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INTRODUCTION

Chagas disease is the most important parasitic disease of the Americas. It causes an estimated 0.67 million disability adjusted life years.¹ Eighteen million people are currently infected, with up to 100 million at risk of the disease. It is caused by Trypanosoma cruzi and is characterized—in its chronic stage—by extensive myocarditis, cardiac arrhythmia, and ultimately death.² Although T. cruzi can be transmitted congenitally or by blood transfusions, most transmission occurs through skin lesions or the mucosa when blood-sucking triatomine bugs deposit their infective feces on the host during or after feeding.²

Because a vaccine against T. cruzi does not exist and because Chagas disease is associated with socio-economic conditions—and poor housing and deficient domestic hygiene in particular—control strategies such as the Southern Cone Initiative³ have mainly focused on insecticide-spraying of houses to control the triatomine vector. The Southern Cone Initiative has been remarkably successful and dramatically reduced the prevalence and incidence of T. cruzi infections in participating countries, namely Argentina, Bolivia, Brazil, Chile, Paraguay and Uruguay, by controlling domestic infestations by Triatoma infestans, the main vector of T. cruzi.⁴ However, concerns exist that after the apparent program success, community participation, and surveillance may be waning, especially as the Ministries of Health have limited financial resources and may be prompted to redirect funds to other infectious diseases such as AIDS or dengue. As monitoring and spraying activities wane, several reports have indicated that, in many endemic rural areas, houses are being re-infested with peridomestic T. infestans and other sylvatic triatomine bugs,⁵ with transmission rates potentially returning to pre-control levels within 3–5 years after cessation of control activities.⁷

Although T. cruzi may be transmitted by a range of triatomine bug species, dogs have consistently been shown to be the main domestic reservoir throughout the endemic range of Chagas disease.⁸ Mathematical modeling predicts that elimination of infected dogs from a household with infected people could be sufficient to almost extinguish transmission of T. cruzi, barring reintroduction of infected dogs or bugs.⁹

We recently showed that single exposure of bugs to dogs wearing deltamethrin-treated dog collars (DTDCs) significantly reduced feeding success of triatomine bugs.¹⁰ The aim of the work presented here was to test the impact of continuous exposure of T. infestans to dogs wearing DTDCs on long-term bug feeding success, survival, and fecundity in experimental huts under natural climatic conditions.

MATERIALS AND METHODS

Study site and protocol. The trial was carried out in a field station run by the Argentinean National Vector Control Program in Punilla, Province of Córdoba (31°14′ S, 64°28′ W) between March 2004 and September 2005. Study location, design of experimental huts, and experimental set-up where previously described.¹⁰

Briefly, 10 mongrel dogs, small to medium sized (7–18 kg) and > 1 year of age, were used in the experiments; all were obtained locally and were vaccinated against rabies, parvovirus, and leptospirosis as well as de-parasitized with mebendazole against possible canine helminth infections before the start of the trial. Seven collared and three uncollared (negative controls) dogs were kept in separate kennels made of chicken wire and a roof, approximately 10 m apart, within the fenced compound. Dogs were fed the same mixture of dog food and given continuous clean water daily. The bugs used in the experiments were T. infestans, second and third generation from bugs collected in Formosa, Argentina. Before the first dog exposure, bugs were starved for 2–3 weeks.

Twenty adult bugs (i.e., 10 male and 10 female), 10 fourth-instar nymphs, and 15 fifth-instar nymphs were introduced into the experimental huts before attaching collars (day 0). Tested DTDCs were impregnated with 40 mg/g deltamethrin.
(Scalibor; Intervet, Boxmeer, The Netherlands), which currently are registered in Europe to protect canines from tick and sand fly bites. According to the manufacturer, the collars continuously release the lipophilic deltamethrin insecticide, which spreads in the dermal secretions over the dog’s body within 2 weeks of application. The manufacturer claims that collars are effective for up to 6 months, which we confirmed in a previous study when measuring the insecticidal content of canine hair after 6-month collar application.10

