

Follow-up of Card Agglutination Trypanosomiasis Test (CATT) positive but apparently aparasitaemic individuals in Côte d'Ivoire: evidence for a complex and heterogeneous population

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Summary

The aetiological diagnosis of human African trypanosomiasis (HAT) is based on the detection of the parasite, but currently available parasitological tests have low sensitivity and are hampered by fluctuating parasitaemia. The identification of seropositive individuals on whom to focus parasitological examination is based on antibody detection by means of the Card Agglutination Trypanosomiasis Test (CATT/*T.b.gambiense*). A complicating phenomenon is the occurrence of serologically positive but parasitologically unconfirmed results (isolated CATT positivity). This work presents a two-year longitudinal serological, parasitological and molecular follow-up of CATT-positive individuals including repeated examinations of each individual, to study the evolution over time of seropositivity at both the population and the individual levels. At the population level, the rate of seropositivity decreased during the first months of the survey, and afterwards showed remarkable stability. At the individual level, the results reveal the extreme heterogeneity of this population, with subjects showing fluctuating results, others with a short transient CATT positivity, and subjects that maintain their seropositivity over time. The stability of seropositivity and the pattern of results obtained with both immunological and parasitological examinations support the view that individual factors, such as immune response to infection, might be involved in the isolated CATT positivity phenomenon.

keywords Trypanosomiasis, CATT, Individual susceptibility, *Trypanosoma brucei gambiense*

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Introduction

Human African Trypanosomiasis (HAT), or sleeping sickness, remains an important public health problem in sub-Saharan Africa. An estimated 55 million people are exposed to the risk of HAT, while only 4-5 million are under surveillance (WHO 1998). A chronic form of HAT, usually caused by *Trypanosoma brucei gambiense* (T. b. g.), prevails in West and Central Africa, whereas an acute form, caused by *Trypanosoma brucei rhodesiense*, occurs in East Africa. The control of the disease is based on medical surveillance, consisting of early detection and treatment of patients, and vector control. As most of the drugs currently available for treatment of sleeping sickness are toxic, a reliable diagnosis is

required prior to treatment. The aetiological diagnosis of sleeping sickness is based on the detection of trypanosomes in lymph node aspirates, blood or cerebrospinal fluid, but currently available parasitological tests have low sensitivity and are hampered by fluctuating parasitaemia (Truc *et al.* 1994; Kanmogne *et al.* 1996). Antibody detection tests are currently used in mass-screening for the identification of seropositive individuals on whom to focus parasitological examinations. The most commonly used test in the field is the Card Agglutination Test for Trypanosomiasis (CATT), developed for *T.b. gambiense* specific antibody detection (Magnus *et al.* 1978).

The existence of seropositive but parasitologically unconfirmed subjects (aparasitaemic individuals, or isolated



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seropositivity) (CATT+/T-) raises the question of overt HAT cases, and may have epidemiological implications, since some of them might develop the disease and contribute to the persistence of disease foci. Presently the treatment of seropositive aparasitaemic individuals is not recommended even though it is sometimes suggested as a possible control strategy in areas with a high prevalence of HAT.

The meaning of the isolated seropositivity phenomenon remains unclear since the respective roles of trypanosome and host have not been examined extensively. First of all it is important to eliminate false seropositivity due to cross-reactivity (animal trypanosome strains or other infectious diseases), or false negativity of parasite detection tests due to subpatent parasitaemia, which may last several months in some individuals (Noireau *et al.* 1988). Lastly, the possibility of an efficient immune response capable of suppressing or even eliminating the trypanosomes, has also to be considered. An individual susceptibility to infection has already been described for both parasitological and bacterial infections (Abel *et al.* 1991; Hill *et al.* 1991; Garcia *et al.* 1998a,b).

Our aim was a longitudinal survey with repeated serological and parasitological examinations, using several laboratory techniques, to study the evolution of the status of seropositive but parasitologically unconfirmed individuals. Since little is known about behavioural, environmental and individual factors potentially influencing seropositivity, our second goal was to investigate such factors.

