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Modelling *Trypanosoma congolense* parasitaemia patterns during the chronic phase of infection in N'Dama cattle

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SUMMARY

*We reanalysed parasitaemia profiles of the trypanotolerant N'Dama cattle (*Bos taurus*), consecutively infected with the same four clones of* Trypanosoma congolense. *Our analysis shows that each individual parasitaemia is characterized by progressively longer intervals between parasites waves. This pattern is most visible during the chronic phase of infection. In addition, the last of the four infections had a significantly larger overall duration of inter-wave intervals. We retrieved these patterns by numerical simulations of a mathematical model, which incorporates assumptions about the molecular basis of antigenic variation and about the anti-parasitic major immune processes. Six potential factors that may determine parasitaemia pattern were studied: carrying capacity of the host environment, intrinsic growth rate of the parasite, affinity maturation of the immune response, immune cell birth and death rate, levels of antibodies to variant surface glycoprotein and levels of antibodies to invariant antigens. Our simulations suggest that the first five factors are not likely to determine the chronic phase parasitaemia pattern whereas the sixth one, namely, antibody response to invariant antigens, yielded profiles consistent with the experimental data. Being cumulative, the immune response to anti-invariant antigens may be increasingly effective as infection proceeds and in successive infections. Comparisons between N'Dama and Zebu and between chronic and acute phases will be needed to make a statement on the role of this phenomenon in trypanotolerance.*

Keywords *trypanotolerance, mathematical model, invariant antigen, antibody response, growth rate, carrying capacity,* Trypanosoma congolense

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INTRODUCTION

African trypanosomes are protozoan parasites transmitted by the tsetse fly to people and to wild and domestic animals in Africa. In addition to the morbidity and mortality caused to human lives (25 000 new infections/year; Kolberg 1994) it causes a severe economical problem in 38 countries, where more than 60 million cattle, on which the food supply in these areas depends, is at risk (Teale 1993). Infection in most of the domestic livestock results in weight loss, impaired immune system and haematopoietic and reproductive disorders, making trypanosomiasis the most important disease affecting livestock productivity. Such livestock breeds (e.g. Zebu, *Bos indicus*), are referred to as *trypanosusceptible*. However, a few breeds of livestock that are indigenous to Africa, such as the N'Dama cattle (*Bos taurus*) of West Africa, are able to tolerate trypanosomes well and in many cases appear to suffer no ill effects from infection. This property, shared by many of Africa's wild ruminants, is known as *trypanotolerance*. (Murray 1987, Teale 1993).

Most Zebu cattle require drug treatment around week six to week seven post infection, to avoid death from anaemia. A minor proportion of these animals (10–30%) will survive the acute phase and enter the chronic phase. In the latter phase the surviving Zebu cattle manifest a much inferior recovery and parasitaemia control than the indigenous N'Dama. It appears, then, that trypanotolerance is characterized not only by surviving the acute phase, but also by the ability to control parasitaemia in the chronic phase. Therefore it seems plausible that factors which determine the characteristic properties of the chronic phase parasitaemia in the natural host may also shed light on trypanotolerance. The latter assumption is one of the motivations for the present study.

Our aim in this study was to investigate the specific pattern of the chronic phase of infection in the natural host, and to identify host mechanisms that may be significant in creating

this pattern. As N'Dama cattle can serve as a good example for a trypanotolerant host undergoing the chronic phase of infection we employed results of field experiments in which N'Dama (and a Zebu breed, called Boran) underwent subsequent infections with the same clones of four different serodemes of *Trypanosoma congolense*, and parasitaemia was monitored thereafter (Paling *et al.* 1991a).

To analyse parasitaemia patterns we employed a new mathematical model, which is an updated version of the model introduced for studying antigenic variation in trypanosomes (Agur, Abiri & Van der Ploeg 1989).

African trypanosomes undergo antigenic variation of their unique cell surface coat protein, the VSG. Every coat usually consists of a single type of VSG, but trypanosomes can change their antigenic identity by switching to the expression of a new VSG-gene, thereby expressing a new coat. Each parasitaemia wave consists of a population of parasites, most of which display one kind of VSG on their surface (SE). As the repertoire of potential VSG coats is very large (about 1000 in *T. brucei;* Van der Ploeg *et al.* 1982), blood-based infections are characterized by relapsing parasitaemia waves, which can progress for long periods of time (see review in Barry & Turner 1991). Antigenic variation was studied by simulations of a mathematical model for a range of parameters, and for different assumptions about the underlying molecular processes. It succeeds in obtaining roughly ordered parasitaemia waves only if it assumes that i) in most of the transitions from the expression of one SE to the next there exist double expressor switch intermediates, expressing simultaneously two VSGs on their coat (DE), and that ii) different DE combinations vary in their susceptibility to the immune response against the older SE in the double coat. Under these assumptions parasitaemia can be ordered *even* if individual parasites switch completely at random. The precision in the order of parasitaemia and in the regularity of waves depends on the proportion of parasites undergoing the DE stage and on the variability in the intrinsic growth-rate of different SEs or DEs (Agur *et al.* 1989).

Indeed, blood form African trypanosomes are able to express two VSGs on their surface at the same time (Baltz *et al.* 1986). Esser & Schoenbechler (1985) suggest that such a dual expression characterizes the transition from the metacyclic stage to the blood stage, but not the blood stage itself. However, the mathematical model's prediction, that in blood the DE stage is practically obligatory, has already been validated (Seyfang, Mecke & Duszenko 1990, Muñoz-Jordán, Davies & Cross 1996). In contrast, the second prediction of the model, namely that the different DEs are differentially susceptible to the immune response against the older VSG on the mixed coat, is as yet to be verified.

MATERIALS AND METHODS

Evaluating parasitaemia

We have plotted as individual profiles the same data which were used for calculating the 'average N'Dama scores' of infection I (IL 1180), infection III (IL 1587) and infection IV (IL 2079. Experimental results of infection II were not available to us (data from Paling, personal communication; see also Paling *et al.* 1991a, b).

To quantify the change in the length of the inter-wave intervals over time we have counted the individual observations in which no parasites were scored. On these counts we have performed analysis of variance (Microsoft Excel ANOVA:Two-Factor with Replication). The original parasite count had been performed daily during the first month post infection and twice weekly thereafter (Paling, personal information). Thus, to exclude any possible variation due to nonuniform experimental data collection we included in the statistical analysis the results as of day 30 of the experiment. Excluding the results of the first month of the experiment also enables focusing the study on the chronic phase of infection.

