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Can we heal Chagas infection?

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HIGHLIGHTS

- A model for the parasite-antibody competition between *T. rangeli* and antibodies.
- The model reproduces experimental data from murine models.
- A preinfection with *T. rangeli* induces a temporary protection against Chagas.
- A preinfection could reduce the in-house vectorial parasitemia.

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ABSTRACT

We present a model for the parasite-antibody dynamical competition between *Trypanosoma rangeli* and its antibodies during the acute phase of an infection in a mammal host. The model reproduces experimental data from murine models found in the literature and allows us to demonstrate that a preinfection with *T. rangeli* induces a temporary protective effect against Chagas disease. As the mammal immune system is able to eliminate a single *T. rangeli* infection, the host high antibody levels, needed to resist the Chagas infection, are reduced with time, returning the system to the initial healthy state. Our results suggest that a preinfection with *T. rangeli* could be used to reduce the in-house vectorial parasitemia through repeated vaccination of domestic animals.

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1. Introduction

Chagas disease is a condition caused by the intracellular protozoan parasite *Trypanosoma cruzi*. It is endemic in Latin America, affecting nearly 10–12 million people and killing more than 15,000 people each year (World Expert Committee, 2002). Nowadays, due to human migration, it is possible to find Chagas cases in some countries of Europe and North America. Usual transmission to humans is through the bite of haematophagus triatomine bugs, but it could also occur by blood transfusion, congenital transplacental, organ transplant and laboratory accidental infection. The parasite *T. cruzi* moves among mammals (reservoirs), humans and triatomine insects (vectors), having three different morphological stages during its life cycle. Under natural conditions, Chagas disease transmission cycle begins when a triatomine acquires the parasitic infection by feeding on the blood of an infected animal or human. Once inside, in the epimastigote stage, the parasite divides rapidly in the insect gut. When the triatomine takes another blood meal, it defecates on the skin of the mammal, depositing parasites (but in the metacyclic trypomastigote stage). *T. cruzi* is introduced into the mammal body by the bite wound, through other cuts and abrasions or through the soft skin of eyes and mouth. Once the parasites are inside, they can penetrate in the host cells, transforming into amastigotes which reproduce by binary fission. After a period of time the cell is full of new parasites and bursts, releasing them into the bloodstream, again in the trypomastigote circulatory stage. In turn, these trypomastigotes can penetrate a new cell or be ingested during other bug bite.

There are others trypanosomes, like *Trypanosoma rangeli*, widespread in Latin American countries. *T. rangeli* is also transmitted by the bite of triatomines, but the contagion is through insect saliva (not faeces), and it is non-pathogenic to a vertebrate host (Guhl and Vallejo, 2003). Due to its innocuity, there are few studies of this parasite, and its life cycle in mammals remains unclear. There are some studies showing the presence of amastigote forms in experimentally infected mice. In particular, Urdaneta-Morales and Tejero (1986) reported intracellular nest, or pseudocysts, containing amastigotes and trypomastigotes of this parasite in heart, liver and spleen of a lactating male white mice (NMRI strain) from a 12-day-old culture of the Dog-82 strain of *T. rangeli*. Osorio et al. (1995) also observed *amastigote-like* forms in an *in vitro* experimental infection of the U937 histiocytic cell line. Remarkably, both studies cited above agreed that observed intracellular forms were

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rare and lacked replication. In contrast, other experiments did not found any intracellular forms. For instance Eger-Mangrich et al. (2001) observed low infectivity but no-intracellular *T. rangeli* replication (Choachi, Macias, and SC-58 clone B1 strains) in Vero cells and murine promonocytes. Añez et al. (1985) observed only circulating blood forms in Albino mice and *Didelphis Marsupialis* bitten four times by *R. prolixus* triatomines infected with the *T. rangeli* parasite. Koerich et al. (2002) developed a method to induce high *T. rangeli* differentiation *in vitro*, testing the infectivity of those culture-derived trypomastigotes in Balb-C mice, and finding just blood-circulating parasites. Considering all these studies, we will assume that *T. rangeli* trypomastigotes reproduce by binary fission in the bloodstream, neglecting the existence of any intracellular form.

Both parasites, *T. cruzi* and *T. rangeli*, share endemic areas and vectors, and the two have a strong antigenic relationship (Basso et al., 1991). As a consequence, mixed *T. cruzi–T. rangeli* infections can occur, making a Chagas disease diagnosis difficult, and a cross reaction between them is possible. Based on this, several researchers, like Basso et al. (1991, 2004, 2007, 2008), Cervetta et al. (2002), Marini et al. (2011), Zuñiga et al. (1997) and Paláu et al. (2003) have proposed a vaccination procedure for Chagas disease. They performed experiments in mice and dogs, using *T. rangeli* epimastigotes to generate protection against a posterior *T. cruzi* infection by inducing an overproduction of antibodies. They found that the *T. cruzi* parasitemia level was lower and lasted a shorter period of time in preinfected animals. The immunization schedule elicited B and T specific responses against *T. cruzi*, associated with high levels of specific antibodies and a particular pattern of cytokines, ending in a strong reduction in the mortality rate among infected mice. More than 95% of vaccinated mice survived an otherwise lethal *T. cruzi* infection, displaying a significantly reduced parasitemia during the acute phase of Chagas disease (Cervetta et al., 2002). The results suggest that it might be possible to reduce the vectorial parasitemia through vaccination of domestic animals. As dogs and poultry are known to play a major role in the domestic cycle of *T. cruzi*, this might represent an appropriate strategy to reduce parasite transmission to humans.

