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DOMESTIC ANIMALS AS POTENTIAL RESERVOIR HOSTS OF *TRYPANOSOMA BRUCEI GAMBIENSE* IN SLEEPING SICKNESS FOCI IN CAMEROON

NJIOKOU F.*, NIMPAYE H.*, SIMO G.**, NJITCHOUANG G.R.*, ASONGANYI T.***, CUNY G.**** & HERDER S.****

Summary:

An explanation of the endemic nature and/or the resurgence of Human African Trypanosomiasis (HAT) in the historic foci in West and Central Africa may be the existence of an animal reservoir. In some HAT foci, pigs were found infected by *Trypanosoma brucei gambiense* but the implication of the other domestic animals was not quite evaluated. This study aims to determine the prevalence of *T. b. gambiense* in domestic animal species (goat, sheep, pig and dog) commonly found in the four active HAT foci in Cameroon (Bipindi, Fontem, Campo and Doumé). Blood samples were collected from 307 pigs, 264 goats, 267 sheep and 37 dogs and used for parasitological (QBC), immunological (LiTat 1.3 CATT) and molecular (PCR) analyses. QBC detected trypanosomes in 3.88 % domestic animals while 22.7 % were sero-positive with LiTat 1.3 CATT tests. Of the 875 animals analysed, 174 (19.88 %) harboured *T. brucei* s.l. DNA, found in each of the four types of animal and in the four localities. The infection rate significantly differed among the animal species ($p < 0.0001$) and localities ($p < 0.0001$). The PCR also revealed *T. b. gambiense* group 1 DNA in 27 (3.08 %) domestic animals. The specific infection rates were as follows: sheep (6.74 %), goats (3.08 %), pigs (0.32 %) and dogs (0 %). *T. b. gambiense* was found in 8 (3.92 %) animals from Bipindi, 15 (4.83 %) from Campo, 4 (2.59 %) from Fontem-Center and none from Doumé. The infection rates significantly differed between the localities, and correlated with the intensity of HAT transmission in the foci.

KEY WORDS : HTA, reservoir, domestic animal, *Trypanosoma brucei* s.l., *T. b. gambiense* group 1, PCR, CATT, QBC, sheep, goats, pigs, dogs, south-Cameroon.

Résumé : LES ANIMAUX DOMESTIQUES, RÉSERVOIR POTENTIEL DE *TRYPANOSOMA BRUCEI GAMBIENSE* DANS DES FOYERS DE MALADIE DU SOMMEIL AU CAMEROUN

Une des explications possibles de la pérennité et/ou de la résurgence des foyers historiques de la maladie du sommeil (THA) en Afrique Centrale et de l'Ouest serait l'existence d'un réservoir animal sauvage et/ou domestique de Trypanosoma brucei gambiense. *Dans certains de ces foyers, le porc a été identifié comme porteur de trypanosomes susceptibles d'infecter l'homme, mais le rôle réservoir des autres animaux domestiques a été peu étudié. Cette étude a pour but de rechercher* T. b. gambiense *chez les animaux domestiques couramment rencontrés dans les quatre foyers actifs de la maladie du sommeil au Cameroun (Bipindi, Campo, Fontem et Doumé). Pour ce faire, des échantillons de sang y ont été prélevés sur 307 porcs, 264 chèvres, 267 moutons et 37 chiens, et analysés à l'aide de tests parasitologique (QBC), immunologique (CATT LiTat 1.3) et moléculaires (PCR à* T. brucei *s.l. et à* T. b. gambiense *groupe 1). Le QBC a permis de mettre en évidence des trypanosomes chez 3,88 % d'animaux échantillonnés alors que 22,17 % étaient séro-positifs avec le test CATT LiTat 1.3. Un total de 174 (19,88 %) animaux sur 875 a été trouvé porteur d'ADN de trypanosomes du complexe* T. brucei *s.l. Ces échantillons positifs ont été observés chez toutes les espèces ainsi que dans toutes les localités étudiées. Ce taux d'infection diffère très significativement entre les espèces animales ($p < 0,0001$) et entre les localités ($p < 0,0001$). La PCR a également révélé la présence de l'ADN de* T. b. gambiense *groupe 1 chez 27 animaux, soit 3,08 %. Il s'agit de 18 (6,74 %) moutons, huit (3,08 %) chèvres, un (0,32 %) porc et aucun chien. Ces infections à* T. b. gambiense *sont observées chez 8 (3,92 %) animaux de Bipindi, 15 (4,83 %) de Campo, 4 (2,59 %) de Fontem-Centre et zéro (0 %) de Doumé. Ces pourcentages diffèrent très significativement entre les localités, et reflètent le niveau de transmission de la THA dans chacun de ces foyers.*

