Open Journal of Veterinary Medicine, 2014, 4, 314-321
Published Online December 2014 in SciRes. http://www.scirp.org/journal/ojvm
http://dx.doi.org/10.4236/ojvm.2014.412038

Molecular Characterization and Prevalence of *Trypanosoma* Species in Cattle from a Northern Livestock Area in Côte d'Ivoire

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Received 15 October 2014; revised 28 November 2014; accepted 12 December 2014

Abstract

Background: African animal trypanosomiasis (AAT) is caused mainly by *Trypanosoma congolense*, *T. vivax*, and *T. brucei brucei* and is the major constraint for livestock productivity in Sub-Saharan African countries. Information about animal trypanosomiasis status in Ivory Coast is missing, especially regarding molecular epidemiology. Therefore, this study intended to apply molecular tools to identify and characterize trypanosomes in Ivory Coast for sustainable control. Methods: 363 cattle blood samples were collected from Ferkessedougou Region in northern Ivory Coast in 2012. Buffy coat technique (BCT) and species-specific PCR assays were used to detect trypanosome species. Results: Out of 363 cattle examined with BCT, 33 were found positive with all trypanosomes species accounting for an average of 9.09% prevalence whereas polymerase chain reaction (PCR) using species-specific primers showed that 81 out of 363 cattle were infected with trypanosomes with an overall prevalence of 22.31%. *Trypanosoma congolense* savanah type, *T. Vivax* and *T. brucei* sl. accounted for 28.39%, 49.38% and 23.45% of the infection rate respectively. No infection with *T. congo* forest type was detected. *T. vivax* infection was the most prevalence in the area investigated compared to the two other trypanosome species. Mixed infections with different trypanosomes species were detected accounting for 7.32% of prevalence. Regarding sex-related prevalence, male cattles were slightly more infected than female but the difference was not significant. Conclusion: Our results showed that there was a high prevalence of AAT in livestock in Ferkessedougou Area. There is therefore a need to strengthen control policies and institute measures that help prevent the spread of the parasites for sustainable control of animal trypanosome in this area.

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How to cite this paper: Kouadio, I.K., Sokouri, D., Koffi, M., Konaté, I., Ahouty, B., Koffi, A. and N'Guetta, S.P. (2014) Molecular Characterization and Prevalence of *Trypanosoma* Species in Cattle from a Northern Livestock Area in Côte d'Ivoire. *Open Journal of Veterinary Medicine*, **4**, 314-321. http://dx.doi.org/10.4236/ojvm.2014.412038

Keywords

Animal African Trypanosomiasis, Molecular Diagnosis, Species-Specific PCR, Côte d'Ivoire

1. Introduction

Animal health and livestock production in Sub-Saharan Africa suffer from high prevalence of trypanosomiasis with estimated annual losses due to direct and indirect consequences of the disease nearby billions of dollars [1].

Regarding the burden and lost occasioned by animal African trypanosomiasis (AAT), the African Union initiated a regional control strategy, the pan Africa tsetse and trypanosomiasis eradication campaign (PATTEC) [2]. Among others, the success of this aim lies on the accurate diagnosis of the disease in each country but this is limited by use of parasitological diagnostic techniques as microscopy due to low sensitivity [3]. Fortunately polymerase chain reaction (PCR) diagnostic assays overcome the low sensitivity limitations of parasitological techniques. PCR assays are powerful marker tools, species-specific and sensitive, for molecular identification and diagnosis of trypanosome species in their mammal hosts and vectors to assess the real prevalence and correlate burden in a given country or region.

Despite campaigns towards trypanosome eradication in Sub-Saharan Africa, there is a lack of research using molecular screening of trypanosomes in Ivory Coast and the latest information about the prevalence of AAT dates about more than two decades and further drug resistance is described [4] [5]. Information on the status of animal trypanosomiasis in Ivory Coast needs to be updated especially in the Northern part which is an important livestock area. This work was therefore designed to investigate the prevalence of trypanosomiasis in Ferkessedougou, a district in northern Ivory Coast, well known for its large population of cattle.