In the last years mathematical modeling had become a useful tool to study the defense mechanisms against parasite/bacteria (Okamoto and Amarasekare, 2012; Arciero et al., 2010; Day et al., 2006). In particular, our group has developed a model to describe the interaction between *T. cruzi* and the immune system (Condat et al., 2003; Sibona and Condat, 2002) during the acute phase of Chagas disease. This model was improved and extended (Sibona et al., 2005; Vega et al., 2011) leading to a good description of the experimental data found in the literature. The model accurately predicts all possible outcomes of the disease: healing, death, and chronic infection, with stationary or quasi-cyclical populations, and allows us to estimate the damage generated by direct parasitic action. For *T. rangeli* instead, this work is the first attempt to reproduce its interaction with the mammal immune system; its aim is to elucidate the parasite dynamics inside the host and build the basis to reproduce Basso's experiments (Cervetta et al., 2002). Here we give a possible explanation to the cross-reaction activity observed between the two parasite populations (*T. rangeli* and *T. cruzi*) through the competition between them and with several antibodies species. Our final goal is to answer the question if *T. rangeli* could be used as a vaccine against Chagas disease.

The rest of the paper is organized as follows. In Section 2, we model the time evolution of the acute stage of a *T. rangeli* infection, based on a previous mathematical model for *T. cruzi* that neglects intracellular replication (Condat et al., 2003). We analyze the properties of this model, showing the time evolution of the populations and the long time steady state values associated with them. Next, in Section 3, we study the heterologous *T. rangeli–T. cruzi* infection, assuming for the evolution of the *T. cruzi* population a model that includes the different stages of the parasite life cycle inside the host (Sibona et al., 2005). We study also, how the phase diagram (in terms of an effective parasite reproduction rate and the antibody generation rate) of a single *T. cruzi* infection is modified by the presence of *T. rangeli*. In Section 4, we examine in detail the acute phase of the Chagas infection for a host pre-infected with *T. rangeli*, showing that the combined model can reproduce the features observed in mixed infections experiments, and confirming Basso's postulates. In particular, we found that a preinfection with *T. rangeli* causes an activation of the immune system (increasing the antibody levels) during a certain period of time. This protective effect disappears when the immune system returns to the initial values, causing the loss of host immunity.

2. *Trypanosoma rangeli*

2.1. The model

This is the first attempt to model a *T. rangeli* parasite infection. The model offers a great opportunity to identify and analyze the different aspects of parasite life cycle inside the host. Considering the absence of evidence of active intracellular replication (Eger-Mangrich et al., 2001; Añez et al., 1985; Koerich et al., 2002), we will work on a previous version of the Chagas infection model (Condat et al., 2003; Sibona and Condat, 2002), which only takes into account the extracellular replication of the parasite. In the present model, the number $m(t)$ of *T. rangeli* parasites increases at a rate κ_m due to reproduction by binary fission, and decreases due to the interaction with antibodies. Assuming that there are N antibody species capable of mediate parasite removal, we quantify the likelihood of antibody-mediated parasite removal upon an encounter through the set of coefficients $\alpha_{i,m}(t)$. The time dependence of the parasite number is then described by the equation

$$\frac{dm(t)}{dt} = \kappa_m m(t) - \sum_{i=1}^{N} \alpha_{i,m}(t) a_i(t) m(t) \tag{1}$$

where $a_i(t)$ is the number of antibodies of species i at time t. This number increases at rate $\gamma_{i,m}$ due to the parasite-induced activation of the immune system associated to the *T. rangeli* presence. However, the production process is not instantaneous and the model considers that there is a delay $\theta_{i,m}$ between the parasite population growth dynamics and antibody creation. The model also assumes that antibody species i has an intrinsic lifetime τ_i and an equilibrium population a_{i0} in the absence of parasites. A relatively large initial population of specific antibodies (larger than a_{i0}) could mean that a previous infection took place in the host and the immune system is already activated. With these considerations the evolution equation for the antibody population is

$$\frac{da_i(t)}{dt} = \gamma_{i,m} m(t-\theta_{i,m}) - \alpha_{i,m}(t) a_i(t) m(t) + \frac{1}{\tau_i}[a_{i0} - a_i(t)] \tag{2}$$

To model the optimization process of the antibodies specificity, we assume that the removal efficiency $\alpha_{i,m}(t)$ is governed by an exponential learning process

$$\alpha_{i,m}(t) = \alpha_{Ai,m} + \alpha_{Bi,m}(1 - \exp[-t/T_i]) \tag{3}$$

This function describes a smooth increase of the antibody efficiency from an initial value $\alpha_{Ai,m}$ to a saturation value $\alpha_{Ai,m} + \alpha_{Bi,m}$ with a "learning time" T_i for each antibody species i.

2.2. Steady states

The model analysis is simplified by considering first the outcomes for a single antibody species at the asymptotic ($t \to \infty$)

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regime. Setting the time derivatives in Eqs. (1) and (2) equal to zero and considering the long time value of the removal efficiency $\alpha_m = \alpha_{A,m} + \alpha_{B,m}$ yields a set of equations defining the steady-state populations, from where four different cases are found.

I. *Healing*. For $\kappa_m < \alpha_m a_0$ and $\kappa_m < \gamma_m$, corresponding to strong induced antibody formation and high removal efficiency, the parasite population disappears while the antibody number goes back asymptotically to its initial value a_0, i.e., the system returns to the initial conditions previous to the infection.