The mathematical model

We adapted the mathematical model put forward in Agur *et al.* (1989) so as to allow for the humoral immune response to invariant antigens of the parasite and for loss of immune memory. In the new model we assume that switches in the expression of different VSG-genes are random, and that 0·99 of variants go through the DE stage. Otherwise this is a conventional logistic-type parasite population growth model with Michaelis-Menten-type immune reactions.

The model assumes that an infection is initiated by injection of two parasites of a single VSG type (SE). Note that this was made for simplicity. In real-life flies infected with trypanosomes emit amounts of parasites that may be larger by orders of magnitude (depending on the species, see Otieno & Darji 1979); according to our simulations, the introduced number of parasites affects parasitaemia profiles only at the very beginning of the infection.

Each SE population grows according to eq. (1)

$$\frac{dv_n}{dt} = v_n\left[r_n\left(1-\frac{V}{K}\right) - (ua_n + u'a_{inv})\right]. \qquad (1)$$

where v_n is the population size of variant n per ml blood, r_n is its growth-rate, V is the total parasite population/ml blood, K is the carrying capacity of the host, in terms of maximum parasitaemia/ml; a_n and a_{inv} are the population size/ml of VSG n-specific antibodies and of the antibodies that bind to the invariant antigens respectively, and u and u' are their

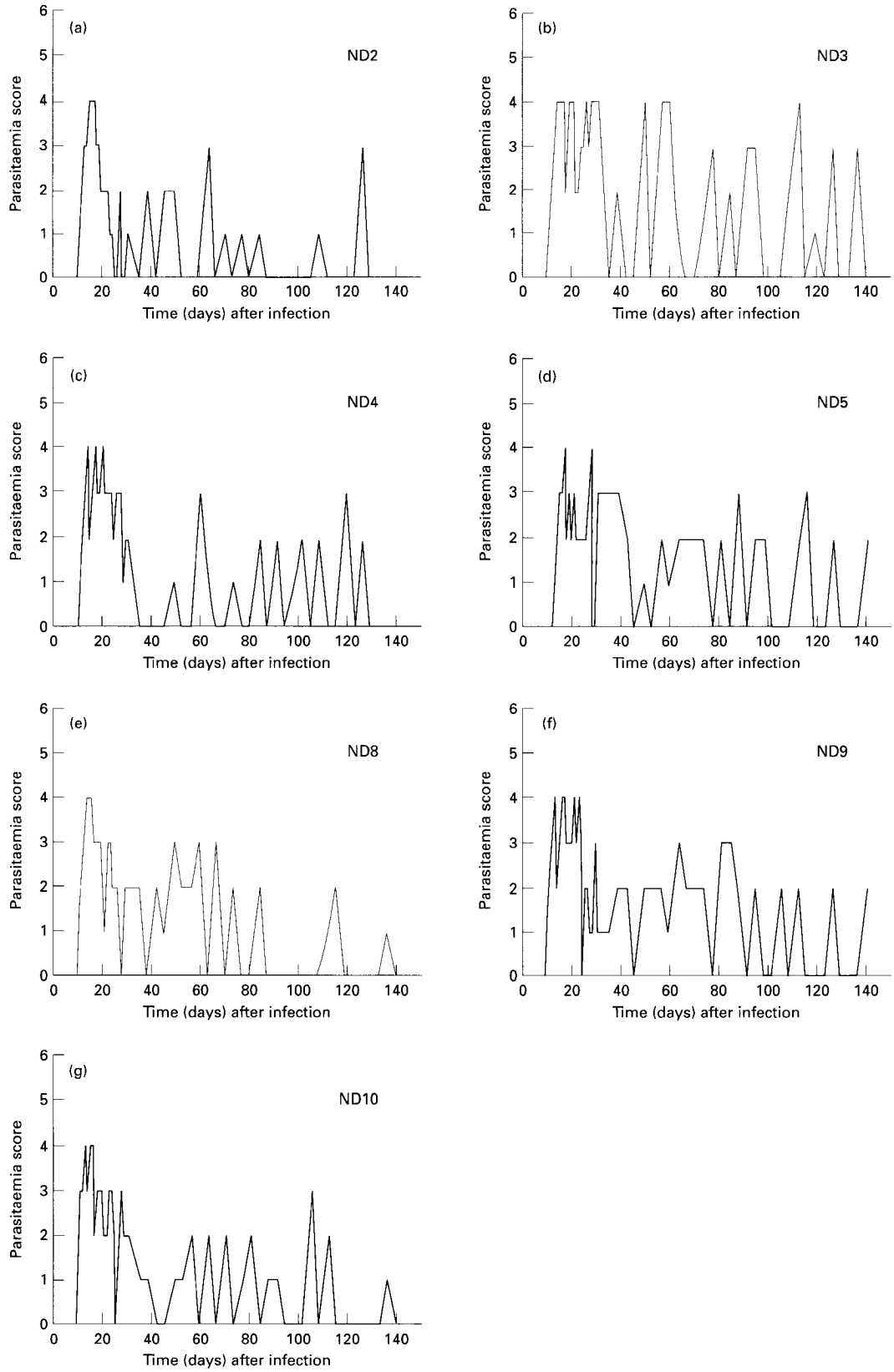

Figure 1 a–g Parasitaemia profiles in 7 N'Dama cattle infected by *Trypanosoma congolense* IL1587 (data from Paling, personal communication).

Table 1 The total length of the intervals between parasite waves in eight N'Dama cattle, which were consecutively infected with four clones of *T. congolense*. The number of observations in which no parasites were detected in blood was monitored for each N'Dama cattle individually in three infections: I (IL 1180), III (IL 1587) and IV (IL 2079) (raw data of the second infection, IL 2642 were not available). To achieve equal resolution monitoring was initiated at day 31 post infection (see text for further details)

Day of infection	*31–60*	*61–90*	*91–120*	*121–150*	Total
IL 1180(I)					
Total Interval	20	33	45	52	150
Average	2·5	4·125	5·625	6·5	4·6875
Variance	2	5·55357143	2·26785714	3·14285714	5·31854839
IL 1587(III)					
Total Interval	23	26	47	41	137
Average	2·875	3·25	5·875	5·125	4·28125
Variance	3·26785714	2·5	2·125	1·83928571	3·82157258
IL 2079(IV)					
Total Interval	17	44	50	60	171
Average	2·125	5·5	6·25	7·5	5·34375
Variance	0·98214286	1·71428571	1·64285714	0·57142857	5·20060484
Total					
Total Interval	60	103	142	153	
Average	2·5	4·29166667	5·91666667	6·375	
Variance	2	3·86775362	1·9057971	2·67934783	

mortality coefficients. A similar equation holds for the DE, $v_{n,m}$, which includes the antibodies against both VSGs, a_n and a_m (Agur *et al* 1989).

