Confirmation of No Influence of *Loa loa* and *Mansonella perstans* on the Card Agglutination Test for Trypanosomosis used for Serological Screening of Human African Trypanosomosis

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Abstract

In central Africa, the geographical distribution of human African trypanosomosis (HAT) due to *Trypanosoma brucei* and *Mansonella perstans* filariases. This study investigated whether the presence of blood borne *M. perstans* and *L. loa* microfilariae interferes with the agglutination reaction of CATT (Card Agglutination Test for Trypanosomosis), used for mass screening of HAT. 146 CATT positive participants and 146 CATT negative persons were not more frequently infected by *L. loa* and *M. perstans* than CATT negative ones. This unique matched case-control study confirmed a previous study and does not bring any evidence of the influence of *L. loa* or *M. perstans* on serodiagnosis of HAT in the field using CATT/ *T. b. gambiense* LiTat 1.3. HAT screening activities can be performed without controlling for filariasis at the same time.

Keywords: Human African trypanosomosis; Microfilariasis; *Loa loa*, *Mansonella perstans*, CATT/ *Trypanosoma brucei gambiense*

Introduction

The card agglutination test for trypanosomosis (CATT/ *Trypanosoma brucei gambiense*) is a serological test that detects *T. b. gambiense* specific antibodies in whole blood, serum or plasma [1]. Easy to use in field conditions, this low-cost test remains the mainstay for mass population screening for the gambiense form of human African trypanosomosis (HAT) since 1978. In case of positive CATT, blood centrifugation and lumbar puncture to obtain cerebrospinal fluid are subsequently performed for confirmation and staging of the infection.

Despite a remarkable specificity, estimated around 97% false positivity to the CATT may occur in case of a transient infection by an animal trypanosome such *Trypanosoma brucei brucei* or in individuals presenting with malaria [2]. It has also been suspected with filarial infections [3].

In 1988, the association between the presence of *Loa loa* and *Mansonella perstans* microfilaraemia and CATT results was investigated in the Republic of Congo as part of a study whose main objective was to compare the performances of the CATT using the antigen consisted of clone trypanosomes of Variable Antigen Type (VAT) AnTat 1.8, isolated from *Trypanosoma brucei brucei* Antigen Repertoire AnFAR 1 [4], and the immunofluorescent antibody test (IFAT) as immunological diagnosis of HAT [3].
Materials and Methods

Study sites

The study was conducted in 2004 in four historical HAT foci: the “Couloir” focus in the Republic of Congo (main locality: Ngabé, located on the bank of the Congo River, about 180 km north of Brazzaville) and three HAT foci in Cameroon: Fontem (Lebialem Division in the South-West Region), Bipindi and Campo (both located in the Ocean Division, in the South Region).

According to the classification developed to define risk for HAT [6], the Couloir (Mpouya-Ngabé) focus was a very high-risk area (number of cases per inhabitant per year ≥ 1/100) whereas the Bipindi and Campo foci were in the moderate risk category (between 1/10,000 and 1/1000), and Fontem was a low-risk area (between 1/100,000 and 1/10,000). The initial surveys to identify CATT positives and CATT negatives subjects were conducted in April and May 2004 in the Campo (nine villages: Afan Essokie II, Bouandjo, Campo-town, Campo-beach, Elobde, Ipono, Mabiogo, Malaba and Mvasse) and the Bipindi foci (seven villages: Bijouka-Centre, Bipindi-Centre, Ebimimbang, Lambi, Mimfombo, Nyamenkoun and Tyango). In the Ngabé focus, the survey was performed between May and July 2004 and included seven villages (Boka Lefini, Boka Sereix, Bokaba, Brick 1, Brick 2, Brick 3, Mouala). Lastly, the Fontem focus was surveyed in July 2004 (eight villages: Azi, Belleh, Fossung, Menji, Mveh, Nchembin, Nchenen and Nsoko). Detailed maps of the Bipindi, Campo and Fontem foci have been presented elsewhere [7-9].