II. *Chronic disease*. If $\alpha_m a_0 < \kappa_m < \gamma_m$ there is strong induced antibody formation but with low removal efficiency, leading to an equilibrium between antibodies and parasites described by

$$m_s = \frac{\kappa_m - \alpha_m a_0}{\tau \alpha_m (\gamma_m - \kappa_m)}, \quad a_s = \frac{\kappa_m}{\alpha_m} \tag{4}$$

III. *Host death*. If $\alpha_m a_0 < \kappa_m$ and $\gamma_m < \kappa_m$, i.e., for weak induced antibody formation, the antibodies, regardless of their efficiency, are not created fast enough to control the infection.

IV. *Inoculation dependence*. For $\gamma_m < \kappa_m < \alpha_m a_0$ the final outcome of the infection will be determined by the size of the initial inoculation. If the initial parasite population is lower than a certain critical value m_c, that depends on κ_m and γ_m, the system returns to its healthy initial condition; otherwise it will be guided to the host death case, i.e. uncontrolled infection.

The stability of all steady-state solutions was proved using the Routh–Hurwitz criterion (Murray, 2002).

2.3. Comparison with experiments

To validate the model and use it to understand the parasite-immune system dynamics inside the host, we must reproduce first some experimental data sets; as, for example, those obtained by Koerich et al. (2002) and Zuñiga et al. (1997) (see Fig. 1). The comparison was made considering three antibody species, IgM, IgG1 and IgG2a, the specific antibodies for *T. cruzi* and *T. rangeli* (Marini et al., 2011). The antibody parameters values (shown in Table 1) were chosen to show the closest visual agreement between the experimental data and the model predictions. Fig. 1(a) shows a comparison between the model and Koerich et al.'s (2002) experimental data for *BALB-C* mice inoculated intraperitoneally with 3×10^5 *T. rangeli* trypomastigotes of the *SC-58* and *Choachi* strains. The resulting parasite parameter values obtained were $\kappa_m = 0.6$ [days^{-1}], and $m_0 = 7365$ [ml^{-1}]. The values of the immune reaction parameters a_{i0}, $\theta_{i,m}$, τ_i, and T_i were kept as in Condat et al. (2003). They where obtained from fits of a similar model describing the evolution of the same antibody populations for *BALB-C* mice.

We must remark that Zuñiga et al. (1997) experiments were performed to investigate the protective effect of *T. rangeli* against a *T. cruzi* infection in mice of different ages and with different inoculum doses. In their work they study the *T. rangeli* parasitic level in 1-month-old mice infected with 3×10^5 trypomastigotes of the *C-23* strain. Following the same procedure as with Koerich's data, we obtained a good agreement with the model, as it can be observed in Fig. 1(b). In this case, the parasite replication rate was found to be $\kappa_m = 0.74$ [days^{-1}]. The antibody parameter values are shown in Table 1, and in Fig. 2 we can observe de sharp peak in the antibody population produced by the *T. rangeli* infection in Zuñiga's experiments.

It must be noted that the values of $\alpha_{i,m}$ and $\gamma_{i,m}$ for Koerich's work are respectively five times and eight times greater than those obtained previously for mice infected with *T. cruzi* (Condat et al., 2003); while for Zuñiga's work were just 0.7 times and 2.1 times bigger, respectively. This similarity between *T. rangeli* and *T. cruzi* parameter values could be due to common biological characteristics among these parasites (They share endemic areas, reservoirs and vectors, and express a high antigenic similarity, Marini et al., 2011).

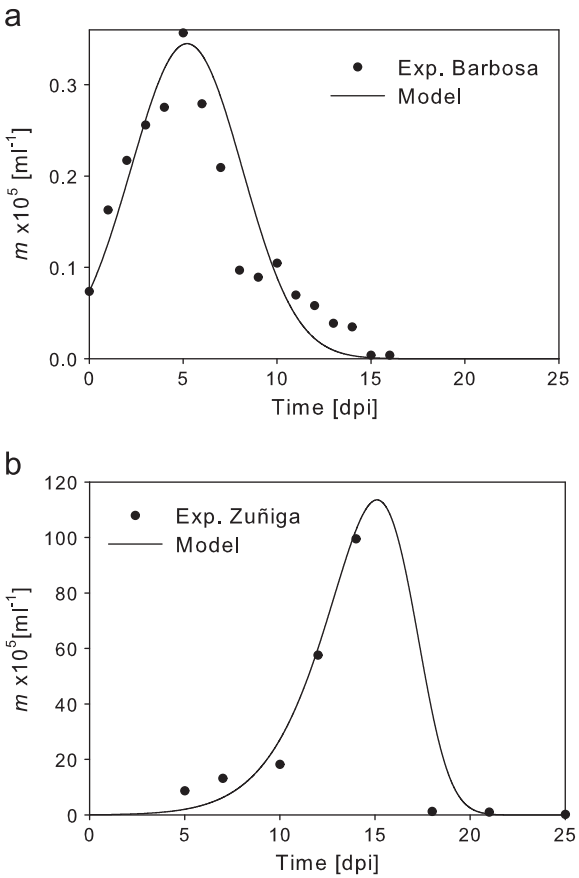

Fig. 1. Comparison of the model with experimental *T. rangeli* population. Data from (a) Koerich et al. (2002), and (b) Zuñiga et al. (1997).

Table 1
Parameters corresponding to the fit of the experimental data from Koerich et al. (2002) and Zuñiga et al. (1997). $\alpha_{i,m}$ is measured in (ml/days) and $\gamma_{i,m}$ is measured in days^{-1}.