2. Methods

2.1. Study Site

The study was carried out from July to August 2012 in five farming villages of the department of Ferkessedougou (9˚5'N - 4˚75'W) in the northern part of Ivory Coast at 650 km from Abidjan, the economical capital (**Figure 1**). Ferkessedougou is covered by arboreous savannah and the climate is Sudanese type (hot and dry).

2.2. Blood Sample Collection and Filed Analysis

363 cattle from both sexs were randomly selected and bled in five cattle herds. 5 ml of blood were aseptically collected from the jugular vein of each animal into an Ethylene diamine tetra acetic acid (EDTA) tube which was then labeled and placed in ice pack. In the field, trypanosomes were check based on Buffy Coat Technique (BCT) [6]: Blood samples from the EDTA tubes were transferred into capillary tubes. One end of each of the capillary tube was sealed with plasticine and spun in a microhaematocrit centrifuge at 1500 rpm for 5 minutes. The haematocrit tubes were then taken and cut at the buffy coat level to release the contents on a clean grease-free microscope glass slide to which a cover slip was placed for examination at 40× objective magnification for motile trypanosomes.

2.3. Molecular Analysis

Further to field analysis, two aliquots of each blood sample were transferred in nunc^{R} cryotubes and taken to the Laboratoire des Interactions Hôte-Microorganisme-Environnement et Evolution (LIHME) at Jean Lorougnon Guédé University for molecular analysis.

Genomic DNA for the screening of trypanosomes was extracted from the blood samples using the salt out method. Briefly, 500 µl of each blood sample were washed four times. Then, 500 µl of the washing buffer, 100 µl of cell lysis buffer, 10 µl of SDS 10% and 10 µl of Proteinase K (concentration 10 mg/ml) were added to pellet and incubated at 56˚C for 30 minutes. Afterwards, DNA was precipitated using ethanol 70%. Pellets were air-dried for about 4 hours, and DNA was re-suspended in 75 ml of Tris-EDTA (TE) buffer and stored at −20˚C until it use for PCR.

