43

Serum Haemolytic Complement Activity and C3 Levels in Bovine Trypanosomosis under Natural Conditions of Challenge – Early Indications of Individual Susceptibility to Disease

E. AUTHIÉ* and T. POBEL

C.R.T.A. B.P. 454 – Bobo-Dioulasso, Burkina Faso (West Africa)

3 0 JAN. 1996

O.R.S.T.O.M. Fonds Documentaire
N° : 43717
Cote : B ex 1.

ABSTRACT

Authié, E. and Pobel, T., 1990. Serum haemolytic complement activity and C3 levels in bovine trypanosomosis under natural conditions of challenge – early indications of individual susceptibility to disease. *Vet. Parasitol.*, 35: 43–59.

Twenty-five Baoule (*Bos taurus*) and 12 Zebu (*Bos indicus*) cattle, which were part of an experiment aimed at characterizing cattle for resistance to trypanosomosis under natural challenge in Burkina Faso, were monitored for complement levels. Total haemolytic activity of the alternative complement pathway and C3 in sera taken weekly were estimated. The results were analysed in relation to the course of the disease, parasitological data, packed red cell volume (PCV) and body weight. All the animals became infected with *Trypanosoma vivax* and/or *T. congolense*. The Zebu had to be treated with Berenil (Diminazene aceturate, Hoechst, W. Germany) after a mean period of 5 weeks of infection, whereas 7 of the 25 Baoule remained in good condition throughout the experiment. The remaining 18 Baoule required treatment after a variable period of infection. There was a decrease in haemolytic complement activity (HC′) as well as in C3 levels, which coincided with the first detection of parasites in the blood. The titres in the Zebu fell to 10–20% of pre-infection level within 2–3 weeks and they showed no tendency towards regaining normal levels. The drop in complement in the Baoule was less pronounced and was in most cases followed by an increase approaching normal values. In these animals, the complement level in early infection was found to depend on the intensity of parasite load and on the control potential of each individual. There was a significant correlation between minimum complement activity (min. HC′), minimum C3 (min.C3) and minimum PCV (min.PCV) in early infection. These three parameters correlated with individual resistance and might, therefore, be useful criteria for the identification of the most resistant individuals within a trypanotolerant breed.

*Present address: International Laboratory for Research on Animal Diseases, P.O. Box 30709, Nairobi, Kenya.

INTRODUCTION

African trypanosomosis impedes the use of 7 million km^2 of land adequate for cattle raising and is a major constraint to livestock development. Since none of the control methods for bovine trypanosomosis is entirely satisfactory, increasing consideration is being given to "trypanotolerant" breeds. This trait is generally attributed to the taurine breeds of cattle in West and Central Africa, namely the N'Dama and the West African Shorthorn, which possess an ability to survive and be productive in tsetse-infested areas without the aid of trypanocidal drugs (Murray et al., 1982). This natural resistance to trypanosomosis however has been reported to be an individual rather than a breed characteristic (Roelants et al., 1983, 1987). The underlying mechanisms are still largely unknown.

Complement is involved in the defense against a number of pathogens and recently there has been an increasing interest in the interactions between complement and protozoa, such as *Babesia* sp. (Mahoney et al., 1980; Goodger et al., 1981), *Leishmania* sp. (Mosser and Edelson, 1985), *Entamoeba histolytica* (Meri et al., 1985; Reed et al., 1986), *Plasmodium* sp. (Cox and Hayes, 1985), *Trypanosoma cruzi* (Kipnis et al., 1985; Sher et al., 1986), *T. lewisi* (Albright and Albright, 1985; Sturtevant and Balber, 1987), *T. brucei gambiense* (Devine et al., 1986) and *T. congolense* (Tabel, 1982; Malu and Tabel, 1986).

Experimental trypanosomosis in susceptible cattle is characterized by a persistent and marked hypocomplementaemia (Kobayashi and Tizard, 1976; Nielsen et al., 1978; Rurangirwa et al., 1980) which is generally attributed to consumption by immune complexes containing parasite antigens.

The objective of the work described here was to investigate the kinetics of serum complement during natural infection with *T. vivax* and *T. congolense* in cattle of differing susceptibility, in order to determine whether there was a relationship to trypanotolerance.

MATERIALS AND METHODS

Experimental design

The present study was part of an experiment conducted by Centre de Recherches sur les Trypanosomoses Animales, (C.R.T.A.) from April to October 1987, aimed at characterizing cattle for resistance to trypanosomosis under natural conditions of challenge. Details of this experiment will be presented elsewhere in a separate publication (Clausen et al., in preparation). One hundred cattle, both Zebu (*Bos indicus*) and Baoule (*Bos taurus*) breed, were transported to Satiri, a bushy area 50 km northeast of Bobo-Dioulasso. Monthly entomological surveys revealed the presence at this site of *Glossina morsitans submorsitans*, *G. tachinoides* and *G. palpalis palpalis* at very variable relative

densities, with infection rates ranging from 4 to 33%. Cattle were monitored for general condition, parasitaemia, haematological parameters and immune response to trypanosomal antigens (Clausen et al., in preparation). For investigations on complement, 25 Baoule aged 18–24 months (12 males and 13 females) were selected and 12 Zebu (10 males, 2 females) of the same age were randomly selected.