Parameter	*T. rangeli*		*T. cruzi*
	Koerich	Zuñiga	Zuñiga
γ_{IgM}	4.75×10^{9}	6.65×10^{8}	4.03×10^{9}
γ_{IgG1}	1.92×10^{11}	2.69×10^{10}	1.63×10^{11}
γ_{IgG2a}	5.9×10^{11}	8.29×10^{10}	5.54×10^{11}
$\alpha_{A;IgM}$	5.2×10^{-20}	1.36×10^{-20}	1.17×10^{-20}
$\alpha_{B;IgM}$	3.76×10^{-20}	9.87×10^{-21}	8.46×10^{-21}
$\alpha_{A;IgG1}$	0	0	0
$\alpha_{B;IgG1}$	1.36×10^{-18}	3.59×10^{-19}	3.07×10^{-19}
$\alpha_{A;IgG2a}$	0	0	0
$\alpha_{B;IgG2a}$	4.64×10^{-18}	1.21×10^{-18}	1.04×10^{-18}

3. The cross reaction

3.1. Adding the T. cruzi infection

As we mentioned in the Introduction, Basso and coworkers (Basso et al., 1991, 2007, 2008, 2004; Cervetta et al., 2002; Marini et al., 2011) showed that *T. rangeli* offers a protective effect against

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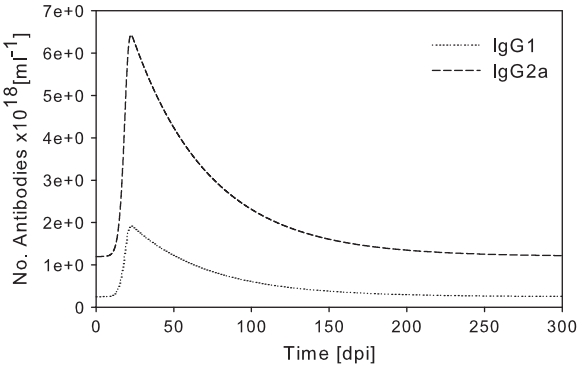

Fig. 2. Evolution of the antibodies population corresponding to the *T. rangeli* infection of Zuñiga experiments (Zuñiga et al., 1997).

T. cruzi infections. In their experiments, they first preinfected mice with *T. rangeli*, introducing the *T. cruzi* parasites a couple of days later. They observed that the *T. rangeli* preinfection induces some immunization, causing a strong reduction in parasitemia and mortality levels when compared with non-vaccinated or non-preinfected mice.

We have previously developed (Sibona et al., 2005; Cossy Isasi et al., 2001) a model to describe the acute phase of the Chagas infection, considering the *T. cruzi* intracellular replication by binary fission of the amastigote stage of the parasite. To do this, we introduced a new population, the number of invaded cells r. Its evolution is given by the equation

$$\frac{dr(t)}{dt} = \xi n(t) - \eta r(t) \tag{5}$$

where the infectivity ξ is the rate at which a circulating parasite penetrates into a host cell to initiate replication, and the cytotoxicity η is the probability per unit time that an infected cell will burst due to the large number of parasites in its interior. Therefore, the circulating parasite production depends now on the infected cell population, and, as a consequence, the circulating parasite evolution equation has to be modified

$$\frac{dn(t)}{dt} = \eta N_r r(t) - \sum_{i=1}^{N} \alpha_{i,n}(t) a_i(t) n(t) - \xi n(t) \tag{6}$$

with N_r being the mean number of trypomastigotes emerging from a cell rupture.

In Vega et al. (2011) we studied in detail a mixed infection of two *T. cruzi* strains and their interaction with one antibody species during the acute phase of Chagas disease. We obtained phase diagrams and steady state populations for both parasites strains and one average antibody species. The situation here is similar, but not the same; we must analyze a mixed infection of two trypanosomes except that this time they have different reproduction processes: the extracellular replication of *T. rangeli* and the intracellular replication of *T. cruzi*. While the parasite evolution equations are described by Eq. (1) for *T. rangeli* and Eqs. (5) and (6) for *T. cruzi*, the antibody time evolution equations have terms for both interactions

$$\frac{da_i(t)}{dt} = \gamma_{i,m} m(t-\theta_{i,m}) + \gamma_{i,n} n(t-\theta_{i,n}) - \alpha_{i,m}(t) a_i(t) m(t) - \alpha_{i,n}(t) a_i(t) n(t) + \frac{1}{\tau_i}[a_{i0} - a_i(t)] \tag{7}$$

where m refers to the *T. rangeli* population, as in Section 2, and n to the *T. cruzi* parasite population. To reproduce the experiments we considered a time shift t^* at the inoculum of the *T. cruzi*. The *T. cruzi* population will be null until the time t^*, being $n(t^*)$ the size value of the inoculation.

We have also assumed the same learning process for the removal efficiency $\alpha_{i,n}$ of *T. cruzi* parasites by antibody species i, as we described in Section 2 for *T. rangeli*. Then the equation system (1), (3) and (5) to (7) is used to describe the mixed *T. rangeli–T. cruzi* infection and the mammal immune system response.