Materials and methods

Area and population

The study took place between February 1997 and January 1999, in the Western-Central part of Côte d'Ivoire, in Sinfra focus, where a control program was conducted from 1995 to 1997. Between 1994 and 1995, more than 70 000 individuals were registered in this area. During mass-screening 50 375 individuals were tested by CATT on whole blood and plasma according to the instructions in the test kit. During the first year, 327 parasitologically confirmed HAT cases were detected, thus yielding an overall observed prevalence rate of 0.65% (in some villages the prevalence was > 1% and even 4% in one village). At the beginning, 160 subjects were recorded seropositive on CATT on whole blood but were apparently aparasitaemic at least once during a previous control program. Our study population consisted of a subgroup of 90 individuals > 8 years whose seropositivity remained strong ($\geq + +$) in both diluted blood (1/4 dilution) and CATT with 5 μ l of plasma. The purpose of the study was explained and informed consent obtained from the study subjects. If a sleeping sickness case appeared in this population, treatment was started immediately.

Biological samples and tests

The selected population was visited by a special team on six occasions in February, March, May and November 1997, and in January and June 1998. During each of these six visits, blood was collected for serological and parasitological tests in the field and serum was prepared and frozen (in liquid nitrogen) for further investigations. A last visit was performed in January 99, to collect blood for PCR analysis. In addition to these visits, the study population was regularly supervised by primary health care agents and, if health problems appeared, seropositive individuals were referred to Sinfra laboratory for HAT control.

For each subject, three CATT tests were performed (on whole blood, on a 1/4 blood dilution in PBS and on 5 μ l of plasma) and parasitological examination was carried out by means of both the Mini Anion-Exchange Centrifugation Technique (mAECT) (Lumsden *et al.* 1981) and the Quantitative Buffy Coat technique (QBC*) (Bailey & Smith 1992). In addition, a vial of the Kit for In Vitro Isolation (KIVI) (Aerts *et al.* 1992; Truc *et al.* 1992) was inoculated with 5 ml of the blood of each subject and followed up as prescribed.

Immune trypanolysis tests (TL) were performed on serum according to Van Meirvenne *et al.* (1995) with LiTat 1.3, 1.5 and 1.6 variable antigen types of *T. b. gambiense*. Serum and cerebrospinal fluid IgM concentration was estimated by titration in the LATEX/IgM (Lejon *et al.* 1998).

PCR was performed on the samples collected in May 1997, January and June 1998, and in January 1999 according to the protocol described by Penchenier *et al.* (1996) using two PCR primers, both specific for *T. brucei* ssp. (Moser *et al.* 1989), TBR1 and TBR2.

Variable of interest

At each examination, a binary variable denoted as Elementary Serological Status (ESS) was defined for each sampled individual. If CATT on 1/4 diluted blood and CATT on plasma were strongly positive ($\geq + +$), the ESS was coded as 1, and conversely in case of weak positivity or obvious negativity of diluted CATT and/or CATT on plasma, ESS was coded as 0. In this work we were also interested in studying the individual serological status and, in order to obtain a unique variable accounting for the overall degree of seropositivity during the follow-up, a binary variable denoted as Individual Serological Status (ISS) was defined as follows. If a subject had all ESSs equal to 1 (all CATT strongly positive) the ISS was coded as 1. If at least one ESS was equal to 0 (at least one CATT weakly positive or negative), the ISS was coded as 0. The first group of individuals (all ESS = 1; ISS = 1) were referred to as seropositive; the second group (at least one ESS = 0; ISS = 0) as seronegative.

No. of measurements j	P_0 §	No. of all subjects with j measurements n_j	Expected no. of subjects with all their ESS = 1 $n_j P_0^j$	Observed no. of subjects with all their ESS = 1
3	0.20	7	1.40	4
4	0.12	9	1.10	5
5	0.07	22	1.57	8
6	0.04	31	1.31	11

Table 1 Observed and expected number of subjects with at least three ESS measurements equal to 1*

*Expected numbers are computed under the hypothesis that a positive ESS occurs at random with the probability P_0 (defined in methods), which is equal to 0.59 in this study.

§ P_0^j is the probability for an individual having j measurements that all his ESS are equal to 1. For example, for three measurements this probability is $0.59^3 = 0.20$, $P < 10^{-9}$ by Pearson chi-square test.

Strategy of analysis and statistical methods

Elementary serological status was used for studying the evolution, by visit, of seropositivity in the population and the stability over time of seropositivity within individuals. Individual serological status was used to test the effect of co-variables on seropositivity, as well as to assess the association and agreement with other biological data, essentially IgM level, Trypanolysis (TL) and PCR results.