Screening strategy and biological examination

All volunteering residents present in the selected villages were examined during daytime. The first step consisted in a careful palpation of cervical lymph node to detect a possible enlargement. A puncture was done whenever a lymph node was larger than 1 cm and the fluid was rapidly examined under light microscopy. All individuals with trypanosomes in the fluid were discarded from the present study and were referred to hospital for treatment where a full clinical examination was done for all patients (data not available). After palpation, a 60 µL fingerplick blood sample was collected from each individual in a heparinized capillary tube to perform immediately a examination to detect trypanosomes [10]. The remaining of the blood sample was centrifuged and the plasma obtained was used to perform CATT titration using twofold dilutions from 1 to 1/32 (CATT-P). Dilutions were made in microtiter plates and the operating fluid were discarded from the present study. After dehemoglobinization, the blood smears were stained with Giemsa stain, examined under a light microscope. All L. loa and M. perstans mf were counted except in the Fontem focus, where only presence/absence of each species was reported. Mf counts are expressed per 75 µL.

Matching procedure and statistical analysis

During the screening phase, 422 persons were WB-CATT positive among 9,617 persons tested (36/3038 including 2 confirmed HAT cases in Bipindi, 61/2647 including 8 confirmed HAT cases in Campo, 204/2504 with no confirmed HAT case in Fontem and 121/1428 including 7 confirmed HAT cases in Ngabe), but only 146 individuals with a WB-CATT positive result were consenting and included in the present study.

Each of the 146 WB-CATT positive individuals was matched on sex, age (± 2 years) and village of residence with a single WB-CATT negative control (randomly chosen when several non-cases were available for matching). A matched case-control analysis was performed to assess whether the presence and intensity of microfilaremia were associated (a) with WB-CATT positivity and (b) CATT-P end dilution titres.

Chi-square tests were used to compare proportions and Wilcoxon signed-rank tests were used to compare mf densities between WB-CATT positive and WB-CATT negative subjects. In addition, we investigated the relationship between CATT-P intensity (end dilution titre) and presence and intensity of microfilaremia amongst WB-CATT positive individuals only. For this step, we performed a logistic regression analysis with the CATT-P titre (CATT-P ≥ 1/16 vs. CATT-P <1/16) as the variable of interest and sex, age and microfilarial status (presence of L. loa and M. perstans mf, and then L. loa mf count and M. perstans mf count), as covariates.

Results and Discussion

During the screening, only 146 individuals of 335 with a positive WB-CATT and no HAT confirmation consented to be included in the study. The original design of the study included a follow-up of CATT titration every 3 months during a 1-year period. The main reason for not consenting was that most individuals with a positive CATT but no parasitological confirmation did not feel ill and refused to be resampled every 3 months. The proportion of individuals showing L. loa microfilaremia ranged from 3.6% in Fontem to 48.3% in Bipindi. The values for M. perstans microfilaremia ranged from 0% in Fontem to 65.5% in Bipindi (Table 1). The proportions of mf carriers were slightly higher in WB-CATT positive individuals than in WB-CATT negative ones for both L. loa (16.4% vs. 13.2%) and M. perstans (14.4% vs. 12.5%) but the differences were not statistically significant (P=0.22 and P=0.32, respectively).

The WB-CATT positive individuals tended to have higher microfilarial counts than WB-CATT negative individuals (Table 2): 39.8 vs. 17.3 for L. loa, 21.2 vs. 7.2 for M. perstans (arithmetic means ±75 µL). But these differences were not statistically significant (P=0.31 and P=0.49, respectively).