Origin and history of animals

Seven of the 25 Baoule selected for investigation on complement had been bought from another infested area (Banfora), 80 km south of Bobo-Dioulasso. Eighteen had been born on the C.R.T.A. farm (20 km north of Bobo-Dioulasso) 17 of which were reared in a fly-proof stable and had previously been experimentally infected at the age of 1 year with a local stock of *T. congolense* (Duvallet et al., 1988). The remaining Baoule was reared on the C.R.T.A. farm under low trypanosomosis risk. As for the Zebu, seven were from the C.R.T.A. farm, while five others were from the experimental area.

Like other animals on the experiment, they were kept on the C.R.T.A. farm for >1 month prior to exposure, where they were treated against endo- and ecto-parasites, and vaccinated against major tropical diseases. All received Berenil (Hoechst A.G., 7 mg kg^{-1}, intramuscularly) 40–60 days prior to their transport to the experimental area (Clausen et al., in preparation).

Cattle management

The selected 37 animals were part of two herds which were grazing throughout the day under herdsmen's supervision and were kraaled at night. Full details of the management and monitoring procedures for the animals are given elsewhere (Clausen et al., in preparation).

Clinical examinations and blood smears for the detection of haemoparasites were made weekly on all animals. At monthly intervals, body weights were recorded and faeces were examined for worm egg counts. Ticks on cattle were manually removed by the herdsmen, three times per week.

Monitoring of trypanosomosis

Blood samples were collected twice weekly for the determination of packed red cell volume (PCV) and parasitaemia. Microcentrifugation and phase contrast buffy coat examination (Murray et al., 1977) were performed on site. In the present study, the same parasitaemia scoring scale as previously used at C.R.T.A. was adopted (Roelants et al., 1983).

Infected animals were treated with Berenil (7 mg kg^{-1}) when their condition had deteriorated to the extent that they were unable to follow the herd for feeding and watering. Treatment was repeated each time a susceptible animal

was found to be reinfected, regardless of its clinical condition. The experiment was terminated after 200 days of exposure and all surviving animals were treated.

Complement assays

Blood for complement assays was collected weekly from each of the 37 animals by jugular vein puncture, kept on ice for transport to the laboratory, where it was allowed to clot for 30 min at 37°C, then centrifuged for 30 min at 4°C (1500×g). Sera were stored at −70°C until used.

Assessment of the total haemolytic activity of the alternative complement pathway (ACP) was made by a haemolytic assay which is described in full elsewhere (Authie and Boulard, in preparation). Briefly, the number of 50% haemolytic complement units per millilitre of serum (HCU 50 per ml) was estimated by an adaptation of the method of Barta and Barta (1972) to a micromethod using rabbit red cells which are natural activators of the ACP and are highly susceptible to bovine complement (Boulard and Bencharif, 1984). Quantitative measurement of C3 was performed by the radial immunodiffusion method (Fahey and McKelvey, 1965) with specific antiserum obtained commercially (Cappel Laboratories, U.S.A.). The C3 concentrations were expressed as percentages of a reference serum pool from three healthy Baoule cattle.

Statistical analysis

HCU 50 ml^{-1} titre determination and statistical analysis were performed using programs created on a PC 1400 pocket computer (Sharp). A one way analysis of variance was used to compare groups of animals. Where statistically significant differences existed at the 5% level, a multiple range test (least significant difference) was used to detect which groups were different. The Student's t-test was used when comparing the two breeds of cattle (Baoule versus Zebu). The relationships between the different parameters were studied by means of linear correlations.

RESULTS

Assessment of resistance

All the animals became infected with trypanosomes, and of the 12 Zebu one died and 11 required trypanocidal treatment after 35±10 days of infection. The Baoule showed various levels of resistance; seven maintained good condition without treatment throughout the experimental period, whereas the remaining 18 had to be treated. Amongst the 18 Baoule that required treatment,

some developed severe disease and received early treatment, while others sur-
vived several months (up to 6 months) before requiring treatment.

To allow statistical analyses, resistance in the Baoule was quantified by
measuring the "duration of the disease", i.e., the time from the first detection
of parasitaemia to treatment or death in the sensitive animals, or to termina-
tion of the experiment in the ones which resisted the disease. The average
duration of the disease in the animals which required treatment was 11 weeks.
Thus the Baoule could be divided into three groups: "highly susceptible Baoule"

TABLE 1

Comparison between groups of cattle for three indicators of resistance

Group of cattle (number of animals)	Duration of the disease[1] (days)		Total survival time[2] (days)		Duration of maintainance of body weight[3] (weeks)	
	Mean	SD	Mean	SD	Mean	SD
Zebu (12)	34.9[a]	10.6	59.7[a,b]	15.4	4.5[a]	3.0
Highly susceptible Baoule (12)	46.4	18.9	98.6[bc]	46.3	5.7[c]	4.7
Baoule of intermediate susceptibility (6)	130.5	41.0	175.0[c]	36.1	15.7[c]	9.0
All susceptible Baoule (18)	71.7[ad]	48.9	121.7[ad]	54.2	8.0[d]	4.6
Resistant Baoule (7)	157.8[d]	26.9	196.0[d]	–	23.4[ad]	6.8

[a-d]Means within an effect with a common superscript differ significantly ($P < 0.02$).
[1]Time from the first detection of parasitaemia to treatment or death in the susceptible animals,
or to termination of the experiment in those which resisted the disease.
[2]Total time an animal could survive under trypanosomosis challenge.
[3]Time each animal could maintain its body weight at $> 95\%$ of its initial value.