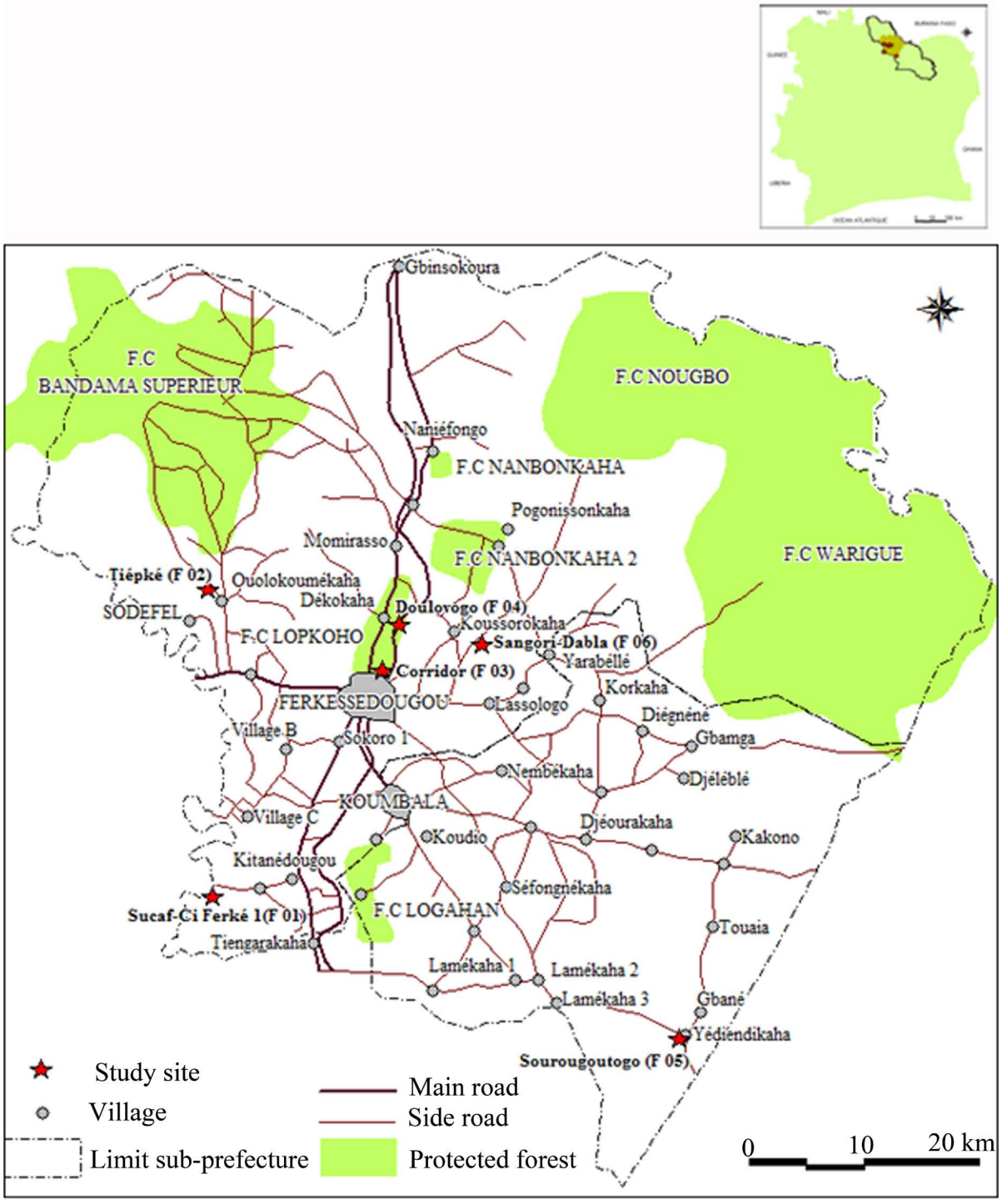

Figure 1. Study locations.

Amplification of trypanosome DNA was conducted using species-specific primer pairs for *Trypanosoma congolense*, *Trypanosoma vivax* and *Trypanosoma brucei* sl. (**Table 1**). The amplifications were conducted in a total volume of 50 µl containing 5 µl of PCR buffer 10× (10 mM Tris-HCl (pH 9.0), 50 mM KCl, 3 mM $MgCl_2$), 15 picomoles of each primer, 200 µM of each of the four deoxynucleotide-triphosphate (dNTP), one unit of Taq DNA polymerase (Appligene-Oncor, USA), sterile water and 5 µl DNA extract. Amplification involved pre-denaturation at 94˚C for 3 min followed by 30 cycles of denaturation at 94˚C for 1 min, hybridization of primers

at 60˚C and elongation at 72˚C for 1 minute, then final elongation at 72˚C for 15 min. PCR products were separated by electrophoreses on a 2% (w/v) agarose gel for 30 min at 100 V. The gel was stained in an ethidium bromide solution for 10 min and visualized under UV light.

2.4. Statistical Analysis

The proportions of animals infected by different trypanosome species were compared between cattle herds and location using Chi-square (X^2) or Fisher exact test according to the Stata 9.2 software [7].

3. Results

3.1. Buffy Coat Technique-Based Diagnosis

Out of 363 cattle examined with BCT, comprising 73 males and 290 females (**Table 2**), 33 were found positive with trypanosomes strains (**Table 3**) accounting for an average of 9.09% prevalence.

3.2. Molecular Diagnosis and Statistic Inferences

PCR using species-specific primers for *Trypanosoma congolense*, *T. vivax* and *T. brucei* sl. showed that 81 out

Table 1. Species-specific primers for PCR amplification.