Specific B cells proliferate according to (2):

$$\frac{db_n}{dt} = r_B b_n \left[\frac{v_n}{v_n + C}\right] - d_B b_n, \qquad (2)$$

where b_n is the population size of B cells specific to VSG n; r_B and d_B are the B cells' birth-rate and death-rate; and C is the size/ml of variant n population at which B cells proliferate at half the maximum-rate; C is a constant.

Specific antibodies are produced and removed according to (3):

$$\frac{da_n}{dt} = c_1 b_n \left[\frac{v_n}{v_n + C'}\right] - c_2 a_n, \qquad (3)$$

where c_1 and c_2 are the rates of antibody secretion and removal, respectively. C' is the size/ml of variant n population at which specific antibodies are produced at half the maximum-rate; C' is a constant.

B cells that produce antibodies against the invariant antigens, b_{inv}, proliferate according to:

$$\frac{db_{inv}}{dt} = r_{inv} b_{inv} \left[\frac{V}{V + C_{inv}}\right] - d_B b_n. \qquad (4)$$

Antibodies against the invariant antigen are produced and removed according to:

$$\frac{da_{inv}}{dt} = c_1 b_{inv} \left[\frac{V}{V + C'_{inv}}\right] - c_2 a_{inv}. \qquad (5)$$

In (4) and (5) C_{inv} and C'_{inv} are the parasite population size/ml at which B cells that carry anti-invariant antibodies, and anti-invariant antibodies are produced at half the maximum-rate; C_{inv} and C'_{inv} are constant. More details can be found in Agur *et al.* 1989.

RESULTS

The parasitaemia in the resistant N'Dama breed

Figure 1 displays parasitaemia profiles of infection III (IL 1587) in individual N'Dama cattle. In this figure it can be readily observed that the most significant property in individual N'Dama parasitaemia profiles is the progressive length of the intervals between successive parasite waves during the five months of each infection.

We have statistically analysed the between-wave intervals in any single parasitaemia wave in individual N'Dama cows. This analysis shows that i) in each of the 3 recorded N'Dama infections the inter-wave intervals were significantly longer as parasitaemia proceeds; ii) the inter-wave intervals in infection IV were significantly larger than in infection III, but no significant difference in interval lengths is observed between the first and the third infection.

Our results may appear somewhat in disagreement with Paling *et al.* (1991a) who used the mean scores of the same parasitaemia for suggesting that N'Damas are characterized by their declining parasitaemia levels, as of day 35 of the first infection. However, it should be pointed out that if

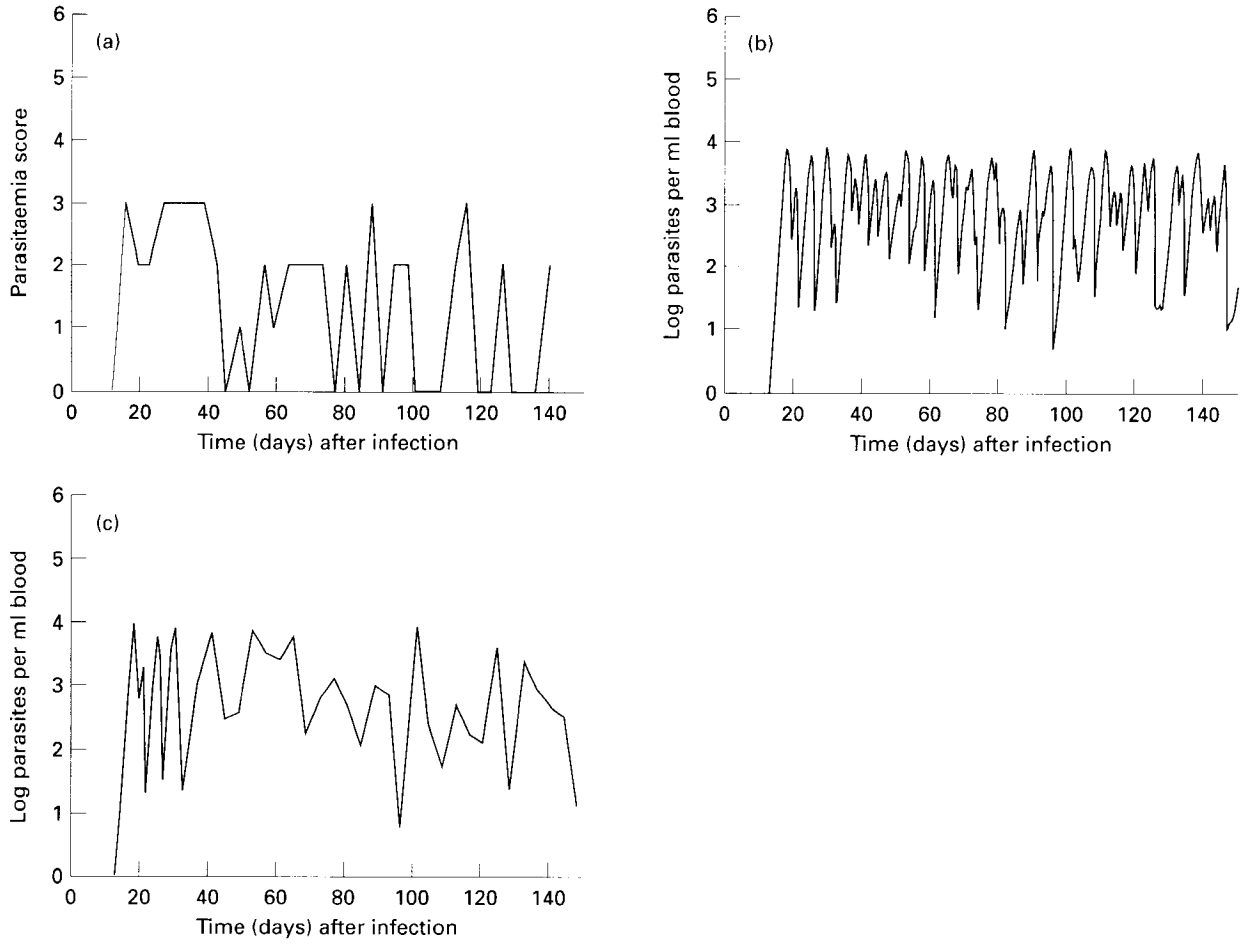

Figure 2 Effect of resolution in data monitoring: a. N'Dama ND5, even resolution (see text); b. simulation results of parasitaemia with even intervals of 6 h between monitoring. c. as in b, but with a monitoring resolution similar to that in the experiment (see text).

the intervals between waves in individual cattle increase as parasitaemia proceeds, parasites' waves are decreasingly likely to co-occur in different cattle. Averaging the group scores when waves coincidence is decreasing appears as lower average scores.