The distribution of CATT-P results according to the end dilution titres (Table 3) was not significantly different between those who were microfilaremic or amicrofilaremic for L. loa (P=0.196). However, CATT-P ≥ 1/16 results tended to be more frequent in L. loa microfilaremic subjects (29.2%) than in L. loa amicrofilaremic individuals (14.8%, P=0.087).
Focus | No. (%) of L. loa mf carriers | No. (%) of M. perstans carriers
---|---|---
Bipindi | CATT Negative 5/14 (35.7%) CATT Positive 9/15 (60.0%) Total 14/29 (48.3%) | CATT Negative 11/14a (78.6%) CATT Positive 8/15 (53.3%) Total 19/29 (65.5%)
Campo | CATT Negative 10/21b (47.6%) CATT Positive 10/22c (45.5%) Total 20/43 (46.5%) | CATT Negative 6/21b (28.6%) CATT Positive 12/22c (55.5%) Total 18/43 (41.9%)
Fontem | CATT Negative 2/83d (2.4%) CATT Positive 3/83e (3.6%) Total 6/166 (3.6%) | CATT Negative 0/83 (0%) CATT Positive 0/83 (0%) Total 0/166 (0%)
Ngabe | CATT Negative 2/26 (7.7%) CATT Positive 2/26 (7.7%) Total 4/52 (7.7%) | CATT Negative 1/26 (3.9%) CATT Positive 1/26 (3.8%) Total 2/52 (3.9%)
Total | 19/144 (13.2%) 24/146 (16.4%) 43/290 (14.8%) | 18/144 (12.5%) 21/146 (14.4%) 39/290 (13.5%)

Table 1: Presence of microfilaremia in the different HAT foci according to the CATT status.

Individuals with CATT-P ≥ 1/16 result harboured higher L. loa microfilaremia (58.9 mf/75 µl) than those with a CATT-P result < 1/16 (23.1 mf/75 µl) but this difference was not significant (P=0.16). M. perstans microfilaremia did not differ significantly in those with a CATT-P result <1/16 (16.1 mf/75 µl) and those with a CATT-P result ≥ 1/16 (6.1 mf/75 µl, P=0.39).

Location | L. loa mf 75 µL of blood | M. perstans mf 75 µL of blood
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Bipindi | CATT Negative 58.2 (121.5) CATT Positive 108.7 (219.0) Total 167.9 (242.4) | CATT Negative 21.6 (26.7) CATT Positive 63.9 (122.4)
Campo | CATT Negative 9.9 (26.4) CATT Positive 35.9 (107.2) Total 45.8 (133.6) | CATT Negative 6.2 (17.9) CATT Positive 16.5 (27.2)
Fontem | CATT Negative NA CATT Positive NA Total NA | CATT Negative NA CATT Positive NA
Ngabe | CATT Negative 0.3 (1.12) CATT Positive 3.1 (14.9) Total 3.4 (16.0) | CATT Negative 0.2 (1.0) CATT Positive 0.2 (1.0)
Total | CATT Negative 17.3 (64.3) CATT Positive 39.8 (129.2) Total 57.1 (193.5) | CATT Negative 7.2 (18.2) CATT Positive 21.2 (66.2)

Table 2: Intensities of microfilaremia (mean ± standard deviation) in each HAT focus according to the CATT status.

Adjusting on individual factors, logistic regressions taking into account presence/absence or number of L. loa and M. perstans mf did not reveal any significant association between L. loa or M. perstans infection and a CATT-P result ≥ 1/16. Thanks to sustained control activities, HAT prevalence appears now very low in most of African countries. A rapid diagnostic test (RDT) [11], is now recommended for passive HAT detection.

However, active medical surveys are ongoing in the Republic of Congo, Central African Republic and Chad, still based on CATT using the LiTat 1.3 antigen. In addition, in case of RDT positive confirmed by parasitology (HAT patient), a small medical survey will be performed in the village of this latter patient using CATT. CATT remains the only serological test for HAT mass screening, using the best reagent based on LiTat 1.3 and not AnTat 1.8 to prevent cross-reaction with a warrant of good specificity.

The CATT using blood showed a specificity of 96.5%, which is consistent with the usually reported values. We compared CATT positive individual with negative ones, and evaluate the influence of L. loa and M. perstans on CATT using whole blood and serum dilution. No significant difference was observed in each comparison, confirming previous results despite the different antigens used in the CATT reagent as mentioned above.
Conclusion

WB-CATT positive persons were not more frequently infected by L. loa and M. perstans mf than WB-CATT negative ones. The distribution of CATT-P dilution titres was not significantly different between those who were microfilaremic or amicrofilaremic. This unique matched case-control study does not bring any evidence of the influence of L. loa or M. perstans on serodiagnosis of HAT in the field using CATT/T. b. gambiense LiTat 1.3. Therefore, HAT screening activities can be performed without controlling for filariasis at the same time.

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