Reithinger, Richard; Ceballos, Leonardo; Stariolo, Raúl; Davies, Clive R; Gürtler, Ricardo E; (2006) Extinction of experimental Triatoma infestans populations following continuous exposure to dogs wearing deltamethrin-treated collars. The American journal of tropical medicine and hygiene, 74 (5). pp. 766-771. ISSN 0002-9637 DOI: https://doi.org/10.4269/ajtmh.2006.74.766

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EXTINCTION OF EXPERIMENTAL TRIATOMA INFESTANS POPULATIONS FOLLOWING CONTINUOUS EXPOSURE TO DOGS WEARING DELTAMETHRIN-TREATED COLLARS

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Abstract. Dogs are domestic reservoir hosts of Trypanosoma cruzi, the etiological agent of Chagas disease. We evaluated the effect of deltamethrin-treated dog collars (DTDCs) over time on the population dynamics of Triatoma infestans, a main T. cruzi vector. Forty founder bugs of mixed life stages were allowed to colonize mud-thatched experimental huts and exposed continuously to either uncollared control dogs (N = 3) or dogs wearing DTDCs (N = 7) for a period of up to 196 days. When compared with bugs exposed to control dogs, bugs exposed to collared dogs were shown to have reduced feeding success (odds ratio [OR] = 0.40; 95% confidence interval [CI], 0.26–0.63; P < 0.001) and lower survival (OR = 0.15; 95% CI, 0.08–0.29; P < 0.001); in fact, all of the bug populations exposed to collared dogs became extinct 77–196 days after study initiation. Bugs exposed to DTDC-wearing dogs were also shown to have a lower fecundity (i.e., number of eggs produced per live female bug: OR = 0.64; 95% CI, 0.51–0.81; P < 0.001) and molting rate to first-instar nymphs (OR = 0.32; 95% CI, 0.13–0.75; P < 0.01) than those bugs exposed to control dogs. DTDCs could represent a novel tool to prevent and control canine and (hence) human Chagas disease.

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 wavering, 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,5,6 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 10 fifth-instar nymphs were introduced into the experimental huts before attaching collars (day 0). Tested DTDCs were impregnated with 40 mg/g deltamethrin.
TRIATOMA Collared dogs

Retrieved bugs 25/451 (5.5%) 8/438 (1.8%)††

Blood 321/348 (92.2%) 255/268 (95.1%) P < 0.01

†† 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.

† 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.

†‡ The both 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. 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.

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 bugs fed was different at tested time-points or varied with life stage. However, the proportion of engorged

<table>
<thead>
<tr>
<th>Exposed*</th>
<th>120</th>
<th>280</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed†</td>
<td>321/348 (92.2%)</td>
<td>255/268 (95.1%)</td>
</tr>
<tr>
<td>Engorged‡</td>
<td>193/348 (55.5%)</td>
<td>130/268 (48.5%)</td>
</tr>
<tr>
<td>Dead§</td>
<td>25/120 (20.8%)</td>
<td>67/280 (23.9%)</td>
</tr>
<tr>
<td>Lost¶</td>
<td>81/120 (67.5%)</td>
<td>214/280 (76.4%)</td>
</tr>
<tr>
<td>Molted#</td>
<td>9/60 (15.0%)</td>
<td>15/140 (10.7%)</td>
</tr>
<tr>
<td>Number of eggs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laid**</td>
<td>451</td>
<td>438</td>
</tr>
<tr>
<td>Developed to first instar††</td>
<td>25451 (5.5%)</td>
<td>8438 (1.8%)</td>
</tr>
</tbody>
</table>

Table 1

Number of bugs and eggs observed during the study period

---

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 (††).
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 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