TABLE 2

Changes in body weight at the time of treatment or at termination of the experiment (200 days
of exposure)

Group of cattle (number of animals)	Pre-infection body weight (kg)		Change during the experiment (kg)	
	Mean	SD	Mean	SD
Zebu (12)	195.3	39.4	−27.4	15.3
Susceptible Baoule (18)	153.7	26.0	−23.6	15.6
Resistant Baoule (7)	141.4	24.5	+8.3	18.1

(HSB) which survived < 11 weeks once infected (12 animals); Baoule of intermediate susceptibility (ISB) which survived > 11 weeks (six animals); resistant Baoule (RB) for which no treatment was required during the 27 weeks of experiment (seven animals), (see Table 1).

Resistance was also assessed by the total time an animal could survive under trypanosomosis challenge without requiring treatment, (see Table 1).

Finally, evolution of body weight was monitored as a measure of resistance which is related to productivity. The sensitive Baoule (SB, which included the HSB and ISB) and the Zebu had lost ~ 15% of their weight at the time of treatment, while five of seven resistant Baoule gained weight during the experiment (Table 2). The time each animal could maintain its body weight at > 95% of its initial value was determined, (see Table 1).

The Baoule which required treatment (SB) were different from the resistant Baoule (RB) for all the three criteria used to measure resistance. Total survival time and duration of maintenance of body weight also gave significant
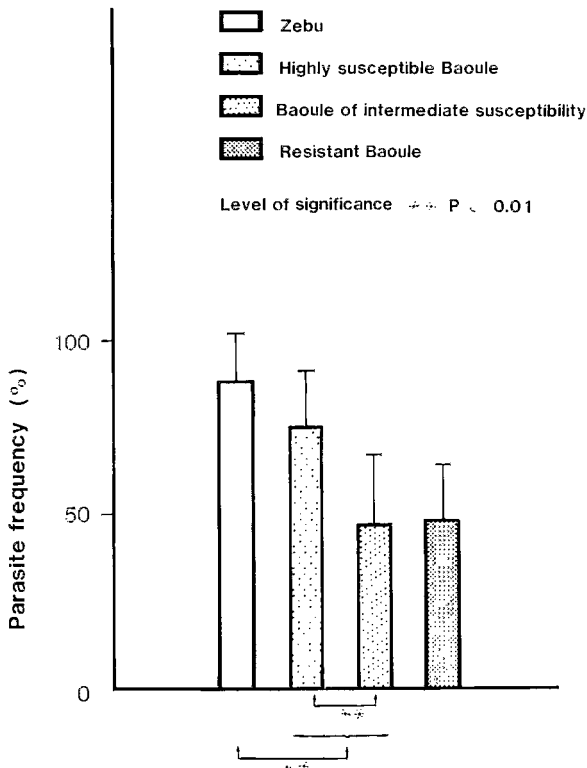