Trypanosome species/subgroup	Primer code and sequence 5'-3'	Product size (bp)
T. c. s.	TCS1 CGA GAA CGG GCA CTT TGC GA TCS2 GGA CAA AGA AAT CCC GCA CA	316
*T. c.*f.	TCF1 GGA CAC GCC AGA AGG TAC TT TCF2 GTT CTC GCA CCA AAT CCA AC	350
T. brucei	TBR1 GAA TAT TAA ACA ATG CGC AG TBR2 CCA TTT ATT AGC TTT GTT GC	177
T. vivax	TVW1 CTG AGT GCT CCA TGT GCC AC TVW2 CCA CCA GAA CAC CAA CCT GA	150

T. c. s. = *Trypanosoma congolense* savanah; *T. c.* f. = *Trypanosoma congolense* forest.

Table 2. Number of animals sampled by site and sex.

Sampling site	Male	Female	Total
Sucaf ci ferke 1	18	42	60
Tiekpe	10	49	59
Corridor ouangolo	8	37	45
Doulovogo	8	52	60
Souroutogo	17	55	72
Sangori-Dabla	12	55	67
Total	73	290	363

Table 3. Microscopy versus PCR diagnosis.

Number of animal tested (N = 363)				
Diagnostic technique	Positive (%)	Negative	X^2	p-value
BCT	33 (9.09)	330	21.2	<0.0001
PCR	81 (22.31)	282		

of 363 cattle were infected with trypanosomes with an overall prevalence of 22.31% in the total bovine investigated. Molecular diagnosis was very significantly different to microscopic observation based on buffy coat technique ($p < 0.001$) (**Table 3**). It appears that PCR was 2.5 times more sensitive compare to BCT. Regarding sex-related prevalence, male cattle were slightly more infected than female (**Table 4**) but the different was not significant ($p > 0.05$). Out of the 81 animals tested positive, *Trypanosoma congolense* savanah type, *T. Vivax* and *T. brucei* sl. accounted respectively for 28.39%, 49.38% and 23.45% (**Table 5**). The site of Corridor ouangolo was the most infected location with 37.78%, followed by Sangori-Dabla with 32.84% and less infected site was Sourougoutogo with 8.33% of prevalence (**Table 6**, **Figure 1**). No infection with *T. congo* forest type was detected. Mixed infections with different trypanosomes species were detected accounting for 7.32% of prevalence.

Table 4. PCR-based prevalence of trypanasomiasis related to sex.

	Results of PCR				
Sex	Number of animals examined	Positive (%)	Negative (%)	X^2	p-value
Male	73	18 (26.02)	55 (73.98)		
Female	290	63 (21.72)	227 (78.28)	0.62	0.43
Total	363	81 (22.31)	282 (77.79)		

Table 5. Prevalence of trypanosomes infection related to species.

Sampling sites	Number of animals examined	Number of positive PCR (N = 81)		
		TCS	TV	TBR
Sucaf ci ferke 1	60	0	3	9
Tiekpe	59	3	6	0
Corridor ouangolo	45	12	1	7
Doulovogo	60	0	5	10
Souroutogo	72	4	2	0
Sangori-Dabla	67	4	23	0
Total	363	23	40	19
Prevalence		28.39	49.38	23.45

TCF: *Trypanosoma congolense* savannah type; TV: *Trypanosoma vivax*, TBR: *Trypanosoma brucei* sl.

Table 6. Prevalence of trypanosomes infection related to sampling sites.

Sampling sites	Number of animals examined	Number of positive PCR (%)
Sucaf ci ferke 1	60	12 (20.00)
Tiekpe	59	9 (15.25)
Corridor ouangolo	45	17 (37.78)
Doulovogo	60	15 (25.00)
Sangori-Dabla	72	6 (8.33)
Sangori-Dabla	67	22 (32.84)
Total	363	81 (22.31)
X^2		20.37
p-value		<0.001

4. Discussion

Achieving the goal of sustainable control of animal trypanosomiasis requires sensitive and specific molecular tools to conduct a rigourous epidemiological study for accuracy assessing the disease prevalence and burden. Still today, several epidemiological studies are based on microscopic observation of trypanosomes [8]-[10]. Our study just showed again that micoscopic observation underestimates the true prevalence of infection. Indeed, only about third of trypanosomes expected are detected by the BCT. Such evidence should help researchers to preferably use molecular diagnosis.