To assess the individual stability of ESS, the following method was used (Garcia *et al.* 1995) (Table 1): Let P_0 be the observed probability of having a positive CATT in the total number of measurements (P_0 = number of positive CATTs/total number of CATTs). According to the hypothesis that a positive CATT occurs at random within individuals (i.e. all measurements are independent), the probability for an individual who had j measurements that all his CATTs are positive is given by P_0^j ; the expected number of such individuals (sampled j times and being always positive) is $n_j P_0^j$, where n_j is the number of subjects sampled j times. These expected numbers were compared with the observed ones by a chi-square test for individuals with more than two measurements. Rejection of the null hypothesis of independence of measurements means that individuals maintain their positive ESS over time.

Explanatory variables tested in this analysis as potentially influencing the IMS were:

- gender;
- age in years;
- social way of life (SWL) defined as living or not in temporary dwellings located in coffee or cocoa plantations with important mixing of population particularly around wells;
- ethnic group: Gouro (natives of the area), Baoule and Bete (from Côte d'Ivoire but allogeous from the area), Dioula, Senoufo, Mossi and other minority groups all

originating from Northern areas or countries with absence or very low level of trypanosomiasis endemicity;

- sleeping sickness case(s) in the family;
- number of years people had lived in the area; and
- occupation and activity divided into three categories: fieldworkers spending most of their time in the forest and the plantations, schoolchildren and teachers with low forest contact, and colleagues (city dwellers).

Univariate analysis to test the effect of categorical variables on ISS was performed by means of Pearson chi-square tests. The McNemar test of symmetry was used to compare results of different tests performed on the same individual. For quantitative variables such as age and IgM titre (after a log-transformation), analysis of variance was used. Multivariate analysis was performed by means of linear regression models. All computations were done using BMDP statistical software (University of California, Los Angeles, CA).

Results

Population

Of the 90 individuals selected at the beginning of the study, 77 were sampled at least twice and represent the population under study. The remaining 13 individuals who refused to continue after the first sampling did not differ significantly on individual variables from the 77 included subjects. The mean age of the latter population was 31.25 years (range = 8-83) with a male:female ratio of 1.4. Concerning SWL, 23.3% of the population has a collective system of land occupation, with important mixing of population; 53.4% lived in Sinfra town with less important forest contact. The remaining 23.3% lived in the forest with the same kind of agricultural activities as the first group, but with a different social organization. Natives of the area represented 31.5% of the included individuals and 28.8% of the population originated

Table 2 Variation, by date of visit, of the proportion of sampled population and of the proportion of positive Elementary Serological status

Visit	Feb 1997	Mar 1997	May 1997	Nov 1997	Jan 1998	Jun 1998	Total
No. of visited subjects	58 (0.75) *	68 (0.88)	66 (0.86)	58 (0.75)	57 (0.74)	59 (0.77)	366
ESS = 0	14	20	29	28	31	27	149
ESS = 1	44 (0.76)	48 (0.71)	37 (0.56)	30 (0.52)	26 (0.46)	32 (0.54)	217 (0.59)

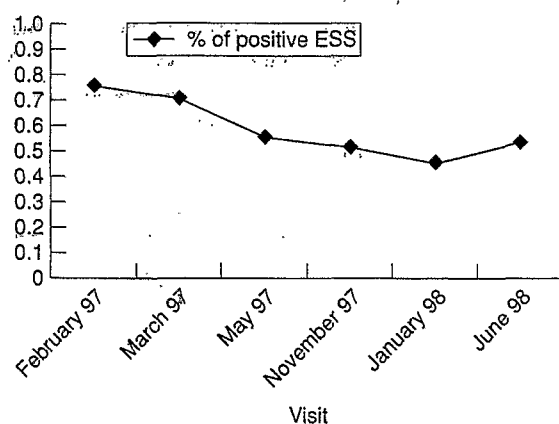
* proportion of the whole population $P < 0.005$.

from Northern areas. The remaining 39.7% represented allogeous people from Côte d'Ivoire. Only 9.8% of the studied population declared having HAT in their family. Fieldworkers represented 52.9% and 38.6% of the population had school-related activities.

Not all individuals were present at each visit and the mean number of examinations per individual was 4.9 (range = 2-6), but no effect of the number of examinations on ISS was detected ($P = 0.07$).