MOTS CLÉS : *fTHA, réservoir, animal domestique,* T. brucei *s.l.,* T. b. gambiense *groupe 1, PCR, CATT, QBC, mouton, chèvre, porc, chien, sud-Cameroun.*

* General Biology Laboratory, Department of Biology and Animal Physiology, Faculty of Science, BP 812, University of Yaoundé 1, Yaoundé, Cameroon.

** Medical Research Center, Institute of Medical Research and the Study of Medicinal Plants (IMPM), Yaoundé, Cameroon.

*** Faculty of Medicine and Biomedical Sciences, University of Yaoundé 1, Yaoundé, Cameroon.

**** Laboratoire de Recherche et de Coordination sur les Trypanosomoses IRD, UMR 177, CIRAD, TA 207/G Campus International de Baillarguet, 34398 Montpellier Cedex 5, France.

Correspondence: Flobert Njiokou, University of Yaoundé 1, Faculty of Science, BP 812, Yaoundé, Cameroon.

Tel.: (237) 77 71 96 31 – E-mail: njiokouf@yahoo.com

INTRODUCTION

Sleeping sickness is caused by a protozoa parasite that belongs to the *Trypanosoma brucei* complex in sub-Saharan Africa. The sub-species *Trypanosoma brucei gambiense* is the causative agent of chronic disease found in Central and West Africa; *Trypanosoma brucei rhodesiense* is the agent of the virulent form in East and Southern Africa while *Trypanosoma brucei brucei* infects only domestic and wild animals (Hoare, 1972). *T. b. gambiense* is divided into two sub-

types, about 80 % presenting a homogenous genetic composition and belonging to group 1 *T. b. gambiense*, while some 20 %, that are very heterogeneous belong to group 2 (Gibson *et al.*, 1986). During the last century, the prevalence of infection in affected zones fluctuated, alternating between high and low transmission periods (Lapeyssonie, 1992; Bouteille *et al.*, 2003). It is possible that a domestic animal reservoir of Human African Trypanosomiasis (HAT) plays a role in the epidemiology of sleeping sickness (Mehlitz, 1986). There have been many studies on the animal reservoir of *T. b. gambiense* (Gibson *et al.*, 1978; Mehlitz *et al.*, 1982, 1985; Komoin-Oka *et al.*, 1984; Guedegbe *et al.*, 1992). Problems of similarity in the morphology of *T. b. gambiense* and *T. b. brucei* have been progressively resolved. Thus, isoenzymes (Gibson *et al.*, 1978; Scott *et al.*, 1983; Nkinin *et al.*, 2002) and amplification of sequences of microsatellite DNA (Herder *et al.*, 2002; Jamonneau *et al.*, 2004; Njiokou *et al.*, 2006) specific to *T. b. gambiense* have confirmed the presence of this parasite in pigs and wild animals. A program for the study of animal reservoirs of sleeping sickness was initiated in Cameroon in 1999; PCR has allowed the identification of many different wild animals (primates, antelopes, rodents and small carnivores) infected with *T. b. gambiense* group 1 in active foci of sleeping sickness in Cameroon (Herder *et al.*, 2002; Njiokou *et al.*, 2006). In non-sleeping sickness zones, the same animal types did not have *T. b. gambiense,* although they were infected by other trypanosomes of the species complex *T. brucei* s.l. (Njiokou *et al.*, 2006). In domestic animals, isoenzymes and PCR revealed the presence of *T. b. gambiense* in pigs in Fontem (Nkinin *et al.*, 2002; Simo *et al.*, 2006).
The present study examines the prevalence of *T. b. gambiense* in the four domestic animal species (pigs, goat, sheep and dogs) commonly found in the four most active sleeping sickness foci in Cameroon.

