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The potential of canine sentinels for reemerging *Trypanosoma cruzi* transmission

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Abstract

Background—Chagas disease, a vector-borne disease transmitted by triatomine bugs and caused by the parasite *Trypanosoma cruzi*, affects millions of people in the Americas. In Arequipa, Peru, indoor residual insecticide spraying campaigns are routinely conducted to eliminate *Triatoma infestans*, the only vector in this area. Following insecticide spraying, there is risk of vector return and reinitiation of parasite transmission. Dogs are important reservoirs of *T. cruzi* and may play a role in reinitiating transmission in previously sprayed areas. Dogs may also serve as indicators of reemerging transmission.

Methods—We conducted a cross-sectional serological screening to detect *T. cruzi* antibodies in dogs, in conjunction with an entomological vector collection survey at the household level, in a disease endemic area that had been treated with insecticide 13 years prior. Spatial clustering of infected animals and vectors was assessed using Ripley's K statistic, and the odds of being seropositive for dogs proximate to infected colonies was estimated with multivariate logistic regression.

Results—There were 106 triatomine-infested houses (41.1%), and 45 houses infested with *T. cruzi*-infested triatomine insects (17.4%). Canine seroprevalence in the area was 12.3% (n=154); all seropositive dogs were 9 months old or older. We observed clustering of vectors carrying the parasite, but no clustering of seropositive dogs. The age- and sex-adjusted odds ratio between

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seropositivity to *T. cruzi* and proximity to an infected triatomine (< 50m) was 5.67 (95% CI: 1.12 – 28.74; $p=0.036$).

Conclusions—Targeted control of reemerging transmission can be achieved by improved understanding of *T. cruzi* in canine populations. Our results suggest that dogs may be useful sentinels to detect re-initiation of transmission following insecticide treatment. Integration of canine *T. cruzi* blood sampling into existing interventions for zoonotic disease control (e.g. rabies vaccination programs) can be an effective method of increasing surveillance and improving understanding of disease distribution.

Keywords

Dog; ELISA; *Triatoma infestans*; *Trypanosoma cruzi*; Sentinel surveillance; Spatial analysis

INTRODUCTION

Chagas disease is a zoonotic and vector-borne disease caused by *Trypanosoma cruzi*, and is arguably the most important parasitic disease in the Americas (World Health Organization, 2008), where 8 million people are infected (World Health Organization, 2014). The parasite is transmitted primarily by triatomine bugs and control programs are focused on reducing or eliminating vector populations through insecticide application (Dias, 2007). The insecticide effect passes after some months and, after this occurs, reinfestation with the vector often initiates (Cecere et al., 2006), and re-emergence of *T. cruzi* transmission may occur (Delgado et al., 2011). Vector reinfestation is a serious problem for Chagas control; a systematic review concluded: “reinfestation of dwelling by native vector species is common, spatially widespread, and temporally persistent” (Abad-Franch et al., 2011). Recurrence of this process of vector reinfestation and transmission re-emergence may threaten current achievements of Chagas disease control programs. Animal sentinels could be used to detect early re-emergence of transmission and thereby signal the need for additional control activities to prevent transmission to humans.

T. cruzi can infect a variety of animals. Several mammal species have been identified as reservoirs and/or carriers of the parasite. Wild mammals such as opossums, raccoons, skunks, armadillos, mice, rats and other rodents have been reported as *T. cruzi* reservoirs (Alvarado-Otegui et al., 2012; Brown et al., 2010; Pinto et al., 2006). Among domestic animals, dogs have been implicated in several studies as reservoirs of *T. cruzi* (Cardinal et al., 2008; Fujita et al., 1994; Gürtler et al., 2007; Jimenez-Coello et al., 2010) and may play an important intermediary role in the transmission of the parasite to humans (Gürtler et al., 2005), particularly in those *T. cruzi* systems where there is a high degree of intradomiciliary transmission. Infection of dogs with the parasite occurs by vectorial transmission as well as by the oral route (dogs eating insects or infected mammals) (Cardinal et al., 2006; Reithinger et al., 2005), triatomine insects show a preference to feed on dogs (Gürtler et al., 2009a), and dogs tend to live more proximate to humans compared to other animals, such as cattle or poultry. These facts make it likely that, in the process of re-emergence of *T. cruzi* transmission, dogs may become infected earlier than humans. Therefore, identification of infected dogs and the factors that promote or hinder their infection can be useful for

preventing human infection. Here we investigate the potential role of dogs as early indicators of reemerging transmission.

