



Molecular characterization of trypanosomes isolated from naturally infected cattle in the "Pays Lobi" of Côte d'Ivoire

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ABSTRACT

Objectives: this study aimed to investigate the prevalence of bovine trypanosomosis in the "Pays Lobi" of Côte d'Ivoire where a lot of trypanosusceptible zebu cattle are now found.

Methodology and Results: Blood samples were collected from 200 male and female cattle sampled in five villages from the department of Bouna. PCR technique was used for the diagnosis of trypanosomes. Out of the 200 animals examined, 41 were infected with trypanosomes, representing an infection prevalence of 20.5 %. The specific diagnosis revealed prevalence rates of 2.5 %, 3 %, 5 %, 10 % for *T. congolense* "Savannah", *T. congolense* "Forest", *T. vivax* and *T. brucei*, respectively. Three mixed infections were found involving *T. congolense* "Savannah" and *T. brucei*, *T. congolense* "Forest" and *T. vivax*, *T. vivax* and *T. brucei*.

Conclusions and applications of findings: The outcome of this study has shown that more attention is needed in controlling trypanosome infection in the "Pays Lobi". Indeed, better understanding of prevalence of sub-clinical infection could help with efforts to control the disease and population-based screening of both animal and insect vectors.

Key words: Prevalence, Trypanosomes, trypanosusceptible, zebu; "Pays Lobi"

INTRODUCTION

In sub-Saharan Africa, diseases transmitted by tsetse flies are a problem for both animal and human health. Animal African Trypanosomosis (AAT) is a vector-borne disease found in tropical regions of Africa (Euzéby 1986). The tsetse fly transmitted trypanosomes, *Trypanosoma brucei*, *T. congolense* and *T. Vivax*, specific to cattle and

other ruminants (Alfredo, 2004; Jay, 2008). Human African trypanosomiasis (HAT), also known as sleeping sickness is an endemic parasitic disease caused by *Trypanosoma brucei sl.*

In Africa, AAT is rampant in 37 countries of sub-Saharan region, an area of approximately 10 million km² (Trail et al. 1985; Erkelens et al.,

2000). AAT remains a major pathological constraint for livestock development in sub-Saharan Africa, in areas that have high forage and agricultural potentials (Panin & Mahabile, 1997). The parasitic disease altered cattle distribution in the region and in addition to causing widespread disease. This disease created local overstocking restricted to Africa where it have been responsible for the exclusion of livestock from large areas of land which are potentially capable of problems in tsetse-free grazing areas. Moreover, ATT enforced nomadism on breeding herds and economic loss in cattle along cattle trade routes in West Africa (Rogers *et al.*, 1996; Wilson *et al.*, 1997). The direct and indirect losses of currencies linked to this pathology are estimated at about 4.5 billion dollars (Mattioli *et al.*, 2004). In tsetse-infested areas, the deficit in meat production is estimated at 30 %, and that of milk production to 40%. Furthermore, a farmer breeds about 2 fold less draught animals and cultivated 3 fold less area (Swallow 1998). In Côte d'Ivoire, except for the forest area (4.3% of livestock) where environmental conditions are a limiting factor for the development of animal husbandry, pre-forest (central) region (17.4% of national herd) and the northern region (78.3% of national herd) appear as livestock regions. However, in some parts of these regions, the prevalence of AAT may reach 25% when there is no control activity. This prevalence is detrimental to farms and animals productivity in this region where breeders from neighbouring sahelian countries and their zebu cattle tend to settle. This is the case of "Pays Lobi" that was literally invaded by zebu cattle. Yet this area of the Northern Region of Côte d'Ivoire has long been

MATERIAL AND METHODS

Study Area: This study was conducted in five (05) farms selected in different villages of the department of Bouna : The village of Kopengué located at 32 km in the west; Tamé-Koulda at 30 km in the north, Niandégué and Bromakoté located at 07 km in the northwest and a camp located near the aerodrome at 4 km in the south of Bouna (Figure 1). Farms were selected by agents of the Department of Animal Production and Fisheries Resources, according to the different geographical areas of the study zone. This