MATERIALS AND METHODS

Study area

The study was carried out in 2003 and 2004 in four active sleeping sickness foci, all situated in the south of Cameroon, but presenting different epidemiological characteristics:
- Bipindi (3° 2' N, 10° 22' E) is situated between Lolodorf and Kribi, 75 km from the sea. It is an historic focus of sleeping sickness, known since 1920, which had a resurgence recently with the diagnosis of 44 patients in 1999 (Grébaut *et al.*, 2001). Domestic animals were bled in Bidjouka, Lambi, Memel, Ebimimbang and Bipindi Centre.
- Campo (2° 20' N, 9° 52' E) is located at the frontier between Cameroon and Equatorial Guinea, and is under the influence of the River Ntem and the Atlantic Ocean. It is a hypo-endemic focus where a few patients are diagnosed each year. Domestic animals were bled in Ipono, Mabiogo, Akak and Campo Centre.
- Fontem (5° 40' N, 9° 55' E) is in the South-West region of Cameroon. It is known as a focus since 1949, but its importance has since significantly reduced. It has a much varied topography, and is divided into three subfoci (North, Centre and South) (Simo *et al.*, 2006). Domestic animals were bled in the Centre at Menji, Fotabong, Fossung, Nsoko and Azi where trypanosomiasis patients are detected each year (reports of PNLTHA). In the northern zone, no case of sleeping sickness was detected during a surveillance campaign conducted by a team from OCEAC (*Organisation de Coordination de la lutte contre les Endémies en Afrique Centrale*) in 1998, although up to 15 % of pigs were found to be infected with *T. b. gambiense* groupe 1 (Nkinin *et al.*, 2002).
- Doumé (4° 16' N, 13° 25' E) is located in the Eastern Region of Cameroon. It is an old sleeping sickness focus, where patients are presently rare. It is a degraded forest zone with several streams and wetlands. Domestic animals were bled in Paki, Medim, Baillon and Loumbou.

Sample collection, immunologic and parasiological analyses

In each focus, one of every three animals was randomly chosen to be included in the study. Bleeding was done through the jugular vein for goats and sheep, sub-clavicle vein for pigs and the saphena for dogs. The blood was collected into EDTA tubes. A LiTat 1.3 CATT (Magnus *et al.*, 1978) was performed on each sample to detect anti-*T. b. gambiense* antibodies, as well as a QBC (Quantitative Buffy Coat) (Bailey et Smith, 1992). The rest of the blood was preserved at 4 °C and transported to the laboratory for molecular analyses.

Extraction and amplification of DNA

In the laboratory, trypanosome DNA was extracted from the blood samples using the Ready Amp Genomic DNA purification system (PROMEGA) kit as described by Penchenier *et al.* (2000). The supernatant containing the DNA was stored at – 20 °C or used directly for PCR.
A first amplification protocol to detect *Trypanosoma brucei* s.l., was proceeded on the extracts of all the samples using the primers TBR1 (5'-GAATATTAAA CAATGCGCAG-3')/TBR2 (5'-CCATTTATTAGCTTTGTT GC-3') (Moser *et al.*, 1989). This was followed by a second amplification on *T. brucei* s.l. samples positive using the primers TRBPA1 (5'-GCGCCGACGATAC CAATGC-3')/TRBPA2 (5'-AACGGATTTCAGCGTT GCAG-3') (Herder *et al.*, 2002), to detect *T. b. gambiense* group 1. Amplifications were proceeded in a final 25 µl volume containing 10 mM Tris-HCL (pH 9),