Many species of triatomine bugs are able to transmit *T. cruzi* (Zeledón and Rabinovich, 1981), but in the southern department of Arequipa, Peru, *Triatoma infestans* is the only insect vector for the parasite (Levy et al., 2006). In the 1990s the Ministry of Health (MoH) of Peru began systematic campaigns of insecticide spraying in areas affected by triatomines (Dias and Schofield, 1999; Náquira and Cabrera, 2009). These campaigns, like many in Latin America, were conducted without comprehensive information on the extent of *T. cruzi* transmission in the area (Dias and Schofield, 1999). In the district of La Joya, Arequipa, our study team found that parasite transmission was interrupted in 1995 (Delgado et al., 2011). However, in the years leading up to 2008, dwellers of La Joya reported vector reinfestation in the area. In 2008, the MoH conducted an insecticide spraying campaign and, in collaboration with the MoH, our study team captured 2,070 triatomine vectors in human dwellings and 7,487 triatomine vectors in peridomestic areas. Remarkably, 96.7% of *T. cruzi*-carrying triatomine insects were captured in peridomestic areas (unpublished data). We also found that out of more than 500 humans 18 years or younger, only 5 were infected in this area (Delgado et al., 2011). These findings suggest that after vector reinfestation was established in La Joya, *T. cruzi* transmission was initiated in the peridomiciliary areas, where several domestic animal species, including dogs, can be found.

The objectives of this study were to (i) estimate the seroprevalence of *T. cruzi* in domestic dogs in an area where *T. cruzi* transmission was reemerging after insecticide spraying, and (ii) to characterize the spatial association of infected dogs with the distribution of *T. cruzi*-infected *T. infestans*.

MATERIAL AND METHODS

Ethical statement

All protocols that involved animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Universidad Peruana Cayetano Heredia.

Location and study population

The study was conducted in a village within the district of La Joya, a district located 30.7 km west (Euclidean distance) of the city of Arequipa, the capital of the department of the same name. La Joya sits at an altitude of 1,617 m, has an average temperature of 18°C (range 10°C to 35°C), humidity ranging between 20% and 85%, and a marked rainy season from December to March. The residents of La Joya raise domestic animals for various uses. In addition to livestock and poultry, many families also keep dogs for protection and/or company, but not for hunting, which might expose dogs to *T. cruzi*-infected animals. The study area is historically endemic for Chagas disease; in 1995 the MoH conducted indoor residual spraying with insecticide for triatomine vector control. Over the 13 years that followed, insect reinfestation in the area led to a re-emergence of *T. cruzi* transmission (Delgado et al., 2011), and the area was treated again in 2008.

Study design and data collection

Canine data—In July 2008, in coordination with the MoH we conducted a cross-sectional study in a village within La Joya district. A house-to-house animal census to collect age and sex of dogs and to assign a unique code to each animal was performed. The inclusion criteria for dogs were (i) to live in a house within the study area, (i) to not have severe health problems, and (iii) permission of the dog's owner to participate in the study. Following the census, field veterinarians collected blood samples (1–3 cc) by phlebotomy from the saphenous or cephalic veins. Blood samples were processed in our local laboratory in Arequipa. Samples were centrifuged, and serum was aliquoted and stored at –20 C. Sera was sent to Lima and serological diagnosis, using an in house enzyme-linked immunosorbent assay (ELISA) previously standardized for dogs in Peru, was performed in the Laboratorios de Investigacion y Desarrollo (Research and Development Laboratories) in Universidad Peruana Cayetano Heredia. The ELISA results were compared to results from an in-house Western-blot. For the in-house ELISA we used an epimastigote alkaline extract (EAE) as antigen from an isolate of *T. cruzi* strain Y. The concentration of antigen fixed in the plate wells was 3.5ug/ml. In each ELISA plate we included the field samples, 7 negative controls and 2 positive controls. The cut off to determine seropositivity was plate specific. We considered seropositive samples those with titers 3 standard deviations higher than the mean of the negative controls in at least two of three runs.