In the 13 Boran animals that were not treated with trypanocides during the experiment, i.e. were less susceptible to the disease, parasitaemia waves were always more dense than in the respective N'Damas. However, as the level of susceptibility in these animals is not clear, we did not feel justified in comparing these results and preferred to focus our analysis on the significant feature in these data, namely, that of decreasing frequency of waves in N'Dama.

Note that the decrease in the peaks observed in Figure 1 may result from the fact that in the first month of the infection, the number of parasites in blood was monitored once daily, and only twice weekly thereafter. One can speculate, then, that in late stages of parasitaemia, when waves were less frequent, the real peaks were increasingly missed, due to the 3, 4-day interval between successive monitoring. To demonstrate this argument we first plotted the parasitaemia profile of an arbitrarily selected N'Dama cattle, ND5, infected by *T. congolense* IL1587 from the original data (Figure 1d). Then we evened the resolution of data monitoring, so that throughout the period of the experiment we present data with a 3 and 4-day interval between records. In the newly made figure the previously appearing peaks on day 16 and on day 27 are not included, so that the curve seems flatter (Figure 2a). We therefore concluded that a high frequency of monitoring is necessary for any quantitative conclusion about the change in parasite levels to be drawn. This point is further illustrated in Figure 2b,c. Here an arbitrary parasitaemia taken from our simulations described below is plotted with six h intervals between monitoring points. Under this resolution of the monitoring the general trend in the plotted profile is non-

decreasing (Figure 2b); the same parasitaemia looks somewhat decreasing when it is plotted with intervals that are similar to those in the field experiments (Figure 2c).

Simulating parasitaemia in the natural host

Using the mathematical model we attempted to retrieve the main characteristic of Trypanosome infection in N'Dama. In particular we aimed at verifying which factors can modulate the length of parasitaemia wave intervals. In our model differences between hosts can take the form of differences in the maximal parasite intrinsic growth-rate, and in the carrying capacity of the host. In addition, hosts may vary in the pre-immune antibody repertoire and in VSG-specific and VSG-nonspecific immune cell proliferation-rate and mortality-rate, as well as in immunoglobulins secretion-rate and in cytokine secretion levels (Sileghem, De Baetselier and Hamers 1985).

Carrying capacity of the host

The carrying capacity measures the maximal density of parasites in blood. A large carrying capacity means that the parasite population can grow to large numbers before density begins to suppress population growth. Displayed below are simulation results of parasitaemia in two hosts, one in which maximal parasite density is 10^7/ml, as reported in mice, and one in which maximal parasite density is 10^4/ml, as in cattle (Paling *et al.* 1991a, Turner, Aslam & Dye 1995). Note that the first isolated wave of parasites, appearing in all our simulations, is in artifact of our arbitrary initial conditions and, hence, should be ignored (see 2·2).

In our results an upper limit of 10^7 parasites/ml leads to a very acute early parasitaemia, with dense high peaks, containing most of the antigenic repertoire (Figure 3a). The sharp decline in parasites' load, observed in Figure 3a, is due to the exhaustion of all effectively growing variants. This effect, which is due to the large numbers of co-emerging new variants is not observed in real-life, as such an acute parasitaemia probably leads to the death of the host (not simulated). In contrast, when the upper limit on parasite density is lower, resembling that in cattle (10^4/ml) an ordered parasitaemia is obtained, which progresses for an extended period (Figure 3b).

Intrinsic growth-rate

Comparing Figure 3b to Figure 4a–f one notes that a significant reduction in the growth-rate of the parasite yields only slightly lower and less dense parasitaemia peaks. In addition, when maximum growth-rate is 0·68, or less, parasitaemia has a variable duration, as the emergence of successfully growing variants is not guaranteed now. Most notably, results in Figure 4 show that, for a given growth-rate, the density of parasitaemia waves remains roughly constant. It should be mentioned here that parasite growth-rate in murine blood is estimated at around 0·1 (Turner *et al.* 1995). However, in our simulations such a low intrinsic growth-rate results in unrealistically short parasitaemias (not shown).

Increasing the variation in the variant-specific intrinsic growth-rate has the effect of upsetting the order of