3.2. Steady-states of a T. rangeli–T. cruzi mixed infection

We study the asymptotic ($t \to \infty$) behavior of the equation system for a single antibody species to obtain the steady state populations, as was done in Section 2 for *T. rangeli*. Since we already know the parameter values for the *T. rangeli* model, including its non-pathologic effects, we do not have to consider all the possible parameter combinations (as was done for a mixed infection of two *T. cruzi* species, Vega et al., 2011), and just consider only those that lead to a healing state, i.e., those satisfying the condition $\kappa_m < \gamma_m$ and $\kappa_m < \alpha_m a_0$ in the case of one antibody species. For this case, we have three possible outcomes, depending on the *T. cruzi* parameters, as follows:

I. *Healing*. If $\kappa_n = (N_r - 1)\xi < \alpha_n a_0$ (a low effective parasite reproduction rate), both parasites populations disappear at long times and the system returns to its initial conditions ($r_s = 0,\ n_s = 0,\ m_s = 0,\ a_s = a_0$) for any γ_n value.

II. *Chronic disease caused by T. cruzi infection*. The *T. rangeli* population disappears, but the system reaches a long-time equilibrium state between *T. cruzi* and antibodies described by

$$r_s = \frac{\xi}{\eta} n_s, \quad n_s = \frac{\kappa_n - \alpha_n a_0}{\tau \alpha_n (\gamma_n - \kappa_n)}, \quad a_s = \frac{\kappa_n}{\alpha_n}$$

The conditions to obtain this state are $\kappa_n > \alpha_n a_0$ and

$$\gamma_n > \gamma_c(\kappa_n) = \frac{B_0 + B_1\kappa_n + B_2\kappa_n^2 + B_3\kappa_n^3}{A_0 + A_1\kappa_n + A_2\kappa_n^2} \tag{8}$$

where A_i and B_i are constants depending on the parameters ξ, α_n, α_m, η, a_0, and κ_m.

III. *Host death*. If $\gamma_n < \gamma_c$ and $\alpha_n a_0 < \kappa_n$. This case is divided into two sub-cases:

III.a Host death due to the unlimited growth of the *T. cruzi* parasite population alone while *T. rangeli* is eliminated. The extra condition for this case is that $\gamma_n > (\alpha_n/\alpha_m)\kappa_m$. While n grows without control, the antibody population reaches the asymptotic value

$$a_s = \frac{\gamma_n}{\alpha_n} \tag{9}$$

III.b If $\gamma_n < (\alpha_n/\alpha_m)\kappa_m$ the host death is due to the unlimited growth of both the *T. rangeli* (m) and *T. cruzi* (n) parasite populations while the antibody population goes to the same value of case III.a.

Again, the stability of the steady-state solutions was proved using the Routh–Hurwitz criterion (Murray, 2002).

From the preceding discussion we can therefore construct a phase diagram in the plane defined by the growth rates γ_n and $\kappa_n = (N_r - 1)\xi$, assuming one average antibody species. This is shown in Fig. 3, where we set the parameter values as $\eta = 1,\ \xi = 10,\ \alpha_n = \alpha_m = 2.5,\ \kappa_m = 4,\ \gamma_n = 6,\ \tau = 10$ and $a_{10} = 2$. Fig. 3(a) shows the phase diagram for a pure *T. cruzi* infection which may be compared with the mixed infection case shown in Fig. 3(b). Here, the boundary between the domains corresponding to cases III and IV (inoculation dependent) was found numerically for two initial parasite population ($n_0 = 1$ and $n_0 = 10$). It is easy to

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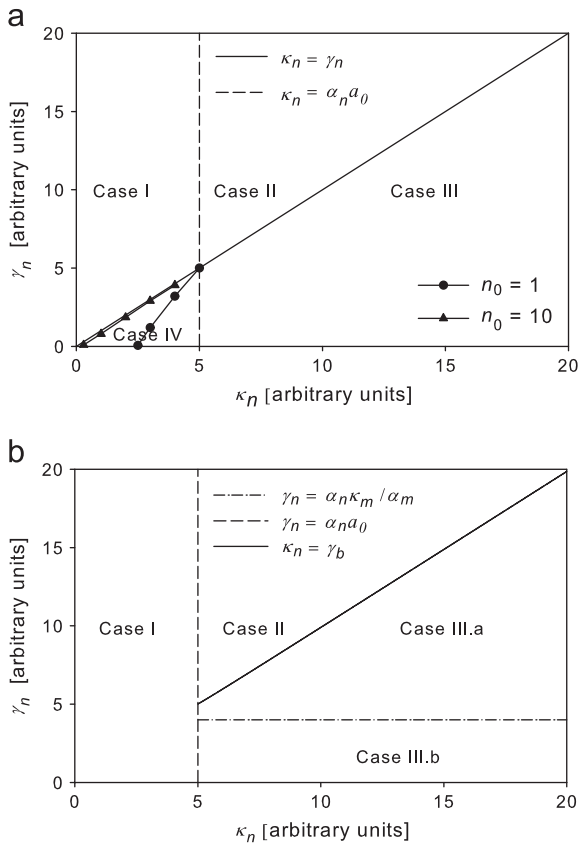

Fig. 3. Phase diagrams describing the outcome of: (a) a pure *T. cruzi* infection, and (b) a mixed *T. rangeli–T. cruzi* infection in terms of the *T. cruzi* effective reproductive rate κ_n and antibody generation rate γ_n. Cases: I. healing, II. chronic, III. host death, IV. inoculation dependence. Case III is divided into two subcases: III.a (III.b) host death by the exponential growth of *T. cruzi* population (*T. cruzi* and *T. rangeli* populations simultaneously).

observe that the presence of the *T. rangeli* parasite modifies some of the boundaries between the different outcome cases. In particular, the border between the chronic (II) and host death (III) cases was $\gamma_n = \kappa_n$ for a single infection (Fig. 3(a)), while for a mixed infection it switches to $\gamma_n = \gamma_c(\kappa_n)$ (Fig. 3(b)). This leads to an increase (decrease) in the chronic (host death) basin, enhancing the chances to survive the infection. Moreover, the inoculation dependent zone disappears, increasing the chances to heal. It is also possible to observe the subdivision of the host death case into the two new subcases (III.a and III.b). The boundary between the healing and chronic cases is unaffected ($\kappa_n = \alpha_n a_0$).