Long-term stability of ESS

Table 2 shows the variation by date of visit of both the percentage of the total population sampled and the proportion of positive ESS. The rate of positivity (i.e. ESS = 1), decreased significantly ($P < 0.005$), and seemed to stabilize around 50% after the third examination (Figure 1). The probability P_0 of having a positive ESS in the total number of measurements was 0.59. Table 1 shows, by number of measurements, the number of individuals having all ESSs positive and the expected numbers computed under the

**Figure 1** Evolution of percentage of positive ESS.

hypothesis of independence as described in the methods. The test result was highly significant ($P < 10^{-9}$), leading to a strong rejection of the hypothesis of random occurrence of a positive ESS within individuals. Even though the rate of positive ESS decreased significantly in the whole population, for some individuals the probability of having all their ESSs positive was higher than under independence hypothesis. Such subjects who maintained their ESS positivity over time were defined, in this study, as seropositive individuals (ISS = 1; $n = 35$), and the question was then: did they have an increased risk of developing the disease?

Factors influencing the ISS

Univariate analysis showed that there was no significant effect of gender ($P > 0.20$), SWL ($P > 0.20$) or ethnic group ($P > 0.30$) on the ISS. Neither the presence of a sleeping sickness case in the family ($P > 0.80$) nor forest-related activities ($P > 0.7$) have a significant influence on ISS. Mean age (SEM) did not differ significantly ($P > 0.80$) between the seropositive and seronegative populations, 32.2 years (3.34) and 31.5 years (3.10), respectively. Mean time (SEM) seropositive subjects lived in the area was 25.9 years (3.92) whereas the seronegative population was present in the area for 23.4 years (3.30) ($P > 0.60$).

Parasite detection

During follow-up no trypanosomes were found in blood by mAECT or by QBC® techniques. No KIVI was found positive. In January 1998 one seropositive subject was found with strong mental confusion without any other clinical sign. No trypanosomes were found in the blood but a lumbar puncture (LP) detected one trypanosome after double centrifugation of cerebrospinal fluid (CSF). This subject was seropositive (ISS = 1 with 5 positive ESS; 5 TL positive; high IgM titre: 8000 (normal = 1000)). Taking this result into account, lumbar puncture and CSF examination was performed on all subjects ($n = 17$) with the same pattern of results (i.e. ISS = 1;

Table 3 Results of immune trypanolysis tests according to individual serological status

	SSI = 0	SSI = 1	Total
TL -	33 (0.47)*	19 (0.27)	52
TL +	6 (0.086)	12 (0.17)	18
Total	39	31	70

*proportion of the whole population.

TL positive and high IgM titre). No trypanosomes were found after double centrifugation of CSF. Unfortunately white blood cell counts could not be performed for technical reasons. As an alternative, IgM in CSF was titrated with the LATEX/IgM but none of the samples showed abnormal IgM concentrations.

Table 3 shows the TL test results according to ISS. Of the seropositive individuals 27% were TL negative and 8.6% of the seronegative subjects, for whom TL results were available, were TL positive ($n = 6$). These proportions differed significantly (McNemar test = 6.76; $P < 0.01$), suggesting that the response to these two serological tests differed in the study population. High serum IgM levels are current in infectious diseases and can be caused by any kind of infection. The mean serum IgM titre was significantly ($P < 0.05$) higher in seropositive individuals, 3678.6 (SEM = 445.6), than in seronegative ones, 2481.5 (355.5), and in TL positive subjects than in TL negative ones, 5000.0 (218.2) and 2459.5 (283.7), respectively. However, when multivariate analysis was performed to test the effect of both TL and ISS on the serum IgM titre, TL remained the only relevant factor influencing IgM in this population ($P < 0.05$).

PCR results

Table 4 shows the number of positive PCR obtained in May 1997, January and June 1998 and January 1999. The overall percentage of positivity was 14.5%, and the positivity rate did not change significantly during follow-up ($P > 0.50$). The

percentage of PCR positivity was significantly higher ($P < 0.02$) for seronegative individuals (SSI = 0) than for seropositive ones (SSI = 1), 28% and 16%, respectively. Of 32 seropositive individuals sampled for PCR tests, five subjects had at least one positive PCR; of these, three had all PCRs positive (2/2, 2/2 and 3/3), whereas of 38 seronegative people sampled for PCR examination, 11 had at least one PCR positive, one of them with all its PCRs positive (4/4). Considering TL results, and disregarding other tests, the percentage of PCR positivity did not differ significantly ($P > 0.2$) between TL-positive subjects (37% of individuals with at least one PCR positive) and TL-negative ones (32%). However, the four individuals who maintained positivity at PCR results were TL positive, and one of them was the subject who developed sleeping sickness.

