

(RESEARCH ARTICLE)



## Trypanosome burden in an emerging livestock breeding area in central-western Côte d'Ivoire

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### Abstract

The Haut-Sassandra region, in the center-west of Côte d'Ivoire, is experiencing a real emergence of cattle breeding thanks to pasture available almost all year round. However, this region, endemic for human African trypanosomiasis, is also teeming with animal trypanosomes, which remains a brake on productivity. In this region, the real parasitic burden due to trypanosomes is not well documented. This study aims to evaluate the serological prevalence of trypanosomes using blood samples collected from 164 cattle originating from 8 farms in the departments of Daloa and Zoukougbeu. Serological tests carried out on all animals revealed an overall prevalence of 27.44% with CATT-B and 57.78% with CATT-P. According to sex, females (CATT-B+ = 28.21% and CATT-P  $\geq 1/4$  = 65.63%) were more infected than males (CATT-B+ = 25.53% and CATT-P  $\geq 1/4$  = 38.46%). The infection rates with CATT-B and CATT-P  $\geq 1/4$  varied significantly ( $p$ -value < 0.05) depending on the departments and study sites. This study revealed traces of trypanosomes in cattle, identifying the cattle in these two departments as animal reservoirs of trypanosomes.

**Keywords:** Trypanosomiasis; Trypanosome; Burden; Serological tests; Livestock breeding.

### 1. Introduction

In the sub-Saharan zone, in Africa, trypanosomes represent a major health concern for both humans and animals [1, 2]. At the animal level, economic losses are estimated at several billion dollars [3]. This disease not only impacts animal welfare but also contributes to food insecurity and limits economic development in affected areas. Moreover, communities living in close proximity to cattle farms may face an increased risk of transmission, either directly through tsetse flies or through risk behaviors such as handling infected animals and/or the lack of preventive measures [4].

This represents a real loss of earnings for developing countries where livestock farming, beyond the cultural aspect, represents a source of income for farmers to support their families. In Côte d'Ivoire, livestock farming was not a priority at independence, given that cash crops, coffee and cocoa, provided the maximum amount of currency. Today, things have changed and Ivorian state encourages livestock farmers to invest in self-sufficiency in animal protein. The Haut-Sassandra region, in the center-west of Côte d'Ivoire, is experiencing a real emergence of cattle farming thanks to pasture available almost all year round. However, this region, which is endemic for human African trypanosomiasis, also abounds with animal trypanosomes, which remains a hindrance in terms of productivity. This region, located in a transitional zone between savanna and tropical forest, provides a favorable habitat for tsetse flies [5]. Additionally, the extensive and poorly structured livestock systems in the area may promote disease transmission. The true parasite burden due to trypanosomes is not well documented.

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This study aims to evaluate the serological prevalence of trypanosomes and determine how human behaviors expose both animals and humans to the risk of infection in the Haut-Sassandra region.

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## 2. Material and methods

### 2.1. Ethics statement

No ethical statement is required by local authorities for domestic animal sampling. Any veterinarian may carry out blood sampling on domestic animals, with the authorization of the owner, as it is performed during prophylaxis or diagnostic campaign. No samples other than those for routine screening and diagnostic procedures were collected. Breeders gave their consent for animal sampling after being informed of the objectives of the study. For cattle care, venous sampling was performed by a veterinary.

### 2.2. Sampling sites

This cross sectional study was conducted in the Haut-Sassandra region, specifically in the departments of Daloa and Zoukougbeu, located in the central-west of Côte d'Ivoire. In this region, cattle farming is thriving and serves as the primary source of meat supply for the area. In Daloa, six (06) farms across different sites were surveyed: N'Drikro, Tagoura, Tagoura Extension (Tagoura\_E), Ceria (Ceria\_E1 and Ceria\_E2), and Toroghue. In Zoukougbeu, farms (Farm\_1 and Farm\_2) were visited. The number of sites depended on the number of farmers who provided their consent for this study.