3.3. Comparison with experiments

As we did in Section 2, to verify the mixed model we have to compare it with experimental data. As was mentioned in the introduction, Zuñiga et al. (1997) performed mixed infection experiments using a *Y* strain of the *T. cruzi* parasite together with a *T. rangeli C-23* strain on male mice of the *Swiss Ico* strain. The *T. rangeli* parasite was introduced when the mice were 15-day-old, and two weeks later they were infected with the *T. cruzi* parasites. All the inoculum doses were of 3×10^5 trypomastigotes. As the model parameters are different for different parasite strain/mammal species combinations, we begin by independently adjusting data for each parasite strain. Fig. 4 depicts Zuñiga et al.'s (1997) data for a pure *T. cruzi Y* strain infection, together with the model results obtained by adjusting the parameters to get the best visual agreement. The value of the parameters related to the intracellular

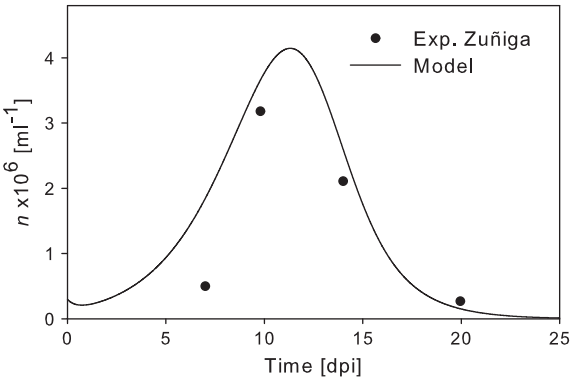

Fig. 4. Comparison of the *T. cruzi* population data from Zuñiga et al. (1997) with the model prediction. The parameter values are shown in Table 1.

The values of the parameters related to the intracellular replication are $N_r = 2.28$, $\xi = 1.05$ [days^{-1}], $\eta = 0.7$ [days^{-1}], $n_0 = 3 \times 10^5$ [ml^{-1}], and the antibody parameter values were those used to fit the *T. rangeli* infection. With this set of parameters, in combination with the set obtained for *T. rangeli C-23* (see Table 1), we could reproduce in Fig. 5 the parasitemia levels for the mixed infection experiment. It is worth mentioning that parameters $\alpha_{Ai,n}$ and $\alpha_{Bi,n}$ had been adjusted with less than $\pm 1\%$ error to show the best possible visual agreement. This error was estimated just looking at the minimum parameter variation needed to observe a divergence between the model and the experiments. In the figure, it is also possible to observe the protective effect that the *T. rangeli* pre-infection offers against the *T. cruzi* parasite. This effect is strengthened by increasing the size of the *T. rangeli* inoculum.

Now that the model has been verified, we can use it to explore new situations, such as to analyze the effect of different *T. rangeli* inocula on the *T. cruzi* infection. This is also presented in Fig. 5, where different curves corresponding to several initial inoculation values are depicted. It is clear that if the *T. rangeli* inoculum increases, there is a decrease in the number of *T. cruzi* parasites due to the immune system over-excitation. As there is an increase in the antibody levels, the host is better suited to attack the new parasites. This is shown in Fig. 6, where the level of antibodies is depicted for a *T. cruzi* infection, with and without a previous treatment. The antibody number for a mixed *T. rangeli–T. cruzi* infection is higher (lower) than for a pure *T. cruzi* infection at long (short) times. The initial higher response leads to a fast circulating parasite reduction, which in turn decreases the whole infection, reducing the cell damage. This can be observed in Fig. 7, where the number of infected cells in a pure *T. cruzi* infection is compared with the case of mice previously immunized with *T. rangeli*. The damage is reduced by six times, given the host the chance to survive the infection or even to heal.

4. Discussion

A model for the interaction between the parasite *T. rangeli* and the immune system is developed for the first time in this work. The model predicts three main different steady states (healing, chronic and death), and the comparison with available data shows good agreement. By constructing a $\kappa_m - \gamma_m$ phase diagram we can analyze the outcomes of the infection according to the values of the *T. rangeli* parasite replication rate and its specific antibodies growth rates. By fitting different experiments (in particular, the results of Koerich et al., 2002; Zuñiga et al., 1997), we confirm that an infection with this parasite falls in the Case I zone of the $\kappa_m - \gamma_m$ phase diagram (Sibona et al., 2005), and therefore the system evolves towards the health steady-state. This agrees with clinical

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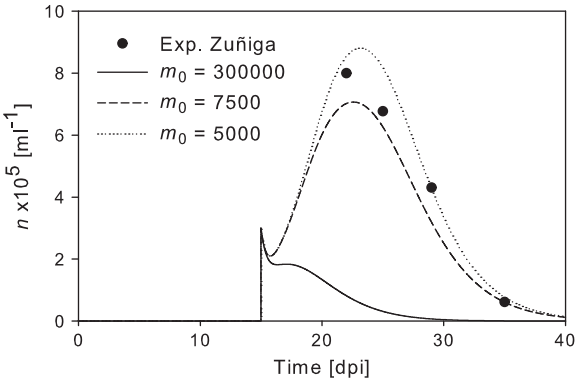

Fig. 5. Evolution of the *T. cruzi* population. Comparison of the model results with the experimental data of the mixed *T. rangeli*–*T. cruzi* infection for different *T. rangeli* inoculation sizes.