50 mM KCl, 3 mM $MgCl_2$, 15 picomoles of each primer, 200 µM of each dNTP, 0.3 units of Taq DNA polymerase (Appligene-Oncor, USA), sterile water and 3 µl of the DNA extract. The amplification protocol included an initial denaturing phase at 94 °C for 3 min 30 sec, followed by 40 amplification cycles, each consisting of denaturing at 94 °C for 30 sec, hybridization of primers at 55 °C (*TBR1/2*) or at 62 °C (*TRBPA1/2*), and an elongation phase at 72 °C for 1 min. A final elongation was achieved at 72 °C for 5 min. The amplification products were separated by electrophoresis on a 2 % agarose gel containing 0.3 µg/µl ethidium bromide for *T. brucei* s.l. or 4 % for *T.b. gambiense* group 1. Amplified DNA fragments were visualised and photographed under UV light.

STATISITICAL ANALYSIS

The proportion of animals positive for CATT, QBC, and PCR were compared between host species and study site using the Chi-square (χ^2) test performed with *Statistix* program.

RESULTS

SEROLOGY

Of 875 animals, 194 (22.17 %) were LiTat 1.3 CATT-positive, with 95 (35.98 %) goats, 60 (19.54 %) pigs, 37 (13.85 %) sheep and two (5.40 %) dogs. All the animal species had trypanosomal antibodies. Therefore, the LiTat 1.3 CATT sero-prevalence differed significantly between the animal species (χ^2 = 29; p = 0.0001). Animals LiTat 1.3 CATT-positive were identified in all the study sites: 44 (14.19 %) in Campo, 80 (39.21 %) in Bipindi, 43 (27.92 %) in Fontem-Centre and 27 (13.04 %) in Doumé and proportion differed significantly between the study sites (χ^2 = 35; p = 0.0001), with the Bipindi and Fontem-Centre having the highest values.

PARASITOLOGY

A total of 34 (3.88 %) domestic animals were QBC-positive. The specific identification of trypanosomes was not possible at this stage. All the four animal species were infected with trypanosomes in all the study sites. The proportion of infected animals was relatively high in dogs (8.1 %) and goats (5.6 %) however, the difference in infection rates between the animal species was not significant (χ^2 = 5.4; p = 0.14) (Table I). Animal infection rates were significantly higher (χ^2 = 24.5; p < 0.0001) in the sleeping sickness foci of Bipindi (8.82 %) and Fontem-Centre (6.49 %) than in the less active places (Table II).

MOLECULAR ANALYSES

PCR revealed the presence of *T. brucei* s.l. DNA in 174 (19.88 %) animals studied, including 82 (30.71 %) sheep, 52 (19.69 %) goats, 27 (8.79 %) pigs and 13

Animals sampled	Number examined	Number QBC-positive (%)	Number CATT-positive (%)	Number PCR-positive (%)	
				TBR	TBG1
Sheep	267	7 (2.62)	37 (13.85)	82 (30.71)	18 (6.74)
Goats	264	15 (5.6)	95 (35.98)	52 (19.69)	8 (3.03)
Pigs	307	9 (2.9)	60 (19.54)	27 (8.79)	1 (0.32)
Dogs	37	3 (8.10)	2 (5.40)	13 (35.13)	0 (0)
χ^2		5.40	29	32.7	19.5
p value		0.14	0.0001	0.0001	0.0002

QBC: Quantitative Buffy Coat; CATT: Card agglutination Test for Trypanosomiasis; PCR: polymerase Chain Reaction; TBR: T*rypanosoma brucei* s.l.; TBG1: *Trypanosoma brucei gambiense* group 1.

