captured locally was a skunk (*Conepatus chinga*) (Ceballos LA and others, unpublished data). Therefore, it is

highly unlikely that dogs and cats became infected by eating *T. cruzi*-infected wild mammals or bugs in this context.²⁴

Like dogs, cats had a much lower prevalence of *T. cruzi* than before the community-wide insecticide spraying.¹⁹ This pattern may also be explained by sustained vector surveillance, very fast population turnover, and low immigration rates. The present survey included nearly twice as many cats as the largest one ever conducted in the Chaco region, and is among the very few studies that used serologic methods to determine cat infection with *T. cruzi*. The only cat found seropositive had permanent residence in a compound where *T. infestans* bugs were found only in 2000 (uninfected) and 2002 (infected with *T. cruzi*). Although the exact moment when the cat became infected can not be assessed, it was an autochthonous case compatible with vector-mediated transmission.

The high concordance between serologic and xenodiagnosis results confirms the sensitivity and specificity of the tests applied to dogs,¹⁶ but more data are needed to assess the performance of the serologic methods applied to cats. The only dog repeatedly seropositive for *T. cruzi* and xenodiagnosis-negative since 2000 constitutes the second such case ever recorded in the study area. A plausible explanation may be an unspecific cross-reaction with *Leishmania* antibodies,²⁶ because this dog came from a distant area with recently reported human cases of leishmaniasis.²⁷ In the Amamá area, no human or dog showed apparent signs of *Leishmania* infection.

Our study demonstrates the effectiveness of dogs as sentinel animals for domestic and peridomestic risk of *T. cruzi* infection during the surveillance phase,^{4,8,28} provided relevant demographic data are collected to exclude introduced or vertically-transmitted cases. Unlike cats, domestic dogs comply with all the ideal characteristics of an animal species as a sentinel of *T. cruzi* transmission: they are susceptible to and have a measurable response to the infectious agent; have a defined territory that overlaps the area to be monitored; are accessible, easy to enumerate and capture, and have an adequate population size that allows representative samples.²⁹ The crucial feature, however, is that dogs become infected with *T. cruzi* before the children cohabiting with them.⁸

The very low prevalence of *T. cruzi* among dogs (and cats) reflects the steady decline in domestic transmission in historically highly endemic rural areas that have undergone continuing vector surveillance. This situation can not be generalized to a district or province-wide level, as indicated by the increased relative odds of *T. cruzi* infection for rural immigrant dogs or for dogs with travel history in comparison to permanent resident dogs. This suggests that active transmission occurred in nearby rural villages under more sporadic, unsupervised vector surveillance. The heterogeneous distribution of infected dogs implies that some dogs make a disproportionate contribution to transmission, which is expected to increase the basic reproductive number (R_0) of infection and, consequently, the efforts required to eliminate the pathogen. House compounds with infected dogs and cats should be targeted for enhanced surveillance, as bugs are more likely to become infected there and trigger local transmission. Sustained, permanent vector surveillance is crucially needed in high-risk areas for Chagas disease such as the Gran Chaco.

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