We used a Western blot test with trypomastigote excreted/secreted antigen (TESA-blot) from an isolate of *T. cruzi* strain Y. We followed standard processes for Western blot testing with the following modifications. We diluted the sample at 1/100 with PBS-Tween 0.3% with milk at 1% and the conjugate Anti-dog IgG, peroxidase labeled, KPL was diluted at 1/2,000 with the same diluent. The revealing gel was chromogenic solution of 4 chloro-alpha-naphthyl with peroxide at 30%.

Entomological data—In August 2008, the MoH carried out a vector control campaign to spray the residual pyrethroid insecticide Deltamethrin 5% wettable powder (K-Othrine, Bayer) at 25mg a.i. per m² in the district of La Joya, Arequipa, Peru. During the application of the insecticide in houses triatomine vectors are repelled by the chemical and emerge from their hiding places. In collaboration with the MoH, field workers followed the sprayers and collected triatomine insects from the study village. Each house was searched for triatomine insects for one person-hour. The rooms or animal pens where the search took place were given a unique code for identification. For purpose of analysis, all vectors caught in a single room or animal pen were assumed to comprise a single colony. Identified vectors were sent to our Zoonotic Disease Field Lab in the city of Arequipa for parasitological analysis. Infection with *T. cruzi* was determined by direct microscopic observation as described previously (Gürtler et al., 1998b; Levy et al., 2006). Briefly, a few drops of triatomine rectal contents were obtained by application of abdominal pressure, diluted with saline solution, and compressed between a glass slide and cover slip. The presence of mobile parasites in all microscopic fields of a 22mm × 22mm slide cover was evaluated at 400× magnification.

Spatial data—The geographic location of the 258 houses in the study area, the rooms within those houses, and the animal pens were mapped comparing satellite imagery in

Google Earth™ (Google, n.d.) to field maps drawn by our fieldworkers. Data from dogs were linked to the house location and triatomines data were linked to the animal pens or rooms where they were found. We implemented a Geographic Information System (GIS) in ArcGIS 9.0 (ESRI, 2013) to link dog and triatomine data and to select datasets for analyses.

Statistical methods

Household locations were mapped to visualize the distribution of sampled, unsampled, and seropositive dogs. The location of pens and rooms where entomological surveys were conducted were also mapped to visualize the distribution of surveyed enclosures, infested enclosures, and *T. cruzi*-infected colonies. The ratios of the spatial intensity of seropositive dogs versus seronegative dogs and positive insect colonies versus negative insect colonies were evaluated with quartic kernels with bandwidths of 100 meters, the average length of a city block in La Joya. Spatial clustering of seropositive dogs and infected insect colonies was assessed using the K function difference (Waller and Gotway, 2004).

Subsequently, we used a GIS to determine which dogs lived within 20m, 30m, 40, 50m, and 60 of *T. cruzi*-infected triatomines. This dichotomous variable (to live or not to live proximate to triatomines infected with *T. cruzi*) under different distances was used as an explanatory variable to examine serologic status of dogs using multivariate logistic regression. We used Akaike and Bayesian Information Criteria (AIC, BIC) for model selection and to determine which of the distance classes used to define the covariate of interest represented the best fit to the data. The total number of triatomines and the number of infected triatomines found in the dog's household were also evaluated as explanatory variables for dog seropositivity to *T. cruzi*. Age and sex of dogs were included in the models as adjusting variables. Multivariate logistic regressions and exploratory spatial data analyses were performed with R (Team, 2013), and all statistical analyses were evaluated with a significance level of 0.05.

