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Vaccination with *Trypanosoma rangeli* reduces the infectiousness of dogs experimentally infected with *Trypanosoma cruzi*

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Abstract

The goal of this work was to test the efficacy of the vaccination with *Trypanosoma rangeli* in dogs. Mongrel dogs received three subcutaneous injections of fixed *T. rangeli* epimastigotes at 6-week intervals. Such immunisation induced antibodies against *Trypanosoma cruzi*. While both control and immunised dogs developed detectable parasitemia, this was lower and shorter in vaccinated animals. Interestingly, feeding of *Triatoma infestans* nymphs on vaccinated and chronically infected dogs led to a sharp reduction in the rate of bug infection. These results suggest that it might be possible to reduce the vectorial parasitemia through vaccination of dogs. As dogs are known to play a major role in the domestic cycle of *T. cruzi*, this might represent a strategy to reduce parasite transmission to humans.

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1. Introduction

Chagas disease occurs throughout Mexico and Central and South America and continues to pose a serious threat to health in many countries of these regions. People infected with the trypanosome parasite may suffer cardiac, gastrointestinal, or neurological damage, although disease manifestations vary widely from one endemic area to another [1]. In Chagas' disease, as in other parasitic diseases, no effective vaccine for humans is yet available in spite of the numerous experimental approaches performed with different antigenic materials, ranging from subcellular fractions to recombinant antigens or even plasmid DNA encoding antigens of *Trypanosoma cruzi* [2–7]. In our laboratory, we have developed a model of mice

vaccination with *Trypanosoma rangeli*, a non-pathogenic *Trypanosomatidae* also infecting people in Latin America [8], that shares many antigens with *T. cruzi* [9,10]. The immunisation schedule elicited B and T specific responses to *T. cruzi*, as well as a particular pattern of cytokines and a strong reduction in the mortality rate among infected mice. Indeed, more than 95% of vaccinated mice survived a lethal *T. cruzi* infection and displayed significantly reduced parasitemia during the acute phase [11], associated with high levels of specific antibodies and elevated serum levels of IL-12 and IFN- γ , low levels of proinflammatory cytokines (IL-6 and TNF- α) and normal levels of IL-10, together with the absence of histopathological lesions [12].

Considering those results and taking into account the important role of dogs as reservoirs in the domestic cycle of Chagas' disease, the goal of the present work was to test the efficacy of the vaccination with *T. rangeli* in dogs.

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2. Material and methods

2.1. Animals

Six 2-month-old mongrel dogs from the same litter weighing 10–12 kg were used. They have been previously immunised following the usual dog immunisation schedule (parvovirus, coronavirus, hepatitis, parainfluenza, adenovirus, hydrophobia, distemper, leptospirosis). An anti-helminthic treatment was also carried out at day 45 to guarantee optimum sanitary conditions. The dogs had not been infected with *T. rangeli*; the presence of this parasite has not been reported in Argentina yet, which can be demonstrated by the absence of antibodies against this parasite. The dogs were treated following the ethical standards for animal testing and experimentation. When the experiments were finished, the dogs received Benznidazol at a dose of 5 mg/(kg day) during 30 days.

2.2. Parasites

T. rangeli epimastigotes were cultured as described in Basso et al. [13]. Parasites were harvested in the exponential phase of growth, washed three times in PBS at 1000 g for 20 min at 4 °C, fixed with glutaraldehyde 0.1% during 15 min at room temperature and 15 min at 4 °C, and washed again. Just before immunisation, epimastigotes (2×10^9 parasites/mL) were emulsified in PBS containing 500 µg/mL of saponin (Sigma, St. Louis, MO, USA).

T. cruzi trypomastigotes of the *Tulahuen* strain were maintained by weekly subinoculations in Balb/c mice. Blood-stream trypomastigotes used for challenging inoculations were obtained by cardiac puncture on day 14 p.i.