### 2.3. Blood sample collection

Under the supervision of a veterinary officer, livestock farmers in the visited areas were informed about the screening of their herds. During the awareness phase, the veterinarian explained the importance of the study and the potential risk associated with African trypanosomiasis. For farmers who gave their consent, 9 mL of blood was drawn from each animal via the jugular vein into an ethylene diamine tetra acetic acid tube (EDTA). The collected blood samples were stored on ice in the field before being transported to the laboratory for serological analyses, including the Card Agglutination Test for Trypanosomosis (CATT) on blood (CATT-Blood: CATT-B) and plasma (CATT-Plasma: CATT-P). A questionnaire was administered to the farmers to gather information on their socio-health status in relation to African trypanosomiasis and their livestock. Animals were randomly selected, and the number of animals sampled depended on their availability.

### 2.4. Serological tests

The blood samples collected from the animals were analyzed using the CATT-Blood and CATT-Plasma tests, as previously described [2]. One milliliter of plasma and 500  $\mu$ L of the buffy-coat were aliquoted from the remaining blood of all cattle and immediately stored at  $-20^{\circ}\text{C}$  for subsequent PCR and trypanolysis tests.

### 2.5. Data analysis

The data obtained were recorded in Excel to produce descriptive statistics. Infection prevalence rates by department, site, and animal sex were expressed as percentage. Logistic regression was used to assess the correlation between prevalence rates, followed by a binomial test. A difference was considered significant in the probability value was strictly less than 0.05 ( $p$ -value < 0.05). All statistical analyses were performed using R software version 4.4.2 [6].

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## 3. Results

### 3.1. Trypanosome burden according to sex

Table 1 present the number of cattle examined and the CATT results (CATT-B test and CATT-P test) based on sex. A total of 164 cattle, including 117 females and 47 males from the departments of Daloa and Zoukougbeu, were tested using CATT serology (CATT-B and CATT-P). Overall, serological analyses based on sex revealed an overall seroprevalence of 27.44% with CATT-B and 57.78% with CATT-P. Among the 47 males tested, 12 (25.53%) and 5 (38.46%) were positive for the CATT-B and CATT-P tests, respectively. Among the 117 females tested, 33 (28.21%) and 21 (65.63%) were positive for the CATT-B and CATT-P tests, respectively. Infection prevalence rates by sex using the CATT-B test ( $p$ -value = 0.8472) and the CATT-P test ( $p$ -value = 0.1114) showed no significant variations.

**Table 1** Number of Cattle Sampled and CATT Results by Sex

Sex	N	CATT-B+ (%)	<i>p-value</i>	N1	CATT-P $\geq$ 1/4 (%)	<i>p-value</i>
Male	47	12 (25.53)		13	5 (38.46)	
Female	117	33 (28.21)	0.8472	32	21 (65.63)	0.1114
Total	164	45 (27.44)		45	26 (57.78)	

N : Sample size, CATT-B : CATT Blood, CATT-P : CATT Plasma : N1 : Number of sample CATT-B positive, % : percentage, + : positive

### 3.2. Trypanosome burden according to department

Table 2 present the results of the CATT-B and CATT-P tests obtained in the departments of Daloa and Zoukougbeu. The seroprevalence recorded in Zoukougbeu (43.24%) is relatively higher than that observed in Daloa (22.83%). The *p-value* (*p-value* = 0.0207) associated with this comparison indicated a statistically significant difference in the proportions of CATT-B positive cases between the two departments. Of the 45 CATT-B+ cattle, 26 tested positive for CATT-P $\geq$ 1/4, with an overall seroprevalence of 57.78%. The variations in seroprevalence observed between the two departments were not statistically significant (*p-value* = 0.5336).

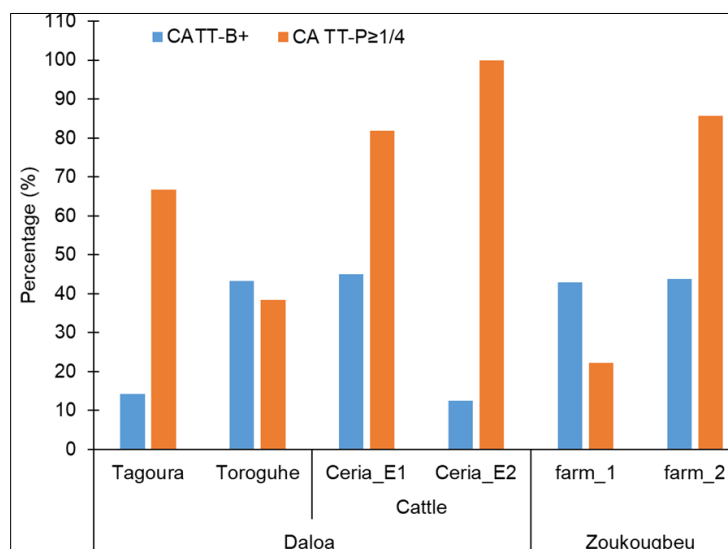
**Table 2** Seroprevalence of trypanosomes by department