RESULTS

One hundred and seventy-five dogs lived in the study area and were distributed across 150 households. We obtained blood samples from 154 dogs distributed in 127 households (sampled dogs = 88.0%; household participation rate = 84.7%). Dog age was negatively associated with participation in blood sampling. The sampled dogs were distributed over the study area and participation did not appear to be aggregated in a particular area in this study. Of 258 households in the study area, 253 (98.1%) participated in the entomological survey.

We found 19 seropositive dogs (seroprevalence = 12.3%), all of them 9-month old or older, 106 triatomine-infested houses (41.9%), and 45 houses infested with *Trypanosoma cruzi*-carrying triatomine insects (17.8%). We found insects in 280 different points in the study area (e.g. guinea pig pen, bedroom). The range of insect counts in these points was 1 – 1414 and the median was 3. The overall distribution of collected, examined, and *T. cruzi*-carrying triatomines by developmental stage can be observed in Table 1. Seropositive dogs and insect colonies infected with *T. cruzi* were concentrated in certain parts of the study area (Figures 1), and there was some overlap of seropositive dogs and *T. cruzi*-infected colonies. An analysis of spatial odds ratios showed that seropositive dogs occur with greater intensity in

certain areas of the field of study compared to the intensity of the seronegative dog population (Figure 1). Also in Figure 1 we observe that the areas of higher risk for dogs are close to or contain infected vector colonies. The clustering level measured by the K-function difference showed no clustering of seropositive dogs (Figure 2). By contrast, insect colonies infected with *T. cruzi* did show significant clustering between 10 and 75 meters (Figure 3). The characteristics of the dogs according to their proximity (within 50m) to *T. cruzi*-infected triatomines are presented in Table 2. Nineteen of the 154 dogs were seropositive (12.3%) and the seroprevalence was higher in dogs within 50 meters of positive triatomines. Dogs proximate to *T. cruzi*-infected triatomines were significantly younger than dogs far from infected triatomines (3 years, 1 month vs. 4 years, 6 months). Overall most dogs were males (73.4%); this proportion did not vary between dogs proximate or far from infected triatomines.

We restricted the regression analyses to dogs 9-months old or older. Based on the AIC and BIC values (Table 3) we report the results of the regression models when a distance of 50m was used as the cutoff for proximity between a seropositive dog and an infected colony. The association between canine seropositivity and presence of positive triatomines within 50 meters of the house was higher in the multivariate analysis (OR=5.82; 95%CI: 1.12 – 28.32) compared to the univariate analysis (OR=4.69; 95%CI: 1.03 – 21.44), but in both cases the effect size was important and the association was statistically significant (Table 4). Dog age was positively associated with seropositivity in the univariate and multivariate model, but this association was only statistically significant in the univariate model (Table 4) where one year of difference in canine age was associated with a 12% increase in the odds of being seropositive. The number of triatomines or number of positive triatomines captured in the dog's household did not show any association with the serologic status of the dog, either in the univariate or in the multivariate model.

DISCUSSION

We document here a relatively high seroprevalence of *T. cruzi* in dogs in a peri-rural area of Arequipa, Peru. There is a wide range in canine seroprevalence in The Americas, and the seroprevalence of 12.3% estimated in the study village in La Joya, Arequipa lays in the middle of that range (Balan et al., 2011; Bonfante-cabarcas et al., 2011; Cardinal et al., 2007; Gürtler et al., 2007; Kjos et al., 2008; Pineda et al., 2011; Rosypal et al., 2007), and was very similar to that estimated by Tustin et al. for humans in the same study area (13.4%) (Tustin et al., 2012). The study area was treated with insecticide in a little-documented campaign in 1995, 13 years before this study was conducted, but only one of the seropositive dogs was 13 year or older, ruling out that the high seroprevalence in dogs was the result of vector transmission prior to 1995. We also considered congenital transmission, but the data suggest that this route of transmission did not explain the seroprevalence level in the study area: all the seropositive dogs were 9-months or older and the odds of being seropositive increased with time after that age, a pattern we would not expect if there was significant congenital transmission (Gürtler et al., 2007). The canine data suggest that vectorial transmission was ongoing in the study area when we conducted the cross-sectional study in 2008.