considered the cradle of trypanotolerant Baoulé breed. Indeed, this region has long been closed to entry of foreign cattle breeds. Nowadays, the cattle herd composition of "Pays Lobi" is strongly influenced by the presence of zebu cattle trypanosusceptible. Indeed, most of the farms in this area are characterized by the presence of local breed (Baoulé), zebu and Méré (animals from crosses between zebu and Baoulé cattle). Furthermore, farmers breed these trypanosusceptible zebu cattle under high trypanocide drug coverage. Nevertheless the wide spread use and the misuse of drug has contributed to the development of drug resistance in the population *T. congolense* parasites (Codjia *et al.*, 1993; Afework *et al.*, 2000). Therefore, it appears necessary to assess the trypanosome risk on cattle farms in this area, where many trypanosusceptible cattle (zebu) are now found. The use of traditional microscopic such as determination of parasite mobility and morphology is sufficient to distinguish the trypanosome species in the mammalian host. However, this method is not accurate enough to provide diagnosis, since many-mixed infections occur in natural conditions (Solano *et al.*, 1995, 1996). The molecular biology method has overcome the limits of sensitivity and specificity of the old methods (Morlais *et al.*, 1998). The polymerase chain reaction (PCR) has been developed to facilitate the detection of many types of parasite (De Bruijn *et al.*, 1993; Laserson *et al.*, 1994), including trypanosomes (Masiga *et al.*, 1992; Majiwa *et al.*, 1994; McNamara *et al.*, 1995). Therefore, this study aims to detect and identify trypanosome infections in cattle from "Pays Lobi" of Côte d'Ivoire, using PCR technique.

selection was based on the following criteria: (i) accessibility of the park, (ii) availability of breeder and (iii) presence of greater than 50 individuals in the herd. The department of Bouna, is located in the north-east of Côte d'Ivoire, near the border with Ghana and Burkina Faso. With an area of 38,000 km², the department of Bouna is one of the gateways to the Comoé National Park. The terrain is flat. However, there are some mountains whose heights, ranging from 500 to 600 meters, which break in places the uniformity

of the landscape. The climate is a sub-Saharan one, and it has four seasons, two rainy seasons from March to June and September to October, respectively. These are interspersed with two dry seasons, which start from November to February and July to August, respectively. Annual rainfall varies between 1100 and 1200 mm with an average temperature of 28 ° C. The vegetation consists of Sudano-Guinean savannah woodlands,

wooded savannas and woodlands. Under the effect of socio-economic activities, the vegetation is increasingly dominated by shrubs and herbs. The drainage system consists of two main rivers that are Comoé and Black Volta. Agriculture and animal husbandry are the main activities of rural people. Indeed, cattle and small ruminants are the second pillar of the region's economy.



Figure 1: geographical distribution of sampling sites

Methods

Sampling: In each farm visited, 40 male and female cattle aged more than a year, were sampled taking into account all the breeds present in the herd. In each farm, the choice of animals was done randomly. From each animal sampled, 5 ml of blood was collected from

the jugular vein into a "vacutainer" EDTA tube (ethylene diamine tetra acetic acid), and stored in ice. Two (2) aliquots of 1 ml were carried out from collected blood. Aliquots were kept on ice in the field before being frozen in the Laboratory for molecular analyzes. Each sampled animal was identified by a code to find the

name of the village, livestock and the serial number of this animal. The same code was carried over the tubes containing the different blood samples. In addition, some information about the body condition of animals sampled was collected.

PCR technique: Genomic DNA was extracted from blood samples collected according to Phenol chloroform method (Maymonna, 1998). PCR technique was applied to determine the presence of genes of different species of trypanosomes. Amplification reaction was performed in a final volume of 50 µl containing 10 mM of Tris-HCL pH 8.3, 50 mM of KCl, 3 mM of MgCl₂, 200 ml of each deoxynucleotide triphosphates (dATP, dGTP, dCTP and dTTP), 20 pmol of each primer, 5 units of Taq DNA polymerase amp (5') and 5µl of extracted DNA (diluted 1/10 or 1/30). The thermal cycler (BIO RAD T100 Thermal cycler) was programmed as follows: an initial denaturation step of 3 min at 94 °C followed by 30 times repeated cycling step

comprising a denaturation phase of 30 sec at 94 °C, a phase of fixing of the primers 30 sec at 60 °C and an elongation phase at 72 °C 1 min and finally a step of 10 min to a final 72 °C extension. The amplification products were subjected to electrophoresis on agarose gel for visualization. The amplification products migrate according to their size. The agarose gel was prepared at 2 % in a 0.5 X TBE buffer and placed in a tank of electrophoretic migration. In each well of the gel produced using a comb, 7µl of each amplification product and a molecular weight marker product (Table 1) were deposited. Then, the generator was powered (100V). Electrophoretic migration takes about 30min. After electrophoresis, the gel is then placed in a tank containing a solution of ethidium bromide (4µg / ml) for 20 min. After rinsing, the gel is placed on a UV transilluminator and photographed to be viewed. The nucleotide sequences of the primers used for PCR are shown in Table 1.