Fig. 1. Comparison between groups of cattle for parasite frequency (number of detected parasitaemia over the number of examinations made following the first positive detection).
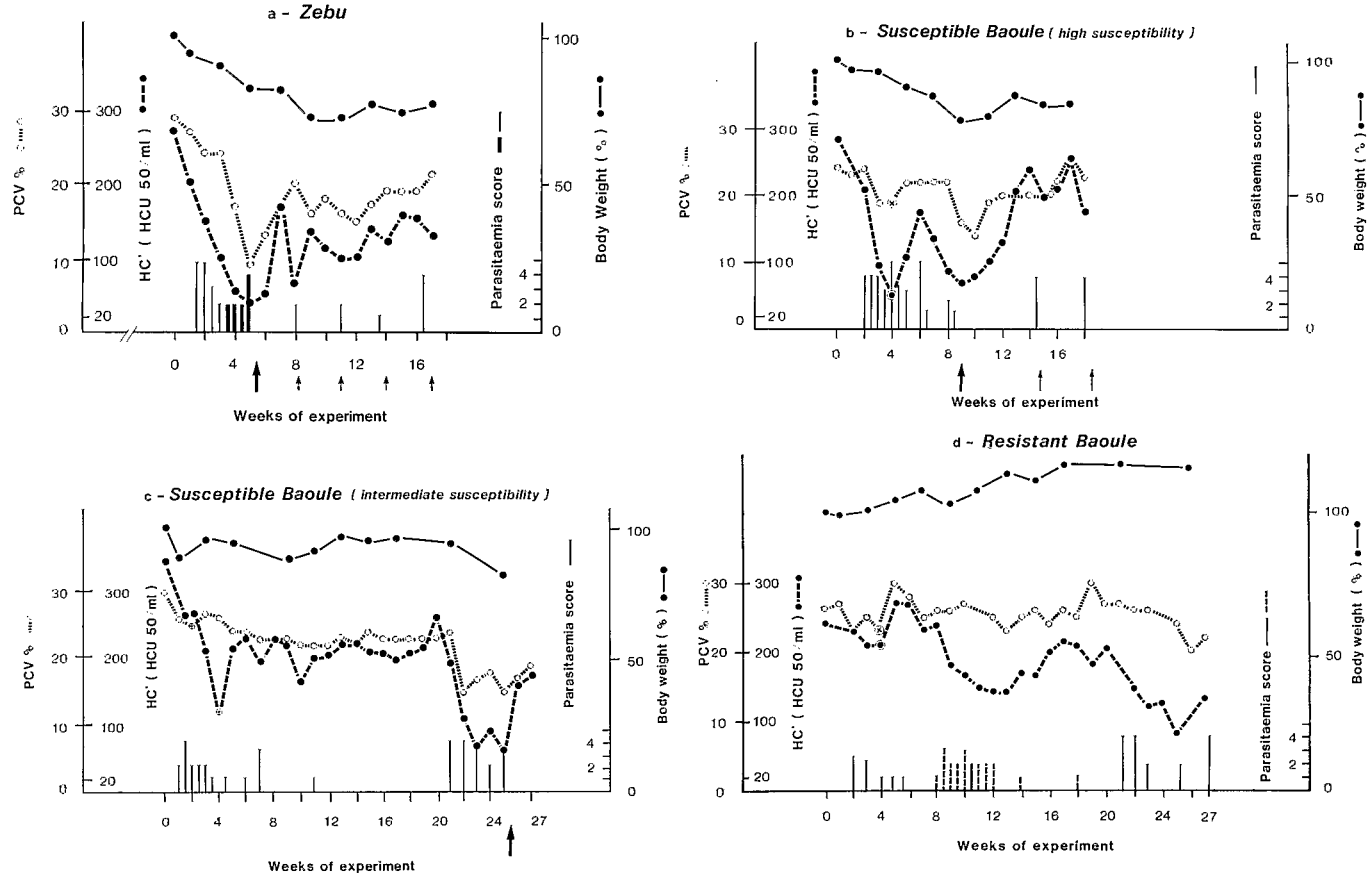
Fig. 2. Changes in haemolytic complement activity (HC'), packed cell volume (PCV) and body weight (% of pre-infection weight) in different individuals). (a) Zebu; (b) highly susceptible Baoule; (c) Baoule of intermediate susceptibility; (d) resistant Baoule. ⇈ treatments (Berenil 7 mg kg^{-1}); | $T.\ vivax$; ¦ $T.\ congolense$; ▌ $T.\ vivax + T.\ congolense$. Parasitaemia score: +1: 10^3–10^5 ml^{-1}; +2: 10^5–10^6 ml^{-1}; +3: 10^6–10^7 ml^{-1}; +4: 10^7–10^8 ml^{-1}; +5: $> 10^8$ ml^{-1}. ○ lowest value of PCV in early infection (min.PCV); ◉ lowest value of complement activity in early infection (min.HC').

differences between HSB and ISB, split from SB on the basis of the duration
of disease (Table 1).

Parasitological data

Trypanosomes were first detected in two Baoule after 11 days of exposure
in the tsetse-infested area. Within 6 weeks, all the Zebu were infected, while
10 Baoule remained aparasitaemic. However no statistical difference was found
between the groups with regard to the pre-patent period, which was highly
variable. Parasites were predominantly *T. vivax* (22 out of 37 were *T. vivax*
alone and 15 were mixed infections, with *T. vivax* first in 14 of them, then *T.
congolense*). The same proportion of mixed infections was seen in both the
Baoule and the Zebu.

Parasite frequency (i.e., the number of times parasitemia was detected over
the number of examinations made following the first detection) was higher in
the Zebu (0.88 ± 0.14) than in the SB (0.66 ± 0.22, $P < 0.01$). There was no
statistical difference between SB (0.66 ± 0.22) and RB (0.48 ± 0.16). Parasite
frequency was higher in the HSB than in the ISB, but no difference was found
between ISB and RB (Fig. 1). There was a negative correlation between par-
asite frequency and the duration of the disease in the Baoule ($r = -0.69$,
$P < 0.001$).

The Zebu had higher levels of parasitaemia than even the highly sensitive
Baoule at the time of first detection ($P < 0.01$), but no difference was seen
between the Baoule sub-groups. The mean levels of parasitaemia detected over
the infection period were 10^5-10^6 ml^{-1} in both Zebu and Baoule, the mean
score being just significantly higher in the Zebu than in all Baoule ($P < 0.05$),
but not different among the Baoule. There was a general relationship between
parasite frequency and mean level of detected parasitaemia ($r = 0.60$, $P < 0.01$).
However unlike parasite frequency, the mean level of parasitaemia did not
correlate with the duration of the disease in the Baoule.

Complement

There was a drop in total haemolytic complement (HC') as soon as trypan-
osomes were first detected in the blood and in some animals a short time before
(Fig. 2a–d). Haemolytic complement activity in the Zebu fell by $80 \pm 12\%$ of
the initial value within 2–3 weeks of infection and showed no tendency towards
recovery. The drop in the Baoule was less severe and was followed by an in-
crease towards regaining normal values, except in the two animals which were

TABLE 3

Mean level of haemolytic complement activity (HC') and mean packed cell volume (PCV) during infection

	HC' level (HCU 50 ml^{-1})		PCV (%)	
	Mean	SD	Mean	SD
Zebu	82.4[a]	29.8	21.7	2.6
Highly susceptible Baoule	137.2[a]	36.0	21.9	2.7
Baoule of intermediate susceptibility	165.8	26.9	23.5[b]	3.5
All susceptible Baoule	146.7	35.4	22.4[a]	3.0
Resistant Baoule	152.4	37.4	26.9[ab]	2.3

[ab]Means within an effect with a common superscript differ significantly ($P < 0.02$).

the most susceptible. At the time of treatment or death, SB had a higher HC' level (83 ± 37 HCU 50 ml^{-1}) than the Zebu (48 ± 22 HCU 50 ml^{-1}, $P < 0.02$). After treatment, HC' started to rise, but reinfection or relapse caused another decrease, although each time it was less pronounced. Complete return to pre-infection levels after treatment required 1–3 months.