Conversely, the human population studied in the same area in 2008 tells a different story (Delgado et al., 2011). Of 125 children under-5 sampled in the study area in La Joya, Arequipa during the same year only 1 was seropositive (Delgado et al., 2011) and the child was born from a seropositive mother. That finding itself could have been enough to identify the area as free of vectorial transmission after the insecticide treatment of 1995. We detected significant clustering of *T. cruzi*-infected colonies, but we did not detect clustering of seropositive dogs, similar to what Delgado et al. (Delgado et al., 2011) found in seropositive humans of the same study area. Also, their results suggest that infection in humans occurred before spraying in 1995, and was not spatially related to the location of infected *T. infestans* in recent times. In contrast, we estimate that the odds of being seropositive to *T. cruzi* for dogs living proximate to infected triatomines was 5.7 times greater than the odds for dogs living far from infected triatomines (after adjusting for age and sex). Moreover, the strong spatial association observed in this study suggests recent transmission in those areas where seropositive dogs were found. Even though only nineteen seropositive dogs were found in the area, the results in the multivariate analysis were statistically significant, suggesting this relationship would hold in similar settings. The process of reemergence of *T. cruzi* could occur under different post-spraying scenarios. These scenarios might range from the full reemergence of *T. cruzi* transmission after neither insects nor infected animals remain in the area, to the reemergence of transmission after elimination of insects, but no control of potential animal reservoir or infected humans. In our study we believe we are in the latter scenario of reemergence of the parasite since no control measures on animal reservoirs or infected humans were recorded by the MoH in the previous years.

Other studies have suggested the potential of dogs as proxies to determine human seroprevalence (Estrada-Franco et al., 2006) and to detect *T. cruzi* transmission in rural (Castañera and Lauricella, 1998; Tenney et al., 2014) and major urban areas (Tenney et al., 2014). Specifically, Castañera et al. (Castañera and Lauricella, 1998) studied the use dogs as indicators of residual insecticide spraying efficacy, linking the reduction of age-seroprevalence with time after the 1st, 2nd and 3rd round of spraying, similar to what is expected when children under 5 years are sampled after insecticide spraying campaigns. Their canine study population was comprised of hunting dogs, seropositive females of reproductive age, and long-range roaming dogs. All these characteristics of a canine population would make it more difficult to use dogs as sentinels for reemerging transmission of *T. cruzi*. In our study area dogs do not hunt, ruling out transmission from eating an infected wild mammal, they do not roam around—or if they do are limited in a very restricted area. The dogs therefore serve as environmental tasters for their surroundings—they find and presumably eat bugs, become infected, and the relationship between age and seroprevalence in the canine population there after carries a useful signal that provides information on the potential for transmission to humans. Dogs in Peru, given their limited range of movement, are thereby as useful as dogs in shelters in Texas in terms of their sentinel value for active transmission of *T. cruzi* (Tenney et al., 2014). In addition, in the study area dogs are typically acquired as puppies and rarely change owners; age is therefore a good surrogate for time of exposure, increasing the usability of the age-seroprevalence structure to make inferences about transmission.

Our study considered proximity to *T. cruzi*-infected triatomines as an explanatory factor for seropositivity in dogs, but the potential of dogs for transmitting *T. cruzi* to nearby triatomine colonies should not be overlooked. The potential role of dogs in the transmission of *T. cruzi* has been suggested by several studies based on seroprevalence results. Serological canine surveys in Paraguay and Argentina have found a higher prevalence of infection in dogs than in other domestic animals (Cardinal et al., 2008; Fujita et al., 1994; Gürtler et al., 2007). As mentioned above, Gürtler et al (Gürtler et al., 2007) found that *T. infestans* strongly prefers to feed on dogs and cats compared to chickens, which would increase the chances of vectors acquiring the parasite in areas with infected dogs. Another study from Argentina reported an association between infected dog ownership and increased risk of Chagas disease in children (Gürtler et al., 2005). Also, it has been reported that dogs living within the same area can be infected with *T. cruzi* from different discrete strains of the parasite revealing the versatility of dogs as reservoirs of the parasite (Enriquez et al., 2013).