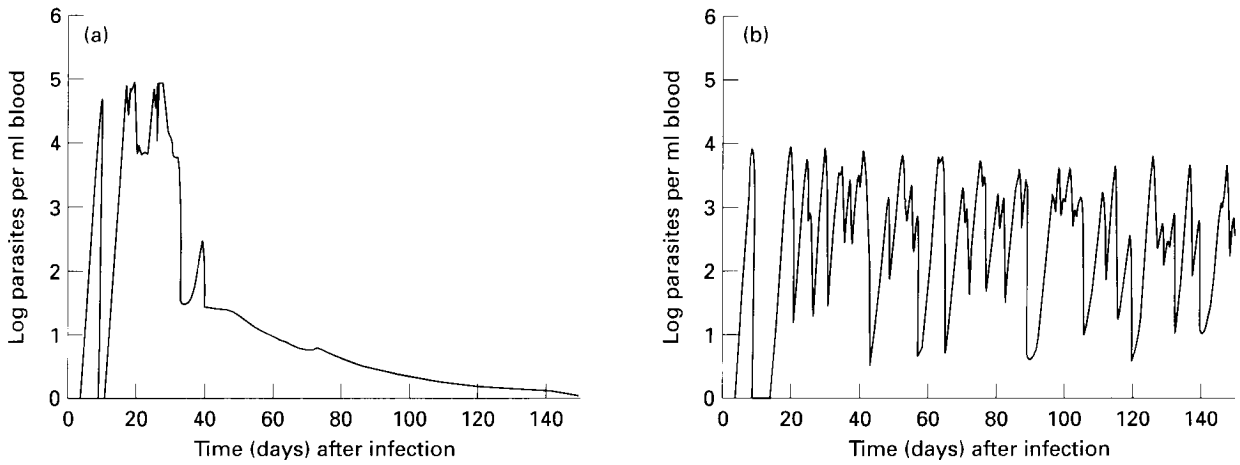

Figure 3 Effect of parasite density, K, on the parasitaemia profile. Simulation of parasitaemia in a single cattle. The following parameters were used: blood volume is 20 litres. Maximum intrinsic growth coefficients are 0·85/generation, and 0·2 /generation, for SE and DE, respectively. Each simulation time unit represents six h (one generation). B cell growth coefficient, r_B, is 0·52/generation. Maximum antibody secretion-rate, c_1, is 170/generation. The time-lag between B-cell stimulation and antibody secretion is 3 days. a $K = 10^7$; b. $K = 10^4$. For other parameters see Agur *et. al.* 1989.

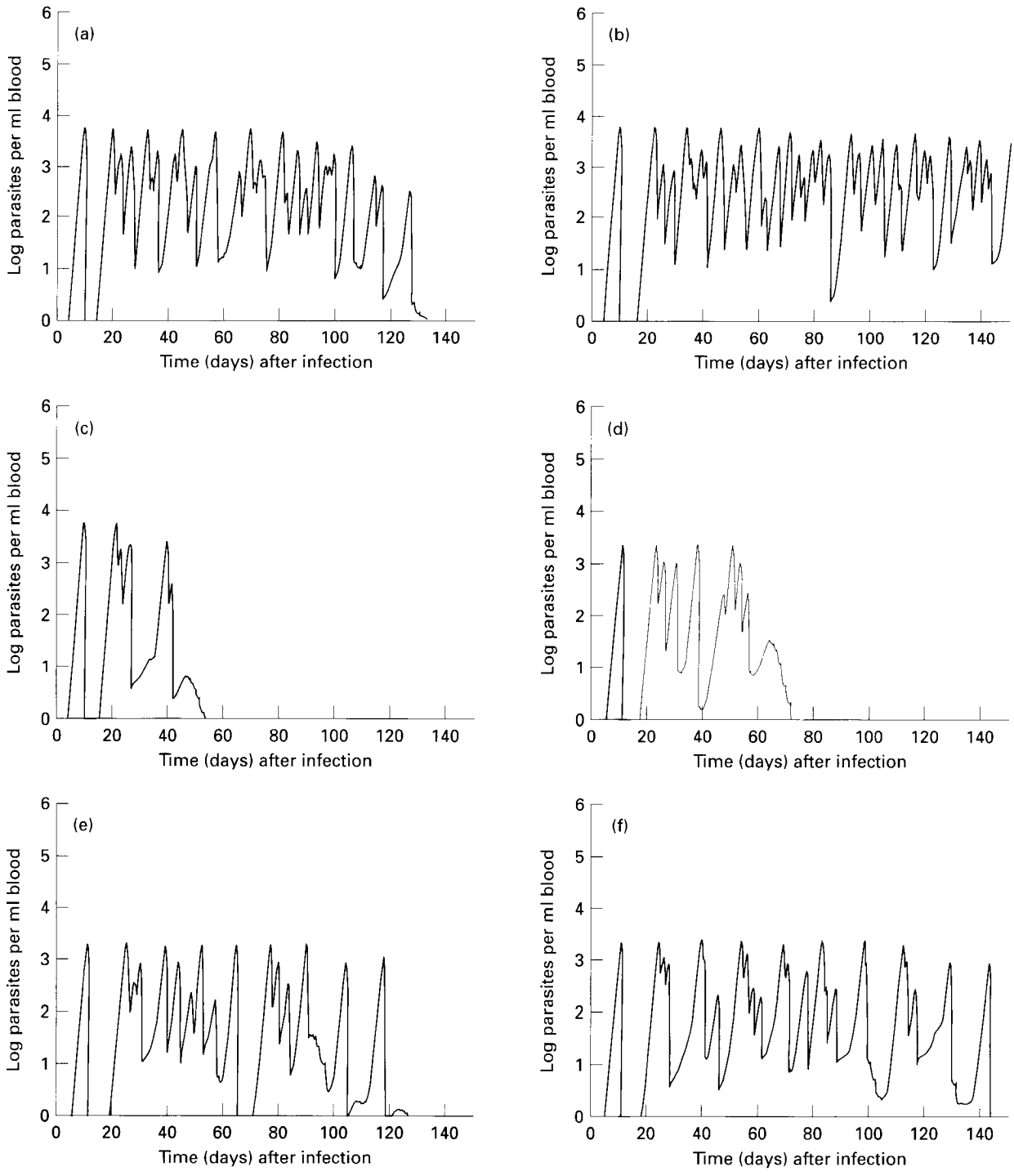

Figure 4 Effect of maximum parasite intrinsic growth-rate, r. Each curve represents a simulation of a single individual; a–c. Maximum growth-rate is, $r = 0{\cdot}68$; d–f. $r = 0{\cdot}51$. For other parameters see Figure 3b.

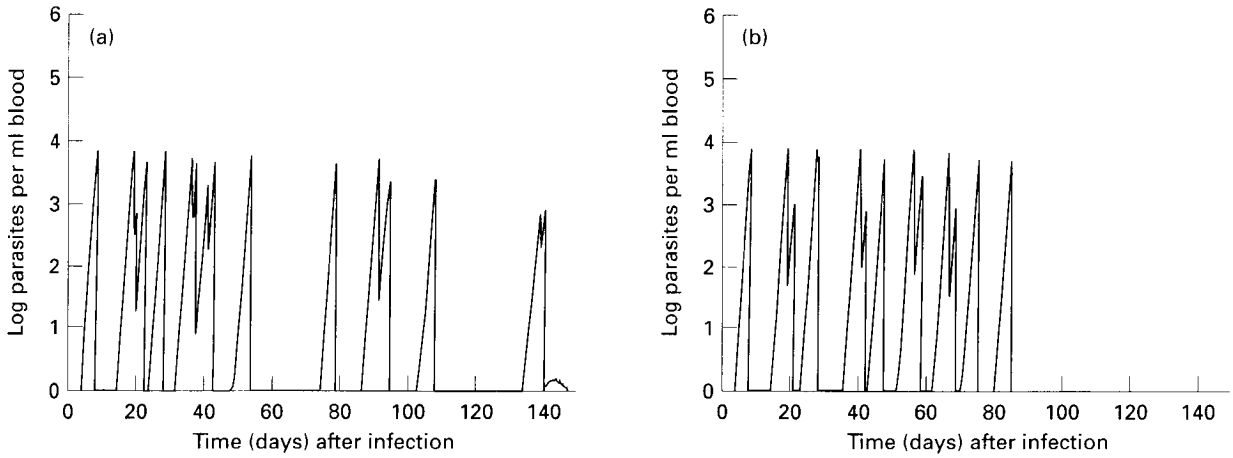

Figure 5 Effect of specific antibody secretion-rate, c_1. a. a hundred fold increase in antibody secretion-rate, $c_1 = 17*10^3/gen$. b. a two hundred fold increase in antibody secretion-rate, $c_1 = 34*10^3/gen$. For other parameters see Figure 3b.