Every night, dogs were walked to the experimental huts, located approximately 30 m from the kennels, and always stationed individually in the same hut. Dogs were exposed overnight (2000 to 0600 hours). Huts were dismantled at day 14, and then at 30-day intervals, with bugs being collected manually to count the number of bugs and eggs present inside the huts.11 Bugs were counted according to their life stage and scored as either dead, lost (i.e., absence of bug cadaver), or alive, and, if alive, as having evidence of a blood meal. Blood-fed bugs found at the second time-point had certainly fed during the first 14 days because all bugs were initially unfed; however, the time when subsequent blood meals were taken could not be identified definitively, because the digestion of a blood meal can take up to 90 days after engorgement.12 Blood meal size of fed bugs was also monitored semi-qualitatively (i.e., by a subjective classification of the size of the bug abdomen after blood ingestion), with bugs being scored as not fed, little fed, medium fed, and engorged.12 Retrieved bugs were color-marked to distinguish present bugs from those that had melted from previous time-points.11 All live bugs and unhatched eggs were returned to source experimental huts 2 days after collection.11

The negative control dogs were exposed to bugs every day as described above to adjust for any background changes in bug survival dynamics over time. Data analysis. General linear models13 in STATA 9.0 (College Station, TX) were used to test whether there was a significant ($P < 0.05$) effect of DTDC on bug feeding success, survival, and egg production in relation to the negative controls (i.e., by analysis of deviance, specifying binomial errors, of the log odds that a bug fed, engorged, or survived, was reduced as a result of the collars). Analyses were carried out on the whole time series, adjusting for time-point and bug life stage and testing for interactions between collar effect and either time or life stage. A Poisson regression was used to compare the number of eggs laid, adjusted by the number of females present. All analyses were clustered by dog to provide robust standard errors. A $\chi^2$ test was used to compare proportions of eggs laid that hatched or bugs that had molted from one-time-point to the next. A Kaplan-Meier survival analysis was carried out to estimate the median survival time of bugs exposed to collared or control dogs.

RESULTS

A total of 120 and 280 bugs were introduced into experimental huts and exposed to control and collared dogs, respectively. No significant difference was observed in the proportion of bugs that fed on exposure to control or collared dogs (Figure 1; Table 1). There was also no evidence that the proportion of fed bugs was different at tested time-points or varied with life stage. However, the proportion of engorged bugs that had fed on collared dogs was significantly lower than on control dogs over the entire 126-day observation period (odds ratio [OR] = 0.40; 95% confidence interval [CI], 0.26–0.63; $P < 0.001$) as well as at all five time-points tested (Figure 1; Table 2). There was no evidence that this effect on bug engorgement varied with life stage.

At day 196, all bug populations exposed to collared dogs had gone extinct, whereas the three bug populations exposed to control dogs were still alive (average reduction in bug survival: 74%; 95% CI, 57–85; $P < 0.001$; Figure 2; Table 2). Of bugs not found alive, 92/400 (23%) were found dead (Table 1). At all tested time-points, bug survival was significantly lower for bugs exposed to collared dogs than for those exposed to control dogs (Table 2). There was no evidence that this effect on bug survival varied with life stage.

Both the proportions of bugs lost or dead throughout the study period were significantly different when comparing bugs exposed to dogs fitted with DTDCs or controls (OR = 2.99; 95% CI, 1.58–5.66; $P < 0.01$ and OR = 3.26; 95% CI, 1.99–5.34; $P < 0.001$, respectively). The effect of collars on the

![Figure 1](https://via.placeholder.com/150)

**FIGURE 1.** Proportion of bugs fed or engorged when exposed to dogs fitted with DTDCs or controls. Bugs were scored quantitatively and qualitatively as described in the Materials and Methods section.