The number of bugs and the number of triatomine insects infected with *T. cruzi* have been described as risk factors for infection with *T. cruzi* in humans (Feliciangeli et al., 2007; Guhl et al., 2005; Hunter et al., 2012; Levy et al., 2011) and dogs (Castañera and Lauricella, 1998). However, in our analysis, these factors showed neither an important effect size coefficient nor a statistically significant association with seropositivity in dogs. The study village is considered a rural town, but the distribution of households does not follow the typical scattered pattern of other rural areas (Song and Kulldorff, 2003). The houses in the study village have an area of 300m² on average and are in close proximity to each other, grouped into blocks that are arranged similarly to urban blocks. The rustic house materials and the poor conditions of the houses are probably not an efficient barrier to the mobility of triatomines between houses (Barbu et al., 2013). A triatomine captured in one house may have originated and fed on humans or animals living in neighboring houses. The strong association between seropositive dogs and triatomine insects infected with *T. cruzi* found in areas surrounding the dog's household reinforces this hypothesis.

There were some confounders that should be evaluated to assess the validity of our estimates. The association between non-participation in sampling and increasing age of dogs could be explained by aggressive behavior in older dogs as reported by field workers, preventing handling and taking of blood samples. Such differential participation by age could have biased our results; however, given the positive association found between age and seropositivity, if all dogs had been sampled, we would expect to see an even greater association, assuming that participation rate is not associated with seropositivity. The high proportion of male dogs in the sample population is explained by anecdotal conversations with local residents who state they prefer male dogs, as they are considered to be better guard dogs and they do not have to worry about unexpected pregnancies in their animals, a common problem in poor areas of Peru where animals are rarely or never spayed. The possible interaction between time of exposure to positive triatomines and proximity to positive triatomines was assessed and age was used as a proxy of time of exposure. We did not observe changes in the estimated coefficients and the addition of this interaction term did not improve the multivariate model (Likelihood Ratio Test post-estimation: $p = 0.6221$). In addition, we may have missed some positive triatomines since we did not conduct PCR to

detect *T. cruzi* infection in triatomines, and the ELISA we used to estimate canine seroprevalence was compared to a TESA-blot assay, a confirmatory test for inconclusive results. We found a concordance of 0.87 using the Kappa test. However, the lack of a gold standard and estimates of sensitivity and specificity are limitations for our results.

Indoor insecticide spraying is the primary intervention for vector control of Chagas disease in the Southern Cone (Cortez et al., 2010; Dias, 2007) and the detection of positive triatomine insects has been used to identify high-risk areas (Guhl et al., 2005). In low-resource areas, which comprise most of the areas affected by Chagas disease, dogs could also be used to detect areas with ongoing or reemerging transmission and prioritize actions for early intervention. Sanitary programs focused on dogs, such as anti-rabies vaccination campaigns, have the advantage of congregating many dogs in a few points where MoH personnel have access to them. Taking blood samples during these campaigns and determining infection in dogs with pooled blood samples could potentially be a more cost effective use of resources.

If dogs are used as sentinels, considerations for handling infected dogs have to be in place to guarantee reduction of transmission. Given the importance of dogs in the epidemiology of Chagas disease and their reservoir competence for *T. cruzi* (Balan et al., 2011; Gürtler et al., 1998a; 1998b) attempts to disrupt the transmission cycle in dogs should be considered. Some of these attempts have found increased mortality of *T. infestans* that feed on dogs treated with topical insecticide (Amelotti et al., 2012; Gürtler et al., 2009b) and reduced feeding and engorging rate in *T. infestans* that live with dogs using deltamethrin-treated collars (Reithinger et al., 2005; 2006). Overall, these studies found high variability in their results and suggest that measures to reduce transmission from and to dogs need more development. Culling infected dogs has been used with the intention of controlling other vector-borne diseases, specifically visceral leishmaniasis (Ribeiro, 2007); however, there is little scientific evidence to support the effectiveness of that practice (Nunes et al., 2008) and currently there is no evidence that it would be useful to control *T. cruzi* transmission.