Table 1: Primers used for PCR amplification of trypanosome

Spfecity	Code	Primer sequence	AP ⁽¹⁾	Reference
<i>T congolense</i>	TCS ₁	5'CGAGAACGGGCACTTTGCGA3'	316	Masiga et al. (1992)
(savannah)	TCS ₂	5'ACACGCCCTAAAGAAACAGC3'		
<i>T congolense</i>	TCF ₁	5'GGACACGCCAGAAGGTACTT3'	350	Masiga et al. (1992)
(forest)	TCF ₂	5'CAACCTAAACCACGCTCTTG3'		
<i>T vivax</i>	TVW ₁	5'CTGAGTGCTCCATGTGCAAC3'	150	Masiga et al. (1992)
	TVW ₂	5'AGTCCAACCACAAGACCACC3'		
<i>T brucei</i>	TBR1	5'CGAATGAATAACAATGCGCAGT3'	164	Masiga et al. (1992)
	TBR2	5'AGAACCATTTATTAGCTTTGTTGC3'		

⁽¹⁾ Expected size of the amplification product (Bp).

Data analysis: Data obtained were recorded in Excel to produce descriptive statistics. These descriptive statistics was employed in analyzing the data. The prevalence rates among localities, type of cattle, and sex of the animals were expressed as percentage of the total number of animal sampled. Chi square test

was used to evaluate association between the prevalence of infection and locality, breed and sex of the cattle studied. A P value of P< 0.05 was considered significant. These analyzes were performed using STATA 9.0

RESULTS

Overall prevalence: The examination by PCR for the presence of at least one of four species of trypanosomes permitted to obtain an average parasitological prevalence of 20.5 % (41/200). No significant difference of infection (p>0.05) was observed among the sampling sites. However, the highest infection was observed in the locality of Kopengué with a prevalence rate of 32.5 % (13/40). The lowest infection was observed in the sampling site near the aerodrome with a prevalence rate of 7.5 % (Table 2). Out of the 200 animals sampled in this study

35 were Méré cattle and 165 zebu cattle. Of the 33 Méré cattle examined, 8 were infected with a prevalence rate of 22.86 %. The prevalence observed in zebu cattle was 20 % (33/165). No significant difference of infection (p>0.05) was observed between these two types of cattle. A total of 177 female were sampled in this study and in total and 20.33 % of them were positive for AAT (36/177). Out of the 23 male examined, 5 were infected with a prevalence rate of 21.74 %. No significant difference (p>0.05) was observed between male and female.

Table 2: Prevalence of trypanosomes depending on sampling sites

Sampling site	Sample size	Infected animals	Prevalence (%)	X ²	P. value
Kopengué	40	9	22.5	6.57	0.160
Tamé-Koulda	40	7	17.5		
Niandégué	40	9	22.5		
Bromakoté	40	13	32.5		
Aerodrome	40	3	7.5		
Total	200	41	20.5*		

*Overall prevalence

Specific diagnosis

Diagnosis of *T. congolense* "Savannah": PCR technique for the specific diagnosis of *T. congolense* "savannah" in the five sampling sites permitted to obtain an overall prevalence of 2.5 %. A heterogeneity in prevalence was observed among the five (05) study sites, although there was no significant difference of infection ($p > 0.05$) with *T. congolense* "Savannah" between these sites. Infection with the parasite was observed in two sites only, in the village of Bromakoté, with a prevalence of 7.5 % and in Tamé-Koulda where a prevalence of 5% was observed. However, there was

no parasitic infection in the three other sites: Kopengué, Niandégué and Aerodrome (Table 3).