### Table 2

<table>
<thead>
<tr>
<th>Study days</th>
<th>Engorgement (95% CI)</th>
<th>Survival (95% CI)</th>
<th>Death (95% CI)</th>
<th>Loss (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>0.52 (0.34–0.90)†</td>
<td>0.29 (0.14–0.69)</td>
<td>0.29 (0.14–0.69)†</td>
<td>3.33 (1.61–6.92)†</td>
</tr>
<tr>
<td>47</td>
<td>0.39 (0.19–0.79)*</td>
<td>0.43 (0.19–0.73)†</td>
<td>0.43 (0.19–0.73)†</td>
<td>3.12 (0.92–10.60)†</td>
</tr>
<tr>
<td>77</td>
<td>0.33 (0.14–0.75)†</td>
<td>0.34 (0.14–0.73)§</td>
<td>0.34 (0.14–0.73)§</td>
<td>2.94 (0.94–9.19)§</td>
</tr>
<tr>
<td>102</td>
<td>0.30 (0.12–0.68)‡</td>
<td>0.31 (0.12–0.68)†</td>
<td>0.31 (0.12–0.68)†</td>
<td>2.88 (0.58–14.32)§</td>
</tr>
<tr>
<td>126</td>
<td>0.00 (0.00–0.00)‡</td>
<td>0.00 (0.00–0.00)‡</td>
<td>0.00 (0.00–0.00)‡</td>
<td>2.53 (0.08–76.35)§</td>
</tr>
<tr>
<td>161</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>196</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Odds ratios derived from logistic regression analyses clustered by dog and adjusted for bug life stage. Significance levels: * \( P < 0.05 \), † \( P < 0.01 \), ‡ \( P < 0.001 \), and § \( P > 0.05 \) (not significant).
Here we report, for the first time, results of such xenointoxication. 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 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 adversely 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 engorge- ment (bugs with smaller blood meals take longer to def- ecate 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

**FIGURE 3.** Number of eggs and bugs present in huts where bugs were exposed to either control (A) or collared (B) dogs. Total number of eggs observed at time t includes the number of new eggs at time t plus the number of eggs remaining from time t minus 1. Number of adult females at time t minus 1 is represented by the broken lines; number of first-instar nymphs that developed from eggs is represented by the solid lines.

**DISCUSSION**

Dogs are the main domestic reservoirs of human T. cruzi infection, with reported infection prevalences as high as 84%. Several studies in Argentina have shown that 1) triatomine bugs feed preferentially on dogs compared with humans (i.e., the ratio of dog to human blood meals detected in bug guts is 2.3–2.6 times the ratio of number of dogs to hu- mans per household); 2) infected dogs are 12 and 100 times more infectious than infected children and adults, respectively; and 3) bug population size and bug T. cruzi infection rates are both positively associated with the number of infected dogs per household. Hence, T. cruzi infection rates in humans are positively associated with the average number of dogs per household. Reducing or eliminating T. cruzi-infected dogs from households was predicted to extinguish transmission of T. cruzi to humans. Because it is acknowledged that residual spraying of peridomestic annexes is rarely effective, the Panamerican Health Organization recommended testing xenointoxication (i.e., the application of insecticide on domestic animals) as a possible strategy to control peridomestic triatomine populations.
the costly monitoring and spraying of houses with residual insecticide is debatable.\textsuperscript{28} Of interest is that deltamethrin-treated collars have also been shown to protect dogs from sand flies and zoonotic visceral leishmaniasis,\textsuperscript{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.\textsuperscript{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 \textit{T. infestans} female may lay up to four eggs daily for 3–6 months), \textit{T. infestans} populations are known to readily recover from insecticide applications.\textsuperscript{3} Ideally, collaring would probably have to be implemented by the onset of spring when 1) bugs recommence feeding and reproducing, 2) \textit{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.\textsuperscript{30} However, this will have to be confirmed in future operational studies and will vary throughout the \textit{T. infestans} range.

Although good, extensive clinical data are scarce,\textsuperscript{30} deltamethrin is a comparatively safe insecticide, with reportedly few systemic side effects that are usually reversible (e.g., neuroexcitation, gastroenteritis).\textsuperscript{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.\textsuperscript{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 \textit{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 \textit{T. infestans} would feed on dogs every 3–5 days in the summer season).\textsuperscript{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., \textit{Rhodnius prolixus}, \textit{T. dimidiata}, and \textit{T. pallidipennis}) and to investigate whether the observed effects are of sufficient magnitude to impact bug densities and/or \textit{T. cruzi} transmission in field conditions.

Received September 19, 2005. Accepted for publication January 13, 2006.

Acknowledgments: The authors thank Isaac Ochoa, Héctor Zamora, and Delmi Canale for logistical support and are grateful to Dr. Rupert Quinell and two anonymous reviewers for comments on the manuscript.

Financial support: This study was funded by the Sir Halley Stewart Trust (Cambridge, UK). R.E.G. is a member of the CONICET’s Researcher’s Career Program. This study was supported by awards from the Agencia Nacional de Promoción Científica y Técnica (Argentina), and University of Buenos Aires. Ricardo E. Gürtler and Leonardo Ceballos were supported by the National Institutes of Health/National Science Foundation Ecology of Infectious Disease program award ROI TW05836 funded by the Fogarty International Center and the National Institute of Environmental Health Sciences (Uriel Kitron and Ricardo E. Gürtler, co-PI).

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