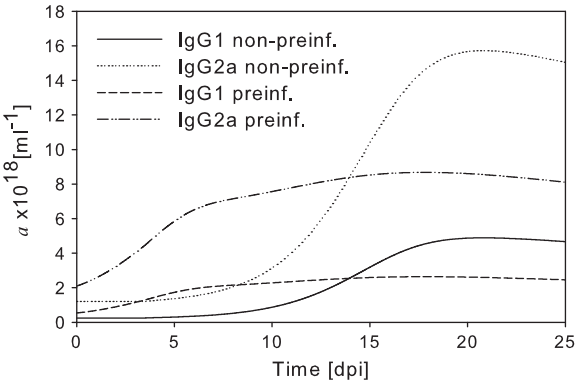

Fig. 6. Comparisons between the model predictions for the antibody species levels in mice non-preinfected and mice preinfected with *T. rangeli*. The data sets used corresponds to the one obtained for Zuñiga experiment.

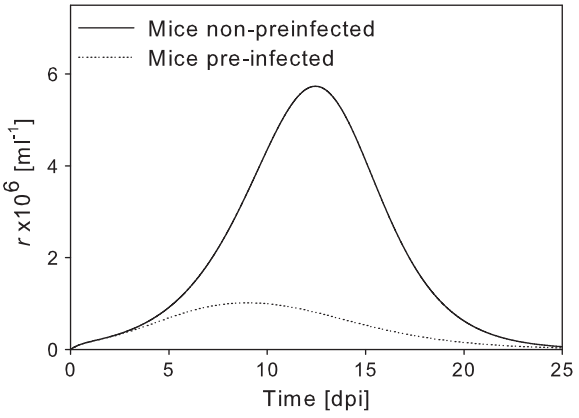

Fig. 7. Comparison between the model predictions of the cellular damage in an animal non-preinfected with one previously vaccinated with *T. rangeli*. The data sets used corresponds to the one obtained for Zuñiga experiment.

observations, for which no chronic disease or long term parasite proliferation have been reported.

The model is extended by adding the interactions with a *T. cruzi* parasite population to analyze the effects of a mixed infection during the acute phase of Chagas disease. A complete analysis shows that, at long times, a chronically ill mammal will only host a single parasite, while both species may coexist during the acute phase. A pre-infection with *T. rangeli* activates the immune system, leading to an increase in the level of antibodies, and providing immunization against a *T. cruzi* invasion. We found that this immunization is just a temporary effect. *T. rangeli* is a non-pathogenic parasite, and then it is eliminated by the antibodies while the host returns to its healthy initial condition ($m_s=0$, $a_s=a_0$). Thereby the mammal host will eventually lose its high antibody levels and a new infection with *T. cruzi* will not meet any enhanced resistance.

The protective effect of a previous *T. rangeli* inoculation over a *T. cruzi* infection can be better understood by analyzing how the $\kappa_n-\gamma_n$ phase diagram is modified. In Fig. 8 we have marked in the phase diagram the positions corresponding to fits to experimental data considering different *T. cruzi* strain-mice species combinations (El Bouhdidi's, et al. 1994; Andersson's, et al. 2003 data). As the data was always adjusted considering several antibodies species (IgM, IgG1 and IgG2a), we may sum up the effects of the three antibody species by considering the effective parameters $\gamma_n=\sum_{i=1}^{3}\gamma_{i,n}$ and $\alpha_n a_0=\sum_{i=1}^{3}\alpha_{i,n}a_{i0}$, which allow us to establish the location of the experiments in the phase diagram. In the figure we can observe that all experiments belong to the chronic case of Chagas disease. However, some of them were deadly to mice due to the high transient parasite populations attained before reaching the asymptotic value.

We must consider also the size of the time shift between the *T. rangeli* inoculation, which primes the immune system, and the introduction of *T. cruzi*. Due to the prior immunization, the antibodies levels rise, modifying the initial condition for *T. cruzi* development. Fig. 8 shows the phase diagram considering as the initial antibody population the value corresponding to the activated state, observed in the simulations at the time shift $t^*=15$ dpi (Zuñiga et al., 1997). We can observe that the time shift causes an expansion of the healing zone by moving the boundary between cases I and II, allowing formerly chronic cases to fall now into a healthy state zone.

Our results can be compared with the findings of Basso et al. (1991, 2004, 2007, 2008); Cervetta et al. (2002); Zuñiga et al. (1997) and Paláu et al. (2003). All of them performed experiments of *T. rangeli* murine infections to observe the evolution of the immune response against a later *T. cruzi* inoculation. All these works showed a decrease in the severity of the disease outcome, a lower parasitic level, survival of all immunized mice and even a complete eradication of the *T. cruzi* population for some cases. We can mention that the only difference between Basso's work and others is that they used dead-fixed forms of *T. rangeli*, while the others use live-trypomastigotes forms of the parasite. Nevertheless, Basso established that the results are very similar Basso (private communication), implying that the effect on the *T. cruzi* infection is due to the immune system excited state and not due to a direct interaction between both parasite species.