Table I. – Results of different tests for the four sampled species.

Study site	Number examined	Number QBC-positive (%)	Number CATT-positive (%)	Number PCR-positive (%)	
				TBR	TBG1
Bipindi	204	18 (8.82)	80 (39.21)	47 (23.03)	8 (3.92)
Campo	310	5 (1.61)	44 (14.19)	97 (31.29)	15 (4.83)
Fontem	154	10 (6.49)	43 (27.92)	24 (15.58)	4 (2.59)
Doumé	207	1 (0.48)	27 (13.04)	6 (2.89)	0 (0)
χ^2		24.5	35.7	46.7	9.9
p value		0.001	0.0001	0.0001	0.019

QBC: Quantitative Buffy Coat; CATT: Card agglutination Test for Trypanosomiasis; PCR: polymerase Chain Reaction; TBR: *Trypanosoma brucei* s.l.; TBG1: *Trypanosoma brucei gambiense* group 1.

Table II. – Number and proportion of animals positive for different tests, by study site.

(35.13 %) dogs. There was a significant difference in PCR results between the animal species (χ^2 = 32.70; p = 0.0001), with sheep and dogs having higher rates than goats and pigs. The number of animal positive for the PCR were 97 (31.29 %) in Campo, 47 (23.03 %) in Bipindi, 24 (15.58 %) in Fontem-Centre and six (2.89 %) in Doumé; with the differences statistically significant (χ^2 = 46.7; p = 0.0001).

Out of the 174 domestic animals positive for *T. brucei* s.l. DNA, 27 (15.5 %) had also DNA specific for *T. b. gambiense* group 1. With respect to the total number of animals studied, this represents a prevalence of 3.08 % *T. b. gambiense* group 1, including 18 (6.74 %) in sheep, 8 (3.03 %) in goats, one (0.32 %) in a pig and none in dogs. These differences between species were statistically significant (χ^2 = 17.6; p < 0.0001). By study site, 15 (4.83 %) animals in Campo, eight (3.92 %) in Bipindi, four (2.59 %) in Fontem-Centre and none in Doumé had *T. b. gambiense* group 1 DNA. The differences were significantly different between the study sites (p = 0.019). However, if we take into consideration only study sites were *T. b. gambiense* DNA were found, the prevalence becomes comparable (p = 0.53). The 147 (84.5 %) positive samples for *T. brucei* s.l. DNA but negative for *T. b. gambiense* group 1 DNA probably contained *T. b. gambiense* group 2 or *T. b. brucei*.

DISCUSSION

The trypanosome infection rates in the domestic animals detected with QBC (3.8 %) corroborates previous finding by other authors in domestic animals (Scott *et al.*, 1983; Noireau *et al.*, 1986; Asonganyi *et al.*, 1986, 1990), and in wild animals (Komoin-Oka *et al.*, 1984; Njiokou *et al.*, 2006).

The proportion of animals LiTat 1.3 CATT-positive were generally higher than for QBC, suggesting that a high proportion of the animals had been in contact with *T. b. gambiense* or other parasites of the *T. brucei* s.l complex, or even *T. congolense* which also give positive results with LiTat 1.3 CATT (Noireau *et al.*, 1986). The results confirm the constant contact between infected Glossina and these animals. Low CATT-positive prevalence in dogs with respect to QBC (8.10 %), may suggest that most of the trypanosomes in dogs do not possess the LiTat 1.3 cross-reacting antigen. This hypothesis is strengthened by the identification of *T. vivax* DNA in some of these dogs (data not shown). Furthermore, these results could be biased due to the low number of dogs sampled compared to other species. PCR detected parasites of the *T. brucei* s.l. complex in 19.88 % of the animals, thus confirming the high sensitivity of the method. The results corroborates those of Simo *et al.* (2006) in pigs and Njiokou *et al.* (2006) in wild animals and are an indication of the high prevalence of trypanosomes of the *T. brucei* s.l. complex in domestic and wild animals in sleeping sickness foci in Cameroon. The presence of *T. b. gambiense* DNA in 6.74 % sheep, 3.03 % goats and 0.32 % pigs in sleeping sickness foci does not only confirm the role of pigs as reservoirs of HAT reported in Cameroon (Nkinin *et al.*, 2002; Penchenier *et al.*, 2005; Simo *et al.*, 2006), but also suggests the implication of other domestic animals like sheep and goats already reported in Congo by Scott *et al.* (1983) and Noireau *et al.* (1989).