parasitaemia and the characteristic structure of peaks, but it does not significantly alter the height of parasitaemia peaks or the rate of their appearance (results not shown). Note also that in real-life infections of different variants of *Trypanosoma brucei* have similar growth-rates, so that assuming a low variation in growth rate is also justified experimentally (Aslam & Turner 1992).

Affinity maturation

We varied the mean and the variance in the time-lag between antigen stimulation of B-cell proliferation and the onset of specific antibody secretion in order to take account of the possibility that the resistant breed is different in the quality of affinity maturation in the humoral immune response. We assumed that the time-lag is either constant, i.e. the same in all VSG-specific responses, or that it is a normally distributed random variable. Results suggest that a shorter time-lag, i.e. a more efficient humoral response, may lead to a less dense parasitaemia and to a meaningful reduction in parasitaemia length (not shown). When variance in this parameter is increased parasitaemia may be somewhat shortened and the intervals between peaks may be larger, but this effect is inconsistent. When the time-lag is larger than 3 days, i.e. efficient immune response is slow to arise (for example, due to a difficulty in isotype switching), parasitaemia is chronic, lasting very long periods (not shown).

Immune cell birth-rate and death-rate

We have simulated varying levels of B-cell replication-rate. Simulation results show that in general the effect of B-cell replication-rate is similar to the above-mentioned effect of the increase in the intrinsic growth-rate of the parasite (not shown). In addition we included in our model specific B-cell mortality rate (Eq. 2). The value of this parameter was assumed to be the same for all specific B-cells. We found that a significant B-cell mortality lengthened parasitaemia, due to the reappearance of early variants (not shown). However, this effect is associated with a very significant increase in basic levels of blood parasites, much above what has been observed in the experiments. For this reason we concluded that B-cell mortality-rate must be smaller than 0·1/parasite/generation, and, hence, this factor probably does not have any meaningful effect on the system under study.

VSG-specific antibody secretion-rate

A hundred fold increase in VSG-specific antibody secretion-rate resulted in narrower parasitaemia peaks and in disappearance of slowly growing variants (Figure 5a). The decrease in the frequency of parasitaemia waves here was due to our assumption that variant-specific intrinsic growth-rate is lower in late appearing variants. The increase in VSG-specific antibody secretion-rate also results in unrealistic parasitaemia: individual waves are discontinuous all along the infection. The reason is that the effect of VSG-specific antibody response is non-cumulative. In other words, large VSG-specific antibody secretion-rates have the same effect throughout the infection; they also result in suppression of lower titres of parasites in between the high peaks. A two hundred fold larger antibody secretion-rate has the effect of shortening parasitaemia: now the decline in parasitaemia waves is so rapid that the probability of a successful antigenic switch is much reduced (Figure 5b).