Diagnosis of *T. congolense* "Forest": From the total of 200 animals examined for AAT, 6 were positive for *T. congolense* "Forest". The comparison of prevalence of the 5 sampling sites showed that there is no significant difference of infection ($p > 0.05$) between them. However, infection with this parasite was observed in two sites; Kopengué and Tamé-Koulda with the same prevalence, 7.5 %, while no animals was infected with this parasite in the three other sampling sites (Table 3).

Table 3: Prevalence of *T. congolense* "Savannah" and *T. congolense* "Forest"

Localities	<i>T. congolense</i> "Savannah"					<i>T. congolense</i> "Forest"				
	Nt	Ni	Prev. (%)	X ²	P	Nt	Ni	Prev. (%)	X ²	P
Kopengué	40	0	0	8.2	0.003	40	3	7.5	9.28	0.06
Tamé ⁽¹⁾	40	2	5			40	3	7.5		
Niandégué	40	0	0			40	0	0		
Bromakoté	40	3	7.5			40	0	0		
Aerodrome	40	0	0			40	0	0		
Total	200	5	2.5 ⁽²⁾			200	6	3 ⁽²⁾		

Nt : Sample size ; Ni : Number of animals infected, Prev. : Prevalence, P: P-value

⁽¹⁾ Tamé-Koulda

⁽²⁾ Overall prevalence

Diagnosis of *T. vivax*: Molecular analysis of the 200 animals for the specific diagnosis of *T. vivax* yielded an average prevalence rate of 5 %. The prevalence rate of 15 % observed in the locality of Kopengué was significantly higher than that obtained in the other sites. However, no infection with *T. vivax* was observed in the locality of Niandégué (Table 4).

Diagnosis of *T. bucei*: Out of the total of 200 animals examined, 20 were infected with *T. bucei*, with a prevalence rate of 10 %. The comparison of prevalence

rate from the different sampling sites through the Chi-square test showed a very highly significant difference ($p < 0.001$) between these sites. No infection was observed in the village of Kopengué and Tamé-Koulda. However, in Niandégué and Bromakoté, a prevalence rate of 22.5 % was observed; 9 animals out of 40 were infected with *T. bucei* (Table 4).

Table 4: Prevalence of *T. vivax* and *T. brucei*

Localities	<i>T. vivax</i>					<i>T. brucei</i>				
	Nt	Ni	Prev. (%)	X ²	P	Nt	Ni	Prev. (%)	X ²	P
Kopengué	40	6	15	11.6	0.02	40	0	0	23.89	0.0008
Tamé ⁽¹⁾	40	2	5			40	0	0		
Niandégué	40	0	0			40	9	22.5		
Bromakoté	40	1	2.5			40	9	22.5		
Aerodrome	40	1	2.5			40	2	5		
Total	200	10	5 ⁽²⁾			200	20	10 ⁽²⁾		

Nt : Sample size ; Ni : Number of animals infected, Prev. : Prevalence, P: P-value

⁽¹⁾ Tamé-Koulda

⁽²⁾ Overall prevalence

Types of infection: Among the 41 cases of infection detected by PCR technique out of the 200 samples of this study, 37 cases (90.24 %) were mono-infections. Four (4) cases of mixed infections were observed. There were two cases of mixed infection involving *T.*

congolense Savannah and *T. brucei*, one case of mixed infection involving *T. congolense* Forest and *T. vivax*, one case of mixed infection involving *T. vivax* and *T. brucei*.

DISCUSSION

The prevalence rate of bovine trypanosomosis of 20.5 % obtained in this study is higher than the 3.9% previously reported in grazing cattle in Ogbomoso, Oyo State (Ameen *et al.*, 2008), 4.69% previously reported in the Oyo State in Nigeria (Fasanmi *et al.*, 2014) and 7.81% previously reported in Woliso Woreda, Ethiopia (Gebreyohannes & Legesse, 2014). However, the 46.8% of bovine trypanosomosis rate reported on the Jos Plateau, Nigeria are higher than what was found in this study (Majekodunmi *et al.*, 2013). This difference could be due to the difference between the number of sampling sites and the sample size. In effect, in this study a total of 200 animals were sampled in five villages, while Majekodunmi *et al.* (2013) have examined 7,143 individual cattle sampled in 30 villages. Furthermore, the absence of a significant difference of infection between the five sampling suggests no discriminatory presence of trypanosomes in all the study area. Moreover, the lowest prevalence rate of trypanosomosis observed in site near aerodrome could be attributed either to (i) a better implementation of preventive and curative trypanocidal treatments in the herds by farmers, (ii) good knowledge of the grazing course by the herdsmen who drive the animals, or (iii) the low level of infestation of the route by the flies. For prevalence depending on type of cattle, no significant difference of infection ($p > 0.05$) was observed between zebu and Méré cattle. However, the observed imbalance in sample sizes of cattle could not allow objective comparisons between these types of cattle.