The mean complement level was calculated over the infection period, i.e., from the first detection of parasitaemia to treatment or to termination of the experiment. Even the most susceptible subgroup of the Baoule (HSB) had a higher mean complement level than the Zebu. No significant difference was seen within the Baoule group (Table 3). The mean level of HC' correlated negatively with parasite frequency ($P < 0.01$) and the mean level of parasitaemia ($P < 0.05$).

The initial drop in HC' had a negative correlation with the duration of the disease in the Baoule ($P < 0.05$). A closer relationship with the resistance of individual Baoules ($r = 0.60$, $P < 0.01$) was shown by analysis of the residual level of complement during the first onset of parasitaemia, instead of the initial drop (see Fig. 2). This residual complement value (min.HC') was nearly twice as high in the ISB (174 ± 72 HCU 50 ml^{-1}) as in the HSB (89 ± 38 HCU 50 ml^{-1}, $P < 0.01$), but no difference was observed between ISB and RB (Fig. 3).

There was a significant relationship between the frequency of parasite detection during the first 3 weeks and min.HC' value, or between the number of positive detections until the minimum value was reached and min.HC' ($P < 0.01$, Fig. 4). This initial frequency did not have any effect on the duration of the disease ($P > 0.05$). Therefore in order to minimize the influence of the parasite load on the fall in complement, we sought to weight min.HC' by a
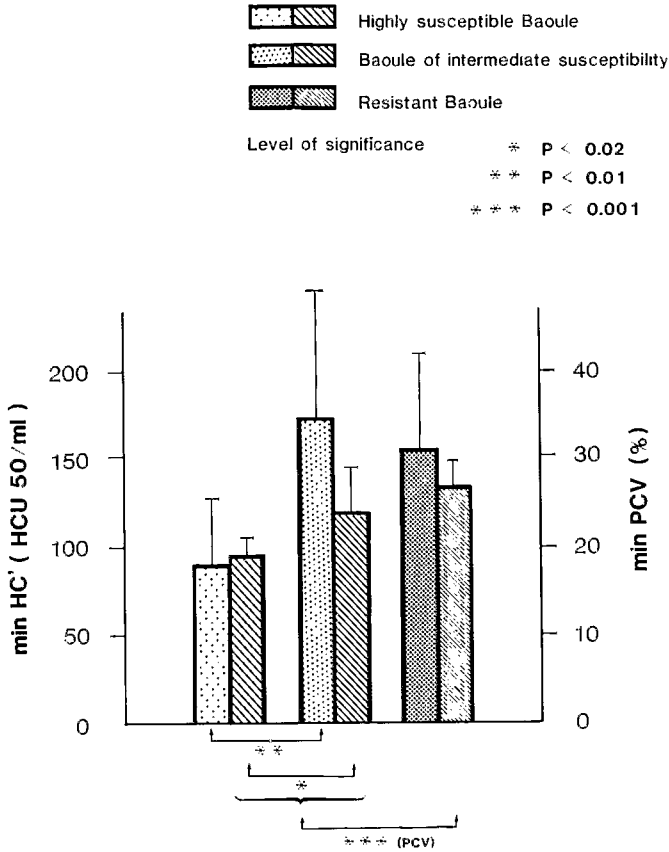
Fig. 3. Comparison between groups of Baoule for the lowest value of PCV (min.PCV, ▧) and haemolytic complement activity (min.HC′, ▨) in early infection.

factor related to the number of positive detections before reaching min.HC′ value (Fig. 4). This corrected minimum haemolytic complement activity (C min.HC′) was even more closely correlated with resistance in the Baoule (Fig. 5: $r=0.75$, $P<0.001$ for duration of the disease; $r=0.5$, $P<0.02$ for duration of maintenance of body weight) and the difference between HSB and ISB was more pronounced ($P<0.001$).

Min.HC′ was reached after a mean duration of infection of 11.6 ± 9.7 days.

Preliminary data had provided an indication that the fall in haemolytic complement was paralleled by a fall in C3 level. C3 was therefore determined in all the Baoule from the onset of infection until the lowest value (min. C3) could be identified. This occurred almost at the same time of minimum complement activity (10.4 ± 9.0 days) and it was closely correlated with min.HC′ ($r=0.93$, $P<0.001$). It was also related to duration of the disease ($r=0.54$, $P<0.01$). Since it was also associated with parasitic multiplication, we weighted it in the
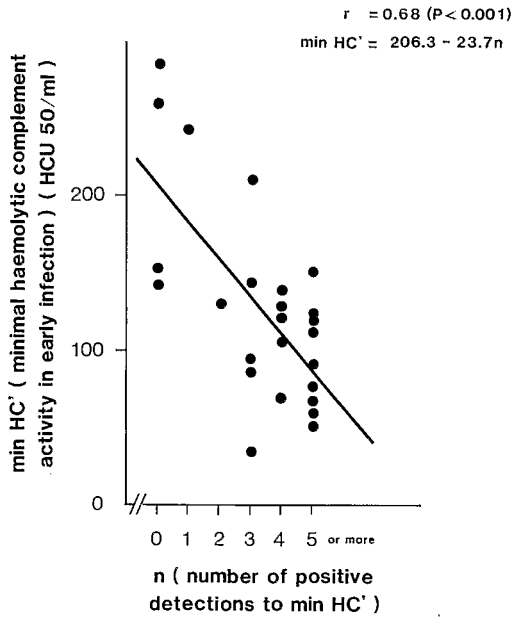
Fig. 4. Relationship between parasite load and initial drop in complement activity in Baoule. Correction of HC' to minimize the effect of parasite load on HC' value:

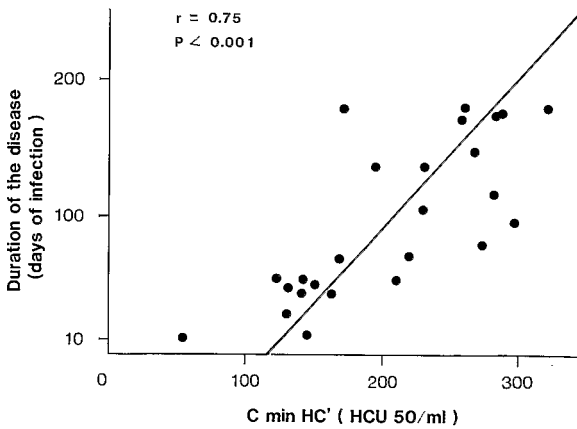$$\text{Corrected HC}' = \frac{206}{206 \times 24n} \times \text{actual HC}'.$$

Fig. 5. Relationship between corrected minimum haemolytic complement activity (C min.HC') and the duration of the disease in Baoule.

TABLE 4

Correlation between parameters of disease in the Baoule

	Abbreviation	PP	DD	DS	DBW	Mean PAR	FQ	Mean PCV	Mean HC	Min.PCV	Min.HC	Min.C3	fq
Pre-patent period (pp)	PP			**									
Duration of the disease	DD			***	***	***	**			***	**	**	
Total survival time	DS				***	**							
Duration of maintenance of body weight	DBW						*		r	*			
Mean level of parasitaemia	Mean PAR						*		*				
Parasite frequency	FQ							**					
Mean PCV										***			
Mean HC level											*		
Min.PCV											**	*	(*)
Min.HC												***	***
Min.C3													***
Parasite frequency during the first 3 weeks of infection	fq												

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; (*)$P < 0.02$ in susceptible Baoule only.

same way as for min.HC' in order to better estimate the host capacity for maintaining C3 level. Corrected minimum C3 (C min.C3) correlated with duration of the disease, although less closely than C min.HC' ($r = 0.60, P < 0.01$). When comparing the groups, min.C3, like min.HC', essentially discriminates between HSB ($19.9 \pm 11.6\%$) and ISB ($51.5 \pm 23.6\%, P < 0.01$).

PCV

PCV in the Zebu dropped continuously and concomitantly with complement activity, until the animals had to be treated. In the Baoule, it decreased slightly at the commencement of parasitaemia and fluctuated during the course of infection, with a tendency towards recovery in the more resistant animals (Fig. 2a–d). At the time of treatment or death, susceptible Baoule had as severe anaemia as the Zebu (PCV = 16 ± 4). Resistant Baoule were superior to susceptible Baoule (HSB and ISB) and to Zebu, with regard to the mean levels of PCV during infection (Table 3). Mean PCV during infection did not correlate with the frequency of parasite detection, nor with the mean level of parasitaemia. It correlated positively with the duration of the disease ($P < 0.01$) and with maintenance of body weight ($P < 0.02$).

In the Baoule, we determined the minimum PCV value (min.PCV) in the same way as for min.HC', taking the lowest value during the first decrease in PCV at the commencement of infection (Fig. 2). This value was observed after a mean time of 9.6 ± 7 days of infection. A negative correlation with the parasite frequency during the first 3 weeks of infection was only observed in the susceptible Baoule ($r = 0.54, P < 0.02$). The percentage change in PCV was significantly lower than the change in haemolytic complement titre, whether at the time of treatment or at minimum values ($P < 0.001$).

As in the case of min.HC', there was a close relationship between min.PCV and the duration of the disease in the Baoule ($r = 0.72, P < 0.001$). There was also a correlation with the duration of maintenance of body weight ($P < 0.02$). Min.PCV was significantly higher in RB than in SB, and in ISB than in HSB (Fig. 3).

Min.PCV and min.HC', as well as min.PCV and min.C3, were significantly correlated ($P < 0.01$ and $P < 0.05$, respectively). Finally, min.PCV and mean PCV were closely related in the Baoule ($r = 0.74, P < 0.001$).

The main correlations between the different parameters are summarized in Table 4.

No statistically significant difference was observed between males and females for any of the parameters examined.

DISCUSSION

From previous studies at C.R.T.A., it was shown that some individuals within the Baoule breed of taurine cattle were able to withstand natural *Glossina* challenge, while others, referred to as "sensitive Baoule", died as soon after infection as Zebu cattle (*B. indicus*) (Roelants et al., 1983, 1987).