3.6. Conclusions

Thirteen years after a little-documented vector control campaign, *T. cruzi*-seroprevalence in dogs was high, suggesting ongoing parasite transmission in the area. By contrast, the patterns of seroprevalence in humans in the same area might be interpreted as evidence that vector-borne transmission had been halted. Dogs may be useful sentinels to detect re-emergence of *T. cruzi* transmission and should be considered in surveillance programs. *T. cruzi*-seropositive dogs are spatially associated with the presence of *T. cruzi*-infected *T. infestans* colonies. *T. cruzi* transmission foci detection, determined by canine serological surveys, could direct targeted insecticide treatment and prompt the testing of potential canine reservoir hosts in the proximity of positive vector colonies. Afterwards, prevention efforts could be directed at interrupting *T. cruzi* transmission from dogs, as the primary reservoir hosts, to humans. Prior studies have explored topical insecticides as means of halting the vector-host (canine) transmission process, though with variable results. Finally, given the strong spatial association between seropositive dogs that are near *T. cruzi*-infected triatomines, canine serological surveys nested within other programs such as rabies-vaccine

campaigns, should be evaluated as cost-effective strategies to identify areas where vectorial transmission is reemerging or high-risk areas within endemic zones (Cardinal et al., 2007).

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Figure 1.
Spatial odds ratios of seropositivity for *Trypanosoma cruzi* in dogs in La Joya, Peru, 2008.
UTM coordinates.

Figure 2.
Clustering of seropositive dogs in La Joya, Peru, 2008.

Figure 3.
Clustering of infected vector colonies in La Joya, Peru, 2008.

Table 1Counts of collected, examined, and *T. cruzi*-carrying triatomines by developmental stage

Stages	Collected	Microscopy examined	<i>T. cruzi</i> -carrying
1 st nymph instar	733	N/A	N/A
2 nd nymph instar	1061	830	62
3 rd nymph instar	2345	2125	286
4 th nymph instar	1568	1483	247
5 th nymph instar	1593	1580	425
Adult female	638	638	191
Adult male	883	882	331
Total	8821	7538	1542

Table 2

Characteristics of dogs and serology by proximity to *T. cruzi*-infected triatomines in La Joya, Peru, 2008

	Dogs	50m of	Dogs > 50m of	Total	p-value
Seropositivity, n	17 (15.2)		2 (4.8)	19 (12.3)	0.08 ^a
Age in years, mean	3.1 (0.3)		4.6 (0.6)	3.5 (0.3)	0.01 ^b
Sex, n (%)					
Males	33 (78.6)		80 (71.4)	113 (73.4)	0.37 ^a
Female	9 (21.4)		32 (28.6)	41 (26.6)	

Evaluated with ^a Chi-squared and with ^b t student tests; n: count; %: percentage; y SE: standard error

Table 3

Akaike and Bayesian Information Criteria for different proximity cut-offs

	20m	30m	40m	50m	60m ^a
AIC	116.24	115.91	113.53	110.51	105.34
BIC	128.36	128.03	125.65	122.63	113.89

^a 60m offered the lowest AIC and BIC but was omitted from the final model due to perfect prediction.

Table 4
Associated factors to seropositivity to *T. cruzi* in dogs 9-month old or older in La Joya, Peru, 2008

	Unadjusted Model			Adjusted Model ^a		
	OR	95% CI	p-value	OR	95% CI	p-value
Live 50m of an infected triatomine	4.69	1.03 – 21.44	0.04	5.82	1.12 – 28.32	0.03
Age in years	1.12	0.97 – 1.29	0.01	1.16	0.99 – 1.43	0.06
Sex (males)	1.08	0.36 – 3.28	0.88	1.15	0.34 – 3.65	0.82
Number of triatomines captured in the household	0.99	0.98 – 1.01	0.47	1.00	0.98 – 1.01	0.57
Number of <i>T. cruzi</i> -infected triatomines in the household	1.01	0.83 – 1.46	0.49	1.14	0.85 – 1.46	0.37

95% CI: 95% confidence interval.

^a Adjusted by sex, age