Discussion

This work presents a two-year longitudinal serological, parasitological, and molecular survey of CATT-positive individuals including repeated examinations of each individual and studying the long-term stability of seropositivity, at both the population and the individual level. At the population level, the rate of positivity decreased during the first months of the survey, and afterwards showed a remarkable stability. At the individual level, our results confirm the extreme heterogeneity of this population, with subjects that maintained their seropositivity over time, others showing important variability and lastly, subjects with a short transient CATT positivity. Evidence exists that *T. congolense* or *T. brucei brucei* infection in animals can induce CATT seropositivity (Noireau *et al.* 1986). *T. congolense* has been suggested to be human serum resistant (Joshua 1989) and recently Truc *et al.* (1998) described a mixed infection with *T. b. gambiense* and *T. congolense* in a patient in Côte d'Ivoire. This patient was only slightly positive in CATT and turned CATT negative shortly after treatment. Although we do not know how long *T. congolense* or *T. b. brucei* can persist in a human being, this period may be long enough to induce a transient CATT positivity. Cohabitation of man and domestic animals harbouring non *T. b. gambiense* trypanosomes could play a role in transient, fluctuating or even long-lasting CATT seropositivity in

	May 1997	January 1998	June 1998	January 1999	Total
PCR positive	12	6	6	8	32
Proportion	(0.19)	(0.12)	(0.11)	(0.16)	(0.145)
PCR negative	51	45	51	41	188
Proportion	(0.81)	(0.88)	(0.89)	(0.84)	(0.855)
Total	63	51	57	49	220

Table 4 Variation by date of visit of the number of positive PCRs

the human population. Eight seropositive adults accepted daily parasitological examination during one week by means of QBC® and mAECT. All remained parasitologically negative (data not shown). Although active surveillance of the study population stopped in June 1998 for parasitological tests and in January 1999 for PCR, all individuals are still under medical surveillance and so far no new case of sleeping sickness has appeared.

This extreme heterogeneity emphasizes the difficulty of determining a simple and standardized control strategy for seropositive though apparently aparasitaemic subjects. The main problem is determining the epidemiological significance of this population, since some of these persons may indeed be infected, and sooner or later play a role in the transmission of disease. Within our study and its 2 years' follow-up (February 1997 – January 1999), one trypanosomiasis case occurred among 77 included individuals, representing an estimated incidence of 0.65 new cases per 100 person-years. According to Van Meirvenne *et al.* (1995), the immune trypanolysis test is highly specific and immune trypanolysis-positive individuals can be considered as being or having been infected with trypanosomes. If we consider that the only subjects really at risk in our study are those who are trypanolysis positive ($n = 17$ disregarding all other tests), the estimated incidence would be much higher (2.3 new cases per 100 person-years). However, our population is highly selected and no quantitative information can be easily derived from it. Furthermore, it is important to note that 8.6% of those subjects were trypanolysis positive but had a negative individual serological status derived from several CATT results. For two of them, CATT was never positive (six measurements) during the follow-up. The mean level of trypanolytic antibody titres did not differ between seropositive and seronegative TL-positive individuals. Use of the IgM titre as a cut-off point to determine a higher risk of developing the disease cannot be proposed without reservation, since our results suggested that observed IgM titre differences are better explained by trypanolysis than by CATT alone. Simarro *et al.* (1999) recently showed that treatment of all CATT positives with an end titre $> 1/8$ can be proposed as a control strategy in high prevalence areas. However, our study took place in a different endemic area and with a strongly selected population that could be compared at the beginning of our work to the one selected at the end of Simarro's study. Although treatment of CATT-positive aparasitaemic people is not a recommended control strategy, it could help in some high prevalence areas at least at the beginning of a control program when the transmission rate is still high. Nevertheless, previous results show that the complexity of this question needs further, both pragmatic and explicative, investigations.

In this survey, primers specific for *Trypanosoma brucei* s.l. (TBR1 and TBR2) were used for PCR, and extractions were