The overall molecular-based prevalence of AAT recorded in the present study, 22.31% is higher compared to what was found 3 decades before [11]. A study conducted in 2010 in Mali, a country which shares the cotton growing areas with the northern part of Ivory Coast gives 30.86% as prevalence [12] which is almost similar to the prevalence we had but in the same country another team found 15.7% as global prevalence [13]. The overall prevalences in those studies were obtained with the BCT and could be revised up if the molecular diagnosis was applied. In addition, the difference in prevalence observed in the same country shows that variations in trypanosome infection rates in different ecological zones and time are common, as previously reported [14]. Therefore, the climatic variations across different geographical regions and type of husbandry practices are factors that can influence survival of both vector and parasite. A statistically significant difference was observed ($p < 0.05$) in prevalence between the 3 trypanosomes species investigated. *T. vivax* was the most prevalent species contrary to studies that have reported *T. congolense* as the predominant species in cattle in various parts of Africa [15]. Our study is in consonant with study conducted in western Kenya [16] were *T. vivax* was found the most prevalent species. The high prevalence of *T. vivax* is consistence with other findings in domestic animals [10] [17] and may result from the level of pathogenicity of this trypanosome, which is generally low and better controlled by animals [18]. The lower prevalence of *T. congolense* with respect to *T. vivax* in animals may result from higher parasitemia in *T. congolense* infections, accompanied by serious anaemia, which leads to the rapid death of animals [19] [20]. Species-specific PCR did not detect *T. congolense* forest type in Ferkesedougou Area and this seems natural considering that it is thought to be a forest sub-groups whereas Ferkessedougou is a savanah region.

The presence of mixed infections emphasizes the need for epidemiologists to focus beyond single species to multiple infections with different parasites and different genotype within species which are a norm under field conditions [21]. Lower trypanosome prevalence found in Sourougoutogo compared to others sites could be due to recent trypanocidal treatment stated by the owners (date not shown).

Even though the prevalence of trypanosomiasis was relatively higher in male cattle than female, there was no significant difference between sex prevalence ($p > 0.05$) and this was in consonant with study conducted in 2011 in south-west Ethiopia [9] contrary to Abenga *et al*. [22] study were sex dimorphism in trypanosomiasis has been reported. Gender should not play a relevant role in influencing the susceptibility of the animals to infection regarding several environment interactions [10].

This molecular epidemiology work confirmed the occurrence of mixed infections in the field, which could not have accurately been detected by the classical parasitological methods although it could influence the severity of AAT.

5. Conclusions

Animal trypanosome prevalence is not insignificant in Ferkessedougou, one of the most cattle production areas in Ivory Coast. Intervention to control the disease by the various stakeholders is therefore highly recommended.

The Pan African Tsetse and Trypanosomiasis Eradication Campaign, PATTEC set up by the African Union has been run for several years in countries that share the same borders with Ivory Coast including Mali, Burkina Faso and Ghana whereas today the Ivory Coast has not yet initiated PATTEC program. Updating data on the epidemiology of animal trypanosomiasis with molecular tools in high potential agropastoral areas for advocacy could help the Department of Animal Production first, to relieve the farmers but also in a context of international dynamic of tsetse populations, to avoid the phenomenon of re-invasion of other countries working to eliminate AAT in their countries infested.

Acknowledgements

This work was funded by the International Foundation for Science (IFS), Karlavägen 108, 5th floor, SE-115 26

Stockholm, Sweden (Fellow ship No. AB/21683R). We are very grateful to the farmers who gave their consent for this study.

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