The sample of 200 cattle of this study consists of 165 zebu cattle, against only 35 Méré cattle. In effect, studies in northern Côte d'Ivoire during the 1980s and analyzing the degree of infection in different genotypes showed that zebu cattle showed infection levels much higher than those of all other genetic, Baoulé, N'Dama and zebu crossbred with (Camus, 1981). Indeed, some studies have provided an exacerbation, the situation of trypanosomiasis with increased blood circulation. In effect, Zebu in farms villagers consist of bullfighting in N'Dama Baoulé (Camus *et al.*, 1981; Sokouri *et al.*, 2009; Bocoum *et al.*, 2012). The result of this study showed that sex prevalence of bovine trypanosomosis was not significantly different between male and female animals. However, this result could not objective because of the great difference between the male sample size (23 individuals) and the female sample size (177 individuals). However, Fasanmi *et al.* (2014) who examined 121 female cattle and 199 male, also reported no significant difference in the infection prevalence of male and female animals. Sex dimorphism in trypanosomosis has been previously reported (Abenga *et al.*, 2004; Agu *et al.*, 1990, Agu & Amadi, 2001). This result is also in agreement with those reported by Sam-Wobo *et al.* (2010). The specific diagnosis allowed the following prevalence; 2.5 % for *T. congolense* "Savannah" and 3 % for *T. congolense* "Forest", 5 % for *T. vivax* and 10 % for *T. brucei*. Thus, the results of this study showed the prevalence of three trypanosomes, *T. congolense*, *T. vivax* and *T. brucei*,

infecting mainly livestock in this region. The prevalence of *T. Congolense* infection in this study is lower than that (27.7 %) reported on the Jos Plateau, Nigeria (Majekodunmi et al., 2013). However, this prevalence is higher than (0.005%) reported in Ethiopia by Gebreyohannes & Legesse (2014). These authors assert that the presence of low prevalence of *T. congolense* is associated with low tsetse infested area. *T. congolense* may enter from endemic area due to animal movement. The prevalence of *T. vivax* infection reported in this study is comparatively lower than those reported on *T. vivax* epizootiology in Nigeria (Agu & Amadi, 2001; Ameen et al., 2008; Fasanmi et al., 2014). According to Fasanmi et al (2014), the higher

prevalence of *T. vivax* observed in Nigeria may be connected with its molecular biology, which may have played a role in conferring it with resistance against both drugs and host defence. This could be the cause of the superiority of *T. brucei* on the other species, with a prevalence rate of 10 %. Moreover, both tsetse species: *G. tachinoides* and *G. p. palpalis* which are able to transmit *T. brucei* (Majekodunmi, 2006) are abundant in the study area and they transmit this species at a higher frequency. The results of this study about mixed infection involving *T. congolense* Savannah and *T. brucei*, *T. congolense* Forest and *T. vivax*, are also reported in Burkina Faso by Solano et al. (1997).

CONCLUSION

This study assessed the trypanosome risk and diagnoses various specific species in the "Pays Lobi" of Côte d'Ivoire, by using the PCR technique. The average prevalence of infection, revealed by molecular analysis, was 20.5 %. Three species of trypanosomes were found in the study area; *T. congolense* (the Savannah type with a prevalence rate of 2.5 % and the Forest type with 3 %), *T. vivax* with a prevalence rate of 5 % and *T. brucei* with a prevalence rate of 10 %. The

outcome of this study has shown that more attention is needed in controlling trypanosome infection in the "Pays Lobi". Therefore, it appears necessary to investigate the prevalence of bovine trypanosomiasis throughout all the "Pays Lobi". Thus, it would be interesting to extend this study to other areas of this region. It also seems necessary to (i) characterize the different species of flies that are present in the region and (ii) assess drug resistance of trypanosomes.

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