2.3. Immunisation and infection of dogs

Dogs were immunised and infected essentially as previously described in the mouse model [9]. Briefly, they received subcutaneous injections of 0.5 mL of the *T. rangeli*–saponin emulsion containing 1×10^9 parasites at days –90, –45 and –15, before being infected at day 0 by intraperitoneal inoculation of 10,000 blood trypomastigotes of *T. cruzi*/kg. Dogs receiving PBS instead of *T. rangeli* emulsion and similarly infected served as controls.

Parasitemia was regularly measured by the method of Pizzi [14]. Briefly, a 5 µL sample of blood was put between a slide and a coverslide (18 mm × 18 mm) and the parasites were counted in 25 fields at 400×. Parasitemia was calculated through the following formula:

$$\text{Parasites/mL} = \frac{N \times np \times 200}{n}$$

where N is the number of total fields, np the number of counted parasites, and n is the number of counted fields

When direct examination yielded negative results, the Strout method was applied. Xenodiagnosis was also per-

formed at days 45 and 60 post-infection (p.i.), with 20 and 30 nymphs (stage IV) of *T. infestans* per dog, respectively. *T. cruzi* parasites were looked for in faeces of nymphs examined individually at days 30 and 60 after feeding. The bugs were obtained from our insectary and they were free of infection with *T. cruzi* and *T. rangeli*.

The levels of specific antibodies against *T. cruzi* were measured by indirect haemagglutination (Wiener Lab) and ELISA (antigen from Wiener Lab and Peroxidase conjugate antidog IgG from Sigma), 15 days after each immunisation dose and at different times-points p.i. In ELISA, the serum of three healthy dogs was used as negative control to calculate a cut-off value (mean of negative controls ± 2S.D.).

At days 0, 25 and 70 p.i., electrocardiographic studies were performed. When the parasitological, serological and electrocardiographic studies were finished, the dogs were treated with Benznidazole at a dose of 5 mg/(kg day) for 30 days.

The weight of dogs was measured every week.

2.4. Statistics

Mann–Whiney U -test was used to determine the statistical significance of the differences in parasitemia between groups. The Odds ratio was calculated to determine differences among the number of positive nymphs through xenodiagnosis performed in control and vaccinated dogs.

3. Results

Mortality in the dogs groups was not observed throughout the experiment. Table 1 shows the parasitemia in both groups of dogs. While both control and immunised dogs developed detectable parasitemia, vaccinated dogs presented lower parasitemia. These findings were statistically significant when the overall results for both groups were compared as a whole at days 15, 25 and 35 p.i. ($\alpha = 0.025$, Mann–Whitney U -test). Moreover, the parasitemic phase was shorter among immunised dogs, since no blood parasites were detected at day 35 p.i. by direct microscopic examination as well as the Strout's concentration method. On the contrary, all of the non-immunised infected dogs still displayed detectable circulating parasites at day 35 p.i.

When xenodiagnosis was performed, the proportion of infected bugs fed on the vaccinated dogs at day 45 p.i. was significantly lower as compared with the control group (Table 1), from 67.5% (27/40) to 28.1% (16/57), respectively (Odds ratio = 0.19; 95% CI: 0.07–0.49; $p < 0.0003$). Effectiveness 81 (51–93). In the xenodiagnosis performed on day 60 p.i., these proportions were 2.6% (2/75) and 21.4% (15/70), respectively (Odds ratio = 0.10; 95% CI: 0.01–0.47; $p < 0.002$). Effectiveness 90 (53–99).

The levels of *T. cruzi* antibodies were measured before and after infection. Table 2 shows that in control dogs antibodies were detected, as expected, only after infection, whereas in immunised animals they were already detected 15 days after

Table 1
Parasitemia of control and immunised dogs infected with *Trypanosoma cruzi*

Dog group	Case	Post-infection day				
		15	25	35	45	60
Control	1	13 ^a	27 ^a	3.4 ^a	8/13 ^b	7/21 ^b
	2	20	7	6.4	10/14	6/25
	3	20	7	6.7	9/13	2/24
					27/40 (67.5%)	15/70 (21.4%)
Immunised	1	17	<3.4	<3.4 ^c	3/17	0/24
	2	17	7	<3.4 ^c	4/17	1/27
	3	10	<3.4	<3.4 ^c	9/23	1/24
					16/57 (28.1%)	2/75 (2.6%)

^a Parasitemia measured by the method of Pizzy ($\times 10^{-3}$ parasites/mL).