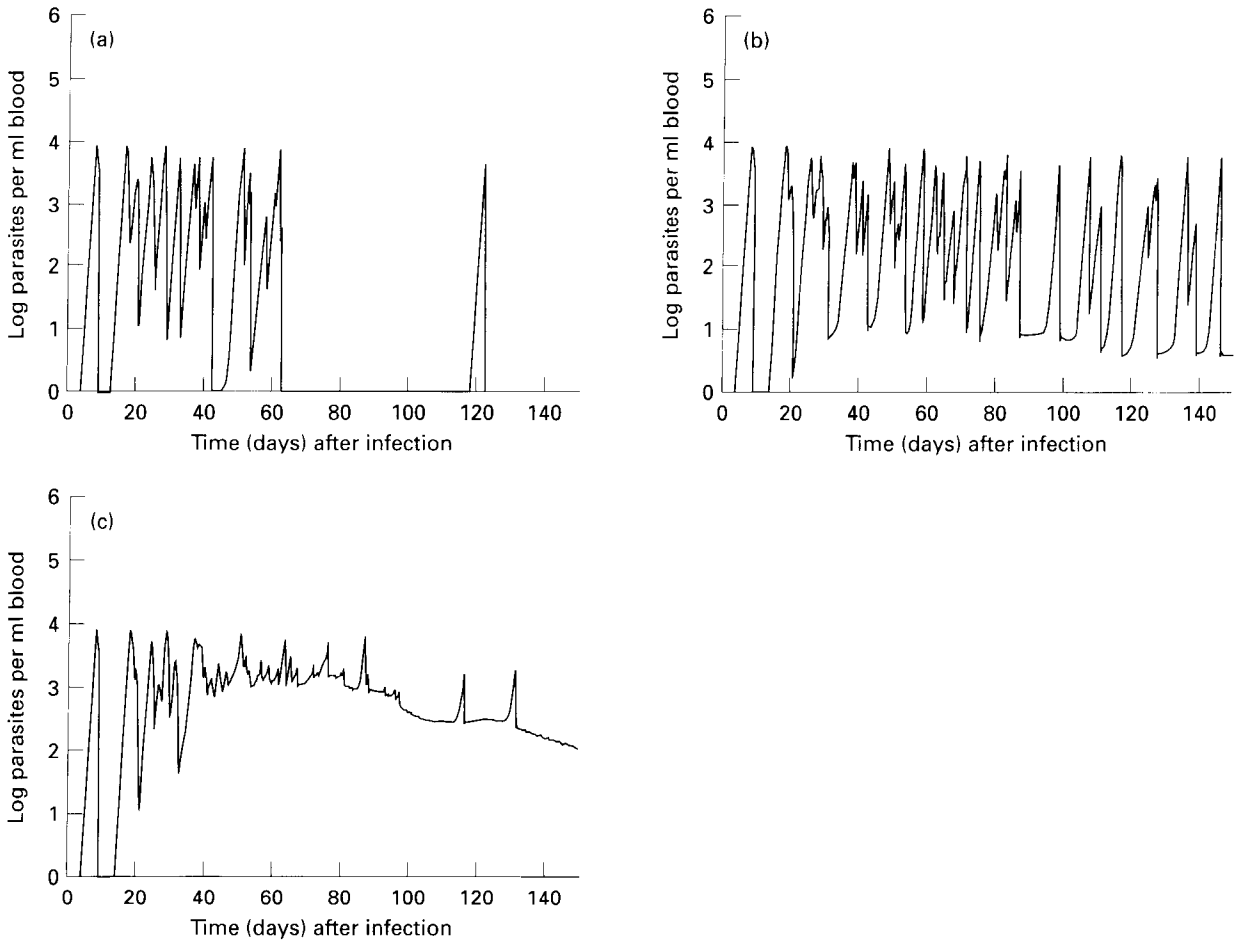

Figure 6 Effect of the efficacy of immune response against invariant antigens, C_{inv}. Initial proportion of anti-invariant B-cells is 0·01. a. $C_{inv} = 10$; b. $C_{inv} = 10^3$; c. $C_{inv} = 10^5$. For other parameters see Figure 3b.

Response to invariant antigens

We considered the response to an invariant parasite antigens, and the possibility that this process acts in synergy with the response against VSGs to generate the observed parasitaemia profiles. When making only a small proportion of the parasite's antigens, the invariant antigen will be presented to the immune cells in a much lower concentration than VSGs. In such a case the non-specific response would be slower than the specific one. We allowed for this effect by varying the efficacy of B-cells that produce antibodies against the invariant antigens (to be denoted *anti-invariant B-cells*). We did so by varying the number of parasites at which the rate of production of anti-invariant B-cells is half of the maximum-rate (Roelants & Pinder 1987). We also varied the initial proportion of anti-invariant B cells.

Results in Figures 6–7 are unique in their ability to retrieve the main features of experimental infections: they show a block of high titres of parasites during the first stage of the infection, and increasingly large intervals between parasitaemia waves thereafter. The magnitude of this effect is a function of the initial proportion of anti-invariant B-cells and of the efficacy of anti-invariant response.

In Figure 6, the initial proportion of anti-invariant B-cells is 0·01, but the efficacy of this response is made to vary. One notes here that if the response to the invariant antigens is unrealistically efficient (20 parasites/ml suffice for maximizing anti-invariant B-cells replication-rate) parasitaemia is relatively short with an eventual emergence of a successful variant (Figure 6a). When 2000 parasites/ml are required for maximizing rate of anti-invariant B cells replication-rate, parasitaemia lasts ca.145 days, waves being increasingly sparse (Figure 6b). When anti-invariant B cells replication-rate is maximized only at 20,000 parasites/ml, parasitaemia stays at unrealistically high levels (Figure 6c).

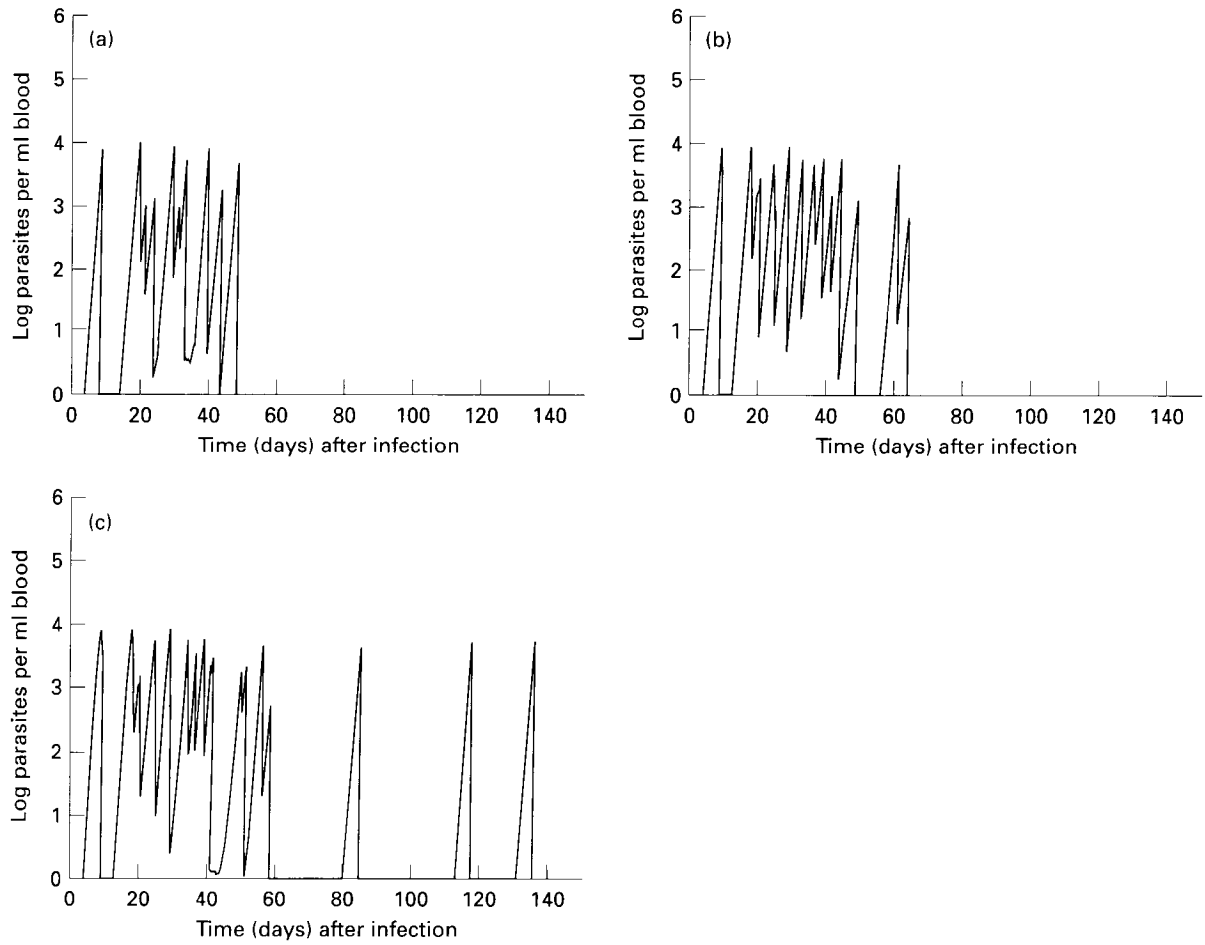

Figure 7 As in Figure 7, but initial proportion of anti-invariant B-cells is .10. a. $C_{inv} = 0$; b. $C_{inv} = 10^3$; c. $C_{inv} = 10^5$. For other parameters see Figure 3b.