We have established that the transition from chronic to health state caused by a *T. rangeli* vaccination depends on the transient level of antibodies in the host at the moment of the *T. cruzi*

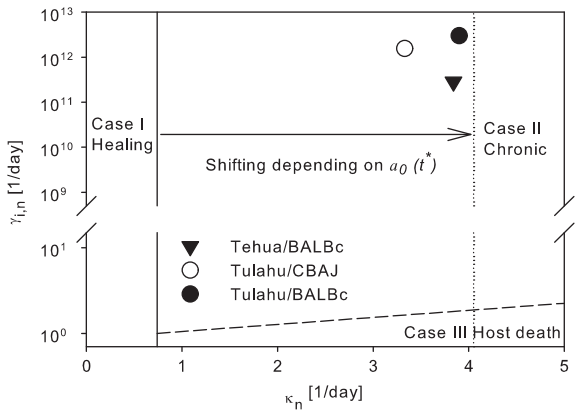

Fig. 8. Transition of the *T. cruzi* chronic cases to a health cases. $N_r=3.47$, N_r ($t^*=22.56$ days) $=14.5$ and $\bar{\xi}=0.3$[1/day]

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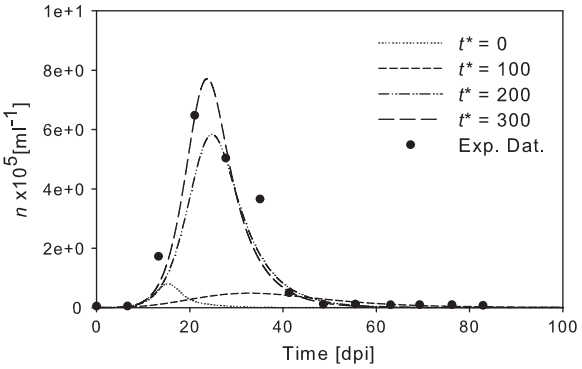

Fig. 9. Evolution of the *T. cruzi* population in a mice preinfected with *T. rangeli*. The re-infection is made at the times (t^*) indicated in the graph.

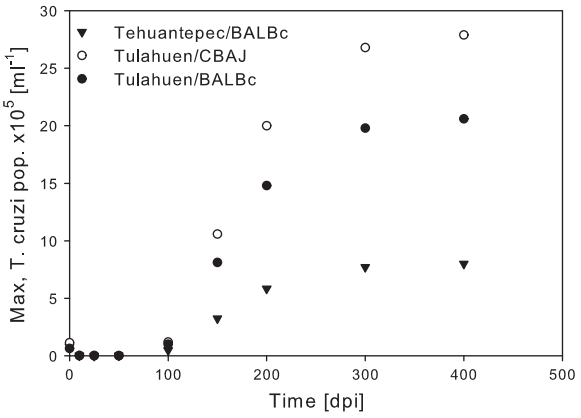

Fig. 10. Maximum parasite level of an infection with *T. cruzi* from El Bouhdidi and Andersson experiments. A pre-inoculation with *T. rangeli* is assumed.

infection. With our model we could answer questions like: does the success of the "vaccine" depend on the time elapsed between both infections? How is the evolution of the *T. cruzi* parasite population modified by infections occurring at different times after the "vaccine" is applied? To answer those questions we performed simulations of the mixed infection for several vaccine-infection time intervals. We report in Fig. 9 the results obtained by using the parameter values adjusted for Zuñiga's experiments with *T. rangeli* and those obtained with El Bouhdidi's data for *T. cruzi*. We observe that for infections occurring at long times after the vaccine is applied, the parasitemia level would be the same as it was in a pure *T. cruzi* infection. The explanation to this is that the first *T. rangeli* infection cannot keep the immune system excited forever. Eventually, the parasites will be eliminated and then the antibody numbers will decrease, returning the system to the initial state ($a_s = a_0$), as it has been shown in Fig. 2. The more time passes between both infections, the less level of antibodies the system would have to fight a later *T. cruzi* infection, allowing the parasite population levels to grow, reaching those of a non-immunization case. This can be observed in Fig. 9, where we have depicted *T. cruzi* infections occurring at different times after the *T. rangeli* "vaccination".

In Fig. 10 we analyze the maximum parasitic level of the *T. cruzi* infection as a function of the vaccine-infection gap time. It can be observed that, for the parameters considered, during the first 4 months after the vaccination, the immune system will reduce the parasite population for more than one order of magnitude, the host having the chance to cure, as it was observed in the $\kappa_n - \gamma_n$ phase diagram. Then, our model suggests that the task to perform could be to vaccinate domestic animals, which serve as reservoirs for the *T. cruzi* transmission cycle, three times per year, reducing in turn the parasite levels in vectors and preventing a possible Chagas disease in-house contagion. It is known that domestic animals increase the risk to human transmission (Cohen and Gürtler, 2001). Moreover, infected dogs and chickens in the household increase both the bug population size and bug prevalence of *T. cruzi* (Gürtler et al., 1997). If in turn domestic animals can reduce the number of in-house bugs, instead of been a *T. cruzi* source, they could transform in an effective strategy to interrupt transmission. Several works have confirmed different degrees of pathogenicity of T. rangeli for its vectors, specially triatomids (particularly from the *Rhodnius* genus) (Guhl and Vallejo, 2003). More experiments, crucially the study of the infectivity of *T. rangeli* in dogs and chicken, are required to confirm our results.

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