The results reported here confirm that wide individual variations in the degree of resistance are seen in trypanotolerant cattle. In this study, even the most susceptible of the Baoule were found to be different from the Zebu. Furthermore, it was possible to delineate within the Baoule a group of animals with intermediate susceptibility, which appears to have been ignored in previous studies.

Because of such variations in trypanotolerance, the individual degree of resistance should be quantitated. Three parameters were examined for their ability to distinguish the different levels of resistance in the Baoule. Total survival time in a trypanosomosis area and the duration of maintenance of body weight are the most objective criteria, and these durations cover the pre-patent period. However the animals having the longest pre-patent period were not the ones which most efficiently controlled their infection and survived longer once infected. Moreover, total survival time correlated better with the duration of the disease ($P < 0.001$) than with the pre-patent period ($P < 0.01$). Therefore duration of the disease appeared to evaluate the degree of resistance the most reliably.

Parasitological and biological parameters were examined for their relationship with the level of resistance by measuring the statistical correlations with the duration of the disease in the Baoule.

Frequency of parasitaemia had a closer association with susceptibility than the mean levels of parasitaemia.

In agreement with studies on N'Dama cattle under experimental infection (Paling, 1987), the ability to control anaemia was found to be independent of the ability to control parasitaemia and mean PCV during infection appeared to reliably reflect the degree of resistance.

The measurement of complement activity gave another reliable indication of the disease status. Haemolytic complement activity and C3 level were depressed very early in infected animals. This decline was continuous in the Zebu, while in the Baoule, complement levels fluctuated during the course of infection depending on the frequency and intensity of parasitaemia. The first minimum value reached in early infection was found to be negatively correlated with the frequency of parasitaemia, and also to be associated with individual resistance. This association was closer when a corrected value which minimized the effect of parasite load had been calculated. Therefore the complement level in infected animals is not merely the consequence of the efficiency in limiting the parasitaemia, but also results from the control potential of each individual on complement level. Minimum complement activity in early infection appeared to be a valuable indicator of resistance in the Baoule. Haemolytic activity of complement showed a better correlation with resistance than the C3 level.

In the same way, minimum PCV in early infection was found to correlate with the degree of resistance, although changes in PCV were much less marked

than changes in complement levels. Paling (1987) has proposed that the lowest PCV reached during an experimental infection between Days 32 and 52 could be used as an indicator for the individual potential to control anaemia. In the present experiment under natural challenge, the first minimum values of PCV and complement were observed 3 weeks after the first detection of parasitaemia. Selecting the animals with min.HC$'$ > 100 HCU 50 ml^{-1} and min.PCV > 23 would have led to the selection of all "resistant" Baoule and four out of six cattle of intermediate susceptibility. As no reliable marker is at present available for the identification of the most resistant animals in trypanotolerant breeds, it is proposed that these two parameters could be used for that purpose, in the form of short-term experimental infections.

In addition to parameters related to resistance and potential markers for selection, results reported here provide some indications on the mechanisms underlying resistance to trypanosomosis.

The main superior traits of the Baoule compared to the Zebu were control of parasite multiplication and the ability to maintain complement level. The course of the disease in infected Baoule was determined by the efficiency of mechanisms controlling parasitaemia, PCV and complement level during the first 3 weeks of infection. When comparing groups of differing susceptibility, it appeared that the resistant Baoule had highly efficient control of all the parameters related to these mechanisms. The Baoule of intermediate susceptibility could control their parasitaemia and maintain complement levels, but they had less efficient control of anaemia than the resistant Baoule. Maintenance of PCV therefore appeared as the main factor determining survival of some individuals for the whole experimental period.

Early and pronounced C3 depletion during trypanosomosis might exert important consequences on the course of infection. It is likely to favour the survival of the parasites and to impair the functional activity of the immune system since C3 is necessary for mounting a normal antibody response (reviewed by Bottger and Suermann, 1987). Interaction with complement is therefore an important aspect in the pathogenesis of trypanosomosis. Not only immune complexes can activate complement via both pathways, but also trypanosomes are able to directly activate complement (Musoke and Barbet, 1977; Nielsen and Sheppard, 1977; Tabel, 1982). We found no evidence that trypanosome-induced degradation of C3 would be different in the Zebu and in the Baoule. Stimulation of C3 synthesis as a control mechanism is more likely to account for the different kinetics observed in Baoule compared to Zebu. In the former, this mechanism would contribute to determine minimal complement activity in early infection, which was found to have a meaningful association with resistance.

REFERENCES

Albright, J.W. and Albright, J.F., 1985. Murine natural resistance to *Trypanosoma lewisi* involves complement component C3 and radiation-resistant, silica-dust sensitive effector cells. Infect. Immun., 47: 176–182.

Barta, O. and Barta, V., 1972. Haemolytic assay of bovine serum complement. J. Immunol. Methods, 1: 363–374.

Bottger, E.C. and Suermann, D.B., 1987. Complement and the regulation of humoral immune responses. Immunol. Today, 8: 261–264.

Boulard, Ch. and Bencharif, F., 1984. Etude comparee des erythrocytes de quatre especes animales dans la determination de l'activite hemolytique des voies classique et alterne du Complement bovin. Comp. Immunol. Microbiol. Infect. Dis., 7: 27–34.

Cox, H.W. and Hayes, M.M., 1985. Reversal of immunosuppression induced with plasma of malarious rats by supplemented complement. J. Parasitol., 71: 50–55.