performed as described by Penchenier *et al.* (1996). The 14.5% overall rate of PCR positivity in our study is higher than the one recently published by Simo *et al.* (1999), using the same method of extraction and the same primers in three foci of Cameroon. These authors found 51 positive PCR (3.8%) in 1343 seropositive individuals, apparently negative in QBC®, mAECT or lymph node puncture, with an important variability according to the focus: 2.9% in Bipindi, 0.5% in Campo and 5.5% in Fontem. Kanmogne *et al.* (1996) reported discordant results in Cameroon since their overall PCR positivity rate was 22.4% (13/58) using phenol extraction (Van der Ploeg *et al.* 1982), but only 3.4% (2/58) using differential lysis methods (Masiga 1994). All 58 tested individuals were strongly positive in CATT and appeared aparasitaemic when blood samples from them were examined after haematocrit centrifugation, mAECT, thick blood films and *in vitro* culture. However, from the 13 PCR-positive subjects, eight presented clinical signs of enlarged glands, facial oedema, headache, tremor, abnormal behaviour, etc., and two of them had positive gland puncture. The same discrepancy according to the focus was denoted by Kanmogne, since 30% of seropositives tested from Fontem were PCR positive whereas this rate decreased to 14.3% in Mbam. Extraction method differences can explain discordant results between these studies as illustrated in Kanmogne's study (Masiga 1994; Weiss 1995), and Smits & Hartskeerl (1995) clearly showed that the quality of DNA limits the efficiency of PCR reaction. Similar differences in sensitivity and specificity occurred in PCR diagnosis of *Plasmodium* infections (Gyang *et al.* 1992).

Differences in these results could not only be explained by extraction methods but probably also by parasite intrinsic properties: Dukes *et al.* (1992) showed that in Cameroon some isolates of trypanosomes do not express LiTat 1.3 antigen. Such parasites could be responsible for CATT-negative infections, with subpatent parasitaemia, detected by PCR. However, an important distinction between all these studies is the definition of seropositive and apparently aparasitaemic individuals. Our longitudinal follow-up shows that, with a very strict definition of seropositive *vs.* seronegative individuals, PCR positivity is more frequent within the seronegative than within the seropositive population, suggesting that, in a cross-sectional study, a positive PCR might appear randomly in a population living in an endemic area. Disregarding the longitudinal aspect of our work, the rate of PCR positivity on CATT-negative individuals decreases significantly from 67% to 33% between the first and the third examination (no CATT was performed in January 1999), emphasizing the importance of repeated analyses. This result can be, at least in part, explained by the lack of specificity of the primers used in all these studies, since TBR1 and TBR2 primers are not specific of *T. brucei gambiense* and can reveal

DNA from *T. brucei brucei* originating from abortive inoculation by tsetse flies. The true value of PCR methods in diagnosis of sleeping sickness remains to be further evaluated, and development of *T. b. gambiense* specific primers would be of great interest.

Sleeping sickness treatment centres in Côte d'Ivoire are rather close to the study area and are involved in the control program established in the region covered by this study, suggesting that the included seropositive individuals probably have never been treated. However, the probability that one of our subjects was a treated sleeping sickness case cannot be totally excluded and, moreover, uncontrolled treatment using traditional medicines could also explain this phenomenon.

Our results are strongly consistent with an important heterogeneity of the CATT-seropositive population. Some trypanolysis-positive individuals with high IgM titre have probably been infected by *T. brucei gambiense* or a related subspecies of trypanosomes. Even though a rather chronic infection course is quite common, the complete absence of disease after 32 months of follow-up seems to require extra explanation, such as a strongly suited and very efficient immune response. This pattern of individual susceptibility to infection and disease has been described for other bacterial (Abel & Demenais 1988) and parasitic (Garcia *et al.* 1998a,b; Garcia *et al.* 1999) infections. While there is evidence for genetic control of either disease resistance/susceptibility (Hill *et al.* 1991; Marquet *et al.* 1996) or immune response (Hill *et al.* 1992), the nature of this control in human infections remains unclear (Beck *et al.* 1995; Migot *et al.* 1995). To our knowledge, in human African trypanosomiasis, although classical epidemiological studies reported some indirect arguments for familial dependencies, shared environment rather than genetic susceptibility has been put forward (Khonde *et al.* 1997). However, involvement of the immune system in pathogenesis, and interactions between the trypanosome and macrophages have been described in animal models (Askona 1985; Olsson *et al.* 1993; Sileghem *et al.* 1989) and humans (Okomo-Assoumou *et al.* 1995; Sternberg 1996). Furthermore, Kemp *et al.* (1997) recently localized genes involved in resistance control to trypanosomiasis in mice.

In conclusion, our study emphasizes heterogeneity of the CATT-positive but apparently aparasitaemic population. Further analyses need to explore every element of this complex phenomenon, focusing not only on testing intrinsic qualities, but also on the interactions between the parasite and the host. All these factors will probably be of great interest for defining a strategy for taking into account this population in control programs.

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