Figure 7 represents simulations that are similar to those presented in Figure 6, except that here the initial proportion of anti-invariant B-cells is ten fold higher. Now the efficacy of the anti-invariant response is clearly reflected in the change in the frequency of parasitaemia waves. This simulation resembles experimental parasitaemia more than any of the above. Thus the latter results seem to suggest that if all other parameters are in the right order of magnitude, then 10%, rather than 1%, of B-cells respond to invariant parasite's antigens.

DISCUSSION

The present study showed that the hallmark of *T. congolense* infection in trypanotolerant cattle is the increasing intervals between parasitaemia peaks. Previously observed properties in N'Dama that were based on analysis of group averages, notably the decreasing parasitaemia levels, seem less significant when individual parasitaemias are considered. We then used mathematical modeling to check which factors are likely to progressively increase the inter-wave intervals in parasitaemia. Six different factors were studied: carrying capacity of the host, intrinsic growth-rate of the parasite, affinity maturation in the host immune system, immune cell birth- and death-rate, level of antibodies to variant surface glycoprotein and level of antibodies to invariant antigens. Simulations of the mathematical model showed that the first five factors are not likely to be responsible for the spacing of parasitaemia waves whereas the sixth one yielded results consistent with experimental data.

More specifically, we showed that differences between hosts in the maximal parasite density, or in parasite's growth-rate, are not likely to play a significant role in determining the parasitaemia patterns observed in N'Damas chronic phase of infection. It appears, then, that our study

does not support the recent speculation that a molecule called trypanosome lytic factor (TLF) may be the basis for differential resistance in cattle (Schmidt 1995). Note that *T. brucei's* resistance to such a human factor has been recently demonstrated (Smith, Esko & Hajduk 1995).

Moreover, we showed that affinity maturation of the immune response, immune cell birth and death rate, levels of antibodies to variant surface glycoprotein are not likely to control the N'Damas chronic phase of infection. These results support the assertion that antibody response to surface-exposed epitopes plays no role in cattle trypanotolerance (Williams *et al.* 1996). In contrast, our results could not exclude the hypothesis that efficacy of the immune response to invariant trypanosome antigens modulates the increase in the duration of the inter-wave intervals. We suggest, then, that the immune response to invariant trypanosome antigens may be involved in the acquired immunity to trypanosomiasis in N'Dama. The explanation of the mathematical results is the following: in our model the number of anti-invariant immune cells produced at any time is proportional to the total number of parasites at that time. As the anti-invariant immune cells have a non negligible longevity and since they are effective against all variants, their overall number, and, consequently, the efficacy of the anti-invariant immune response, tends to increase over time. This is not so for the VSG-specific response, where the efficacy of subsequent individual VSG-specific responses are not additive.

Our results support Authié *et al.* (1993a,b) who observe in N'Dama a relatively high IgG1 response to two non-VSG proteins. One protein, immunodominant in *T. congolense* (69 kD), is similar in several ways to heat-shock proteins and is present in all stages of the life-cycle of the parasite. It is thought that heat-shock proteins act as molecular chaperones, facilitating the correct folding of newly synthesized proteins. As such they may have a vital role in the synthesis of VSG molecules. If this is the case, then immune reaction against the 69 kD protein must interfere with the ability of trypanosomes to maintain an intact surface. The second protein, a 33 kD, is a cysteine protease, an enzyme which helps to break down proteins, and may facilitate secretion of lymphokines.

Olsson *et al.* (1991) suggest that *T. brucei* induces production of IFN-γ, which, in turn, promotes parasite growth, either directly, by increasing the growth-rate of the parasite, or indirectly, via its effects on the immune system. However, as was already stated, our results suggest that a higher growth-rate of the parasite, lower rates of B-cells production or lower rates of VSG-specific antibody secretion are not likely to be responsible for the N'Dama decreasing frequency of parasitaemia waves.

Moreover, our analysis has revealed that in N'Dama the overall duration of inter-wave intervals in the last of the four infections (by clone IL 2079) is significantly larger than in the previous ones; no significant statistical difference was observed between the overall duration of the inter-wave intervals of the third and the first infections. This result may indicate that previous experiences of different clones of *Trypanosoma congolense* can alter the intra-host dynamics of the current infection. It is interesting to speculate that the interaction is mediated by immune responses to shared non-VSG epitopes. This is consistent with the theory that dominant antigens will not be shared between strains or clones, but that these may nonetheless be loosely coupled by weak responses against conserved antigens (Gupta *et al.* 1996).

Our model refers to the cellular immune response only in an indirect manner. The main reason for that is the need to keep it tractable to analysis. The existence of a more complex mechanism than those described above, and the involvement of other factors, such as antigen presenting cells or T-cell help cannot be ruled out by our model. Nevertheless we believe that the conclusion of the present paper, notably, that the immune response to invariant antigens of the parasite has a cumulative effect on parasitaemia patterns, point out a robust property of infection in trypanosomiasis and is not dependent on the assumptions concerning mechanisms underlying these responses.

In order to discriminate between the various processes that may lead to the patterns of increase in the inter-wave period, we have extended and modified a previous mathematical model of antigenic variation in trypanosomes. A prediction of the original model that the course of antigenic variation must involve double expressor switch intermediates has received considerable experimental support (see above). Nonetheless, it will be interesting to check to what degree the conclusions of the current exercise depend upon this assumption.

The mathematical methods employed here permitted us both to speculate on the qualitative nature of this immune response and also to quantify it: our simulations suggest that 10% of the hosts B-cells respond to invariant antigens. It would be interesting to check experimentally whether the differences between resistant and susceptible cattle may be ascribed to such quantitative aspects of the immune response.

It might be possible to further verify our theoretical conclusions in an experimental set-up in which antibody response to an invariant antigen could be increased without affecting the VSG-specific response. This can be done, for example, by immunizing the cattle with a trypanosome lysate before infection. An experiment of this kind will be an important step towards understanding trypanotolerance in cattle.

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