Devine, D.V., Falk, R.J. and Balber, A.E., 1986. Restriction of the alternative pathway of human complement by intact *Trypanosoma brucei* subsp. *gambiense*. Infect. Immun., 52: 223–229.

Duvallet, G., Ouedraogo, A., Pinder, M. and Van Melick, A., 1988. Observations following the cyclical infection with *Trypanosoma congolense* of previously uninfected Baoule and Zebu cattle. In: Livestock Production in Tsetse Affected Areas of Africa. (I.L.C.A./I.L.R.A.D., 1988). Proceedings of a meeting, 23–27 November 1987, at Nairobi, Kenya, pp. 318–325.

Fahey, J.L. and McKelvey, F.M., 1965. Quantitative determination of serum immunoglobulins in antibody-agar plates. J. Immunol., 94: 84–90.

Goodger, B.V., Wright, I.G. and Mahoney, D.F., 1981. Changes in conglutinin, immunoconglutinin, complement C3 and fibronectin concentrations in cattle acutely infected with *Babesia bovis*. AJEBAK, 59: 531–538.

Kipnis, T.L., Krettli, A.V. and Da Silva, W.D., 1985. Transformation of trypomastigote forms of *Trypanosoma cruzi* into activators of alternative complement pathway by immune IgG fragments. Scand. J. Immunol., 22: 217–226.

Kobayashi, A. and Tizard, I.R., 1976. The response to *Trypanosoma congolense* infection in calves. Determination of immunoglobulins IgG₁, IgG₂, IgM and C3 levels and the complement fixing antibody titres during the course of infection. Tropenmed. Parasitol., 27: 411–417.

Mahoney, D.F., Wright, I.G. and Goodger, B.V., 1980. Changes in the haemolytic activity of serum complement during acute *Babesia bovis* infection in cattle. Z. Parasitenkd., 62: 39–45.

Malu, M.N. and Tabel, H., 1986. The alternative pathway of complement in sheep during the course of infection with *Trypanosoma congolense* and after Berenil treatment. Parasite Immunol., 8: 217–229.

Meri, S., Richaud, C. and Linder, E., 1985. Complement activation by antigenic fractions of *Entamoeba histolytica*. Parasite Immunol., 7: 153–164.

Mosser, D.M. and Edelson, P.J., 1985. The mouse macrophage receptor for C3bi (CR3) is a major mechanism in the phagocytosis of *Leishmania* promastigotes. J. Immunol., 135: 2785–2789.

Murray, M., Murray, P.K. and McIntyre, W.I.M., 1977. An improved parasitological technique for the diagnosis of African trypanosomiasis. Trans. R. Soc. Trop. Med. Hyg., 71: 325–326.

Murray, M., Morrison, W.I. and Whitelaw, D.D., 1982. Host susceptibility to African trypanosomiasis: trypanotolerance. Adv. Parasitol., 21: 1–68.

Musoke, A.J. and Barbet, A.F., 1977. Activation of complement by variant specific surface antigen of *Trypanosoma brucei*. Nature (London), 270: 438–440.

Nielsen, K. and Sheppard, J., 1977. Activation of complement by trypanosomes. Experientia, 133: 769–771.

Nielsen, K., Sheppard, J., Holmes, W. and Tizard, I., 1978. Changes in serum immunoglobulins, complement and complement components in infected animals. Immunology, 35: 817–826.

Paling, R.W., 1987. Studies using N'Dama at I.L.R.A.D. Presentation for the scientific seminar on the occasion of the official opening of the International Trypanotolerance Centre, Banjul, The Gambia, 23–27 March 1987.

Reed, S.L., Curd, J.G., Gigli, I., Gillin, F.D. and Braude, A.I., 1986. Activation of complement by pathogenic and non pathogenic *Entamoeba histolytica*. J. Immunol., 136: 2265–2270.

Roelants, G.E., 1986. Natural resistance to African trypanosomiasis. Parasite Immunol., 8: 1–10.

Roelants, G.E., Tamboura, I., Sidiki, D.B., Bassinga, A. and Pinder, M., 1983. Trypanotolerance. An individual not a breed character. Acta. Trop., 40: 99–104.

Roelants, G.E., Fumoux, F., Pinder, M., Queval, R., Bassinga, A. and Authie, E., 1987. Identification and selection of Taurine cattle naturally resistant to trypanosomiasis. Acta Trop., 44: 55–66.

Rurangirwa, F.R., Tabel, H., Losos, G. and Tizard, I.R., 1980. Hemolytic complement and serum C3 levels in Zebu cattle infected with *Trypanosoma congolense* and *Trypanosoma vivax* and the effect of trypanocidal treatment. Infect. Immun., 27: 832–836.

Sher, A., Hieny, S. and Joiner, K., 1986. Evasion of the alternative complement pathway by metacyclic trypomastigotes of *Trypanosoma cruzi*: dependence on the developmentally regulated synthesis of surface protein and N-linked carbohydrate. J. Immunol., 137: 2961–2967.

Sturtevant, J.E. and Balber, A.E., 1987. *Trypanosoma lewisi*: restriction of alternative complement pathway C3/C5 convertase activity. Exp. Parasitol., 63: 260–271.

Tabel, H., 1982. Activation of the alternative pathway of bovine complement by *Trypanosoma congolense*. Parasite Immunol., 4: 329–335.