<p>| Table 1 |</p>
<table>
<thead>
<tr>
<th>Number of bugs and eggs observed during the study period</th>
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<tbody>
<tr>
<td>Control dogs</td>
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<tr>
<td><strong>Number of bugs</strong></td>
</tr>
<tr>
<td>Exposed*</td>
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<tr>
<td>Fed†</td>
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<tr>
<td>Engorged‡</td>
</tr>
<tr>
<td>Dead§</td>
</tr>
<tr>
<td>Lost¶</td>
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<tr>
<td><strong>Number of eggs</strong></td>
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<tr>
<td>Laid**</td>
</tr>
<tr>
<td>Developed to first instar††</td>
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</table>

Total number of bugs exposed to control and collared dogs at day 0 (*); cumulative proportion of bugs that fed (†) or were engorged (‡) during the study period; proportion of founder bugs that were collected dead (§); or were unaccounted for (lost) (¶); proportion of fourth- and fifth-instar nymphs that molted to adult stages during the study period (††); total number of eggs laid during the study period (**) and proportion that developed into first-instar nymphs (††).
proportion of bugs lost or dead varied significantly with time (Table 2). Additionally, the effect of the collars on the proportion of bugs dead was also shown to vary with life stage: whereas there was no significant effect of DTDCs on fourth-instar nymph mortality, mortality of fifth-instar nymphs (OR/95% CI, 3.21; 1.16–8.89; P < 0.05) and male (OR/95% CI, 15.05; 4.29–57.75; P < 0.001) and female adults (OR/95% CI, 3.65; 1.94–6.87) was significantly higher when exposed to dogs with DTDCs compared with controls.

A Kaplan-Meier survival analysis of the data accounting for the bugs that were lost between time-points showed that the median survival time of bugs exposed to control and collared dogs was more than 196 (interquartile range: 161 to >196) and 126 (102–196) days, respectively (log-rank test, P < 0.001).

The average number of new eggs laid per surviving female was 3.91 and 5.93 for populations exposed to collared and control dogs, respectively. Exposure to collared dogs was found to have reduced the number of eggs observed per female bug alive, by an average of 36% throughout the study period (95% CI, 19–49%; P < 0.001; Figure 3). Three of seven bug populations exposed to collared dogs failed to produce any eggs beyond day 77 because of the absence of an adult female.

The proportion of fourth- and fifth-instar founder bugs that molted was not significantly different between bug populations exposed to collared dogs or control dogs (Table 1). However, bug populations exposed to control dogs yielded a significantly greater proportion of eggs that produced first-instar nymphs than populations exposed to collared dogs (Yates-corrected χ² test; OR = 3.15; 95% CI, 1.34–7.69; P < 0.01; Table 1; Figure 3). For both bug populations, none of the recruited first-instar nymphs molted to second instars during the course of the study.

No dogs had visible side effects from wearing DTDCs; potential locomotive and dermal side effects can occur, but subside on collar removal.14
Here we report, for the first time, results of such xenoinoculation. Using experimental conditions mimicking ecological characteristics found in a typical domestic environment in Chagas disease-endemic areas, we show that bugs continuously exposed to dogs wearing DTDC have reduced survival and fecundity, ultimately causing the extinction of exposed bug populations. The results reported here contrast our previous experiments where a single exposure of bugs to dogs fitted with DTDC failed to impact bug survival in a significant way, and confirms that exposure to collars significantly reduces bug feeding success (i.e., the proportion of bugs that fully engorge). Furthermore, for the first time, we show that bugs exposed to collared dogs have reduced fecundity and molting rates. This is likely to be caused by the observed reduction in the degree of engorgement as well as sub-lethal effects caused by the insecticide exposure.

A caveat of our study is that we only can interpret our data in terms of bug survival rather than mortality. Throughout the 196-study period, some bugs were lost (i.e., they were neither recovered alive nor dead after nightly exposure to dogs). Because of the experimental setup, we can exclude that these missing bugs escaped from the huts. It is likely that these bugs were preyed on by dogs, as previously observed, especially if affected (e.g., paralysis) by sub-lethal doses of the insecticide. However, we do not know when these bugs went missing between time-points and whether the bugs were alive or dead. It is clear that whereas the former would have overestimated bug mortality because of DTDCs, the latter would have underestimated it. Although the proportion of lost adult females was higher in collared (i.e., 47/70) than control dogs (i.e., 18/30; not significant) throughout the study period, we do not think that this may have averseally impacted bug reproduction—on the contrary, bug density has been shown to be negatively correlated with number of eggs per female. Molt ing rates would have been underestimated if molted bugs were among the bugs that were lost.