The relative importance of the role of different animal species as reservoir hosts is complex. In this study, pigs are less infected by *T. brucei* s.l. than dogs, sheep and goats; yet, pigs are less infected by *T. b. gambiense* than goats and sheep. Further, analysis of blood meals shows that Glossina feed more on pigs than on the other domestic animals (Laveissière *et al.*, 1985; Simo *et al.*, 2008). These results can be explained not only by differences in the susceptibility of the animals, but also by their lifecycles. Whereas dogs, sheep and goats are maintained for many years in the foci and have prolonged contact with Glossina with possible infection, pigs hardly ever live for longer than one year. Young pigs 1 to 2 months old are usually reared from the beginning of the years and generally sold for various financial and ceremonial needs by September and December. In addition, pigs have a self-cure capacity and so can eliminate a *T. b. gambiense* infection (Penchenier *et al.*, 2005).

Comparison of study sites shows that animals from Bipindi and Fontem had the highest proportion of positive results with QBC and CATT, suggesting a greater frequency of contact with Glossina and trypanosomes, as observed by Simo *et al.* (2006) for pigs, and by Morlais *et al.* (1998) for Glossina in Fontem. The high prevalence of *T. brucei* s.l. and *T. b. gambiense* in animals in the sleeping sickness foci of Campo and Bipindi corroborate the results of a medical surveillance team that identified many patients in these foci (rapport PNLTHA, 2007, 2009).

The low number of positive results obtained in Doumé may be a reflection of the low transmission rate of HAT, since only three patients have been identified since 2000 by the National Program against HAT (PNLTHA). Further, this should be considered together with the very low apparent density (number of flies/trap/day) of the unique vector *Glossina fuscipes* in Doumé (0.11) as compared to the relatively higher density for the *Glossina palpalis palpalis* vector in Bipindi (1.89), Campo (2.06) (Mbida Mbida, 2006) and Fontem (4.96; Njitchouang *et al.*, personal communication). In addition, the frequency of transmission of trypanosomes in each locality is not stable; it can vary according to the frequency of diagnosis and treatment of patients which drastically reduces the human reservoir population; it can also vary according to the frequency of the cam-

paign against Glossina, which also reduces the transmission of all Glossina-transmitted trypanosomes. Diagnosis and treatment, and vector control explained the huge decrease of *T. b. gambiense* group 1 prevalence in wild animals in Bipindi from 10 % in 1999 to 0.1 % in 2001 (Njiokou *et al.*, 2006). The absence of *T. b. gambiense* infected pigs in Fontem-Centre in this study, compared to 15 % reported in Fontem-North in 1999 (Nkinin *et al.*, 2002) could also result from the reduction in transmission because of the annual medical surveillance organized in the focus by the PNLTHA since 2000.

This study has shown that, in addition to pigs already known to be reservoir hosts of *T. b. gambiense* group 1 in sleeping sickness foci in Cameroon, sheep and goats may also be infected in natural conditions. Although the importance of these animals as possible reservoir hosts for *T. b. gambiense* group 1 still need to be further explored, it is worthy of note that the prevalence of *T. b. gambiense* in the animals in the different study sites seems to reflect the general level of transmission of HAT. This probably indicates the implication of the animals infected with *T. b. gambiense* in the epidemiology of HAT in Cameroon.

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