^b Number of infected bugs/number of bugs still alive at the time point after feeding on dogs.

^c Negative results even after blood concentration by micro-Strout method.

the first vaccination dose, and were thus present at the time of infection. The titres rose until day 35 p.i. in both groups of dogs, being slightly but not significantly higher in immunised ones.

The animal's weight was not modified throughout the study. The electrocardiographic studies revealed no alterations compatible with chagasic cardiomyopathy. Indeed, only respiratory sinus arrhythmias were observed in all dogs without differences between both groups.

4. Discussion

The results of this study confirm our previous laboratory findings [9,10], which demonstrated that *T. rangeli*, a parasite that is not pathogenic for humans [2,15], is a good agent to induce a protective immune response against *T. cruzi* infection. In recent years, other authors have also reported a protective response induced by *T. rangeli* by using other strains and employing different experimental model [16,17]. The protective effect is based on the high antigenic similarity between *T. cruzi* and *T. rangeli* [18]. Besides, as autoimmunity is one of the postulated mechanisms in the

pathogenesis of chronic Chagas' disease [19,20], the immunisation with non-pathogenic parasites should not induce deleterious autoimmune response. Our protocol of immunisation with fixed epimastigotes of *T. rangeli* induced an antibody response against *T. cruzi* in dogs. This was associated with a shorter acute parasitemic phase of weaker amplitude. More interestingly, although dogs were not fully protected by this vaccination, their lower parasitemia led to a lower rate of infection of the vector *T. infestans* when they feed on the blood of chronically infected dogs. In a field trial, Basombrio et al. [21] reported similar results obtained by vaccination with attenuated *T. cruzi*.

Our model of vaccination with a parasite non pathogenic for man, might represent a strategy to reduce the transmission rate of *T. cruzi* in the domestic cycle. Indeed, dogs seem to play a major role as reservoirs for the transmission to human [22–24]. Although it would be interesting to analyze the efficacy of vaccination in longer periods of time, the results are promissory and encourage the prosecution of this line of research.

In summary, the present work shows that immunisation of dogs with *T. rangeli* epimastigotes reduced parasitemia and infectiousness to the vectors when dogs were challenged with

Table 2
T. cruzi-specific antibody levels in control (C) and immunised (I) dogs

Dog	Pre ^a		Post 1 ^b		Post 2		Post 3		15 p.i.		25 p.i.		35 p.i.	
	HAI ^c	ELISA ^d	HAI	ELISA	HAI	ELISA	HAI	ELISA	HAI	ELISA	HAI	ELISA	HAI	ELISA
C 1	–	–	–	–	–	–	–	–	8	+	8	+	16	+
C 2	–	–	–	–	–	–	–	–	16	+	16	+	32	+
C 3	–	–	–	–	–	–	–	–	16	+	16	+	32	+
I 1	–	–	–	+	–	+	8	–	16	+	16	+	32	+
I 2	–	–	8	+	8	+	16	+	32	+	32	+	64	+
I 3	–	–	8	+	8	+	16	+	32	+	32	+	64	+

^a Sera taken before the immunisation schedule.

^b Post 1: sera taken after first immunisation dose. Post 2: sera taken after second immunisation dose. Post 3: sera taken after third immunisation dose.

^c Result of haemagglutination (1/antibody titre).

^d Results of ELISA (– and + indicate an absorbance lower or higher than the cut-off value).

T. cruzi. Such immunisation tool might be combined with treatments as proposed by other authors [25] to effectively reduce the infection rate among dogs, and consequently, the vector-borne transmission of human Chagas' disease.

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