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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

Pion SD[1], **Njiokou F**[2], **Boussinesq M**[1], **Simo G**[3] **and Truc P**[4*]

[1]*French National Research Institute for Sustainable Development IRD, Centre of Montpellier, Montpellier, France*

[2]*Laboratory of Parasitology and Ecology, Department of Biology and Animal Physiology, Faculty of Science, University of Yaoundé, Cameroon*

[3]*Unit of Parasitology and Molecular Entomology, Department of Biochemistry, Faculty of Science, University of Dschang, Dschang, Cameroon*

[4]*French National Research Institute for Sustainable Development IRD, Campus International de Baillarguet, Montpellier, France*

***Corresponding author:** Philippe Truc, French National Research Institute for Sustainable Development IRD UMR 177 INTERTRYP IRD CIRAD, Laboratory for Research and Coordination on Trypanosomoses, TA A-17/G, Baillarguet International Campus 34 398 Montpellier Cedex 5, France, Tel: +33-611888828; Fax: +33-467593894; E-mail: philippe.truc@ird.fr

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Abstract

In central Africa, the geographical distribution of human African trypanosomosis (HAT) due to *Trypanosoma brucei* gambiense overlaps that of *Loa loa* 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 age and sex matched CATT negative subjects were recruited in three sites in Cameroon and one in the Republic of Congo. CATT positive 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* microfilaremia 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 AnTAR 1 [4], and the immunofluorescent antibody test (IFAT) as immunological diagnosis of HAT [3].

A total of 115 subjects infected with *L. loa* (defined by a history of Calabar swelling or of eyeworm, or the presence of blood microfilariae) and/or *M. perstans* (defined by the presence of blood microfilariae) and who were not exposed to the risk of HAT were tested with CATT using serum. Among these individuals, 29.6% were positive at the CATT, whereas none of the 105 individuals included in a "healthy patients" group (i.e., without HAT, filariasis, schistosomiasis or toxoplasmosis) were CATT positive. *Prima facie*, these results suggest that a filarial infection can induce a positive CATT result. However, the presence of microfilariae was not significantly associated with CATT positivity but no definitive conclusion could be drawn.

The main objective of the present study was to provide additional information on the possible false positivity of the CATT using another cloned VAT, LiTat 1.3 derived from *T. b. gambiense* antigen repertoire LiTAR 1, in the presence of microfilarial infection. This choice resulted from comparative studies on the distribution of several IsoVATs [4,5], in *T. b. gambiense* repertoires of different geographical origin. We assessed whether the presence of microfilarial infection influenced the CATT results by using i) whole blood and ii) an appropriate case-control design: we compared the proportion of subjects with *M. perstans* and *L. loa* microfilariae (mf) between CATT positive and CATT negative controls matched on age, sex and community of residence.

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, Ebodje, 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 Serieux, 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, Nchenfen 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 fingerprick blood sample was collected from each individual in a heparinized capillary tube to perform immediately a whole blood CATT (WB-CATT). When the WB-CATT was found positive, an additional 5 mL blood sample was collected by venous puncture in a heparinized vacutainer tube. Three hundred µL of this sample were processed using the mini-anion-exchange centrifugation technique (mAECT) 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 procedures were performed. All these tests were performed the same day in the field. People in whom trypanosomes were found by the mAECT were also referred to hospital for treatment. All individuals found negative at the mAECT were left untreated.

Filarial infections were searched in 75 µL thick blood smears. 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 77 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 mf/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		
	CATT Negative	CATT Positive	Total	CATT Negative	CATT Positive	Total
Bipindi	5/14[a] (35.7%)	9/15 (60.0%)	14/29 (48.3%)	11/14a (78.6%)	8/15 (53.3%)	19/29 (65.5%)
Campo	10/21[b] (47.6%)	10/22[c] (45.5%)	20/43 (46.5%)	6/21[b] (28.6%)	12/22[c] (55.5%)	18/43 (41.9%)
Fontem	2/83[d] (2.4%)	3/83[e] (3.6%)	6/166 (3.6%)	0/83 (0%)	0/83 (0%)	0/166 (0%)
Ngabe	2/26 (7.7%)	2/26 (7.7%)	4/52 (7.7%)	1/26 (3.9%)	1/26 (3.8%)	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%)

[a] 1 thick blood smear unreadable

[b] 1 thick blood smear unreadable; 3 thick blood smears *L. loa* positive without density for *L. loa* but with density for *M. perstans*

[c] 2 thick blood smears *L. Loa* positive without density for *L. loa* but with density for *M. perstans*

[d] 3 thick blood smears *L. loa* positive without density for *L. loa*

[e] 3 thick blood smears *L. loa* positive without density for *L. loa*

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	
	CATT Negative	CATT Positive	CATT Negative	CATT Positive
Bipindi	58.2 (121.5)	108.7 (219.0)	21.6 (26.7)	63.9 (122.4)
Campo	9.9 (26.4)	35.9 (107.2)	6.2 (17.9)	16.5 (27)
Fontem	NA	NA	NA	NA
Ngabe	0.3 (1.12)	3.1 (14.9)	0.2 (1.0)	0.2 (1.0)
Total	17.3 (64.3)	39.8 (129.2)	7.2 (18.2)	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.

		CATT-P end titre results						
		1	1/2	1/4	1/8	1/16	1/32	Total
Bipindi	Loa mf -	0	1	2	1	0	2	6
	Loa mf +	0	3	1	2	0	3	9
Campo	Loa mf -	0	4	2	3	1	2	13
	Loa mf +	0	2	1	4	2	1	9
Fontem	Loa mf -	8	26	27	15	2	2	80
	Loa mf +	0	1	2	0	0	0	3
Ngabe	Loa mf -	3	2	7	3	5	4	24
	Loa mf +	0	0	0	1	1	0	2
All foci	Loa mf -	11	33	38	22	8	10	122
	Loa mf +	0	6	4	7	3	4	24

Table 3: Distribution of CATT-P end titre results in subjects with and without *Loa loa* microfilaremia.

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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