It is envisaged that the epidemiologic impact of tested DTDCs on canine (and possibly human) Chagas disease incidence could possibly be 2-fold. First, in terms of triatomine bug abundance, which will affect contact rates of uninfected or infected bugs to susceptible or infectious dogs and humans, continuous exposure to DTDCs leads to reduced bug survival, bug molting rates, and bug fecundity. Second, in terms of T. cruzi transmission dynamics, it is expected that DTDCs will significantly affect transmission of T. cruzi to and from dogs, the main domestic reservoir, by reducing bug engorgement (bugs with smaller blood meals take longer to defecate and thus would be less likely to transmit T. cruzi parasites). Crucial to the effectiveness of the intervention will be that there will be no dramatic change in bug host preference caused by the use of the collar—this will have to be tested before mass use of DTDCs in an operational intervention campaign. Also, any strategy to mass use DTDCs should include monitoring of potential insecticide resistance to deltamethrin, because continuous exposure could potentially lead to the development of such resistance in bugs.

Tested collars could not only be a potential tool to prevent Chagas disease in endemic areas of human disease but also in areas where human disease is scarce and Chagas disease is mainly of veterinary importance (e.g., in the United States, where autochthonous canine T. cruzi infections are regularly reported). Whether collars could be a sole alternative to
the costly monitoring and spraying of houses with residual insecticide is debatable.28 Of interest is that deltamethrin-treated collars have also been shown to protect dogs from sand flies and zoonotic visceral leishmaniasis,14 a disease endemic throughout Latin America and that also causes significant human morbidity and mortality because domestic dogs—as for Chagas disease—are the main reservoir.29 It could be envisaged that these collars could be implemented as an integrated control tool for both diseases, thereby increasing the intervention’s cost-effectiveness.

To maximize effectiveness of DTDCs on triatomine bugs, the timing of collar application on dogs may be crucial. Because of their comparatively high reproductive potential (e.g., an engorged T. infestans female may lay up to four eggs daily for 3–6 months), T. infestans populations are known to readily recover from insecticide applications.3 Ideally, collaring would probably have to be implemented by the onset of spring when 1) bugs recommence feeding and reproducing, 2) T. cruzi transmission increases steeply, and 3) pyrethroid insecticides are expected to be more effective because of the inverse relationship between temperature and insecticide efficacy.30 However, this will have to be confirmed in future operational studies and will vary throughout the T. infestans range.

Although good, extensive clinical data are scarce,30 deltamethrin is a comparatively safe insecticide, with reportedly few systemic side effects that are usually reversible (e.g., neuroexcitation, gastroenteritis).31 It is heavily used in agriculture and public health to control crop pests or vectors of disease, and the consensus is that the gain in reduction of disease morbidity and mortality caused by its use outweigh the potential adverse events experienced by people exposed to it.32 As with any potentially toxic product, care should be taken to minimize required contact (e.g., not letting young children play with the collar, touch it, or put it in their mouth).

In conclusion, our work presented here shows that tested DTDCs could be a promising tool to protect dogs from T. cruzi infection and thereby reduce transmission of Chagas disease to humans, as long as exposure of bugs to dogs wearing DTDCs is continuous (e.g., typically T. infestans would feed on dogs every 3–5 days in the summer season)12,15. Our work presented here also provides a platform to investigate whether our findings can be extrapolated to other domestic Chagas disease vectors (e.g., Rhodnius prolixus, T. dimidiata, and T. pallidipennis) and to investigate whether the observed effects are of sufficient magnitude to impact bug densities and/or T. cruzi transmission in field conditions.

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