Department	N	CATT-B+ (%)	<i>p-value</i>	N1	CATT-P $\geq$ 1/4 (%)	<i>p-value</i>
Daloa	127	29 (22.83)		29	18 (62.07)	
			0.0207			0.5336
Zoukougbeu	37	16 (43.24)		16	8 (50)	
Total	164	45 (27.44)		45	26 (57.78)	

N : Sample size, CATT-B : CATT Blood, CATT-P : CATT Plasma, CATT-B+, N1 : Number of sample CATT-B positive, % : percentage, + : positive

### 3.3. Seroprevalence of trypanosomes according to sampling site

The CATT-B+ and CATT-P $\geq$ 1/4 results by site are presented in Figure 1. Fisher exact test revealed that the CATT-B+ prevalences, ranging from 12.5% to 55%, varied significantly between study sites (*p-value* = 0.0208). Additionally, the test showed that the CATT-P $\geq$ 1/4 prevalences, ranging from 22.22% to 100%, also varied significantly between sites (*p-value* = 0.0175).



**Figure 1** Serological results according to study sites

### 3.4. Significance of trypanosome burden between strata

The results from the logistic regression model showed that the variables site ( $p$ -value < 0.0001) and department ( $p$ -value = 0.0090) have a significant influence on the prevalence obtained with the CATT-B (Table 3). However, the regression performed with CATT-P showed that only the village ( $p$ -value < 0.0001) has a significant influence on the results of this serological test (Table 3).

**Table 3** Quasi-logistic regression model on the variables department, village and sex of the cattle tested

		Df	Deviance	Resid. Df	Resid. Dev	F	Pr (>F)
	NULL			163	192.72		
	Departement	1	5.643	162	187.08	6.9936	0.0090 *
CATT-B+	Site	6	38.609	156	148.47	7.9744	1.685e-07 *
	Sex	2	0.534	154	147.94	0.3307	0.718943
	NULL			163	146.71		
	Departement	1	5.643	162	145.83	1.0256	0.3128
CATT-P $\geq$ 1/4	Site	6	38.609	156	114.40	6.1178	9.016e-06 *
	Sex	2	0.534	154	113.23	0.6849	0.5057

Df: Degrees of freedom, Deviance: model deviance, Resid. Df: residual degrees of freedom, Resid. Dev: residual deviance; F: F statistic, Pr: probability associated with the F statistic, \* : significative

At the department level, to understand the influence of this factor, a binomial test was performed to compare the proportion of positives in Daloa against a significance threshold of 0.5. The results obtained from this test showed that the proportion of positives in the CATT-B test is significantly ( $p$ -value = 0.0362) higher in the department of Daloa (64.44%) compared to the department of Zoukougbeu (35.56%). At the site level, the pairwise comparison test using the pairwise proportions comparison for CATT-B described in the matrix below (Table 4) showed significant variations between the sites Tagoura\_E and Toroguhe ( $p$ -value = 0.0044), Tagoura\_E and Ceria\_E1 ( $p$ -value = 0.0006), Tagoura\_E and farm\_1 ( $p$ -value = 0.0087), and between Tagoura\_E and farm\_2 ( $p$ -value = 0.0110). Similar, the pairwise comparison test using the pairwise proportions comparison for CATT-P described in the matrix (Table 5) showed significant variations between the sites Tagoura\_E and farm\_2 ( $p$ -value = 0.0443), and between Tagoura\_E and Ceria\_E1 ( $p$ -value = 0.0019).

**Table 4** Pairwise comparison of proportions CATT-B+

	Ceria_E1	Ceria_E2	NDrikro	Tagoura	Tagoura_E	Toroguhe	Farm_1
Ceria_E2	0.46382						
NDrikro	0.25130	1					
Tagoura	0.34280	1	1				
Tagoura_E	0.00057	1	-	1			
Toroguhe	1	1	0.64079	0.99060	0.00441		
Farm_1	1	1	0.79669	1	0.00865	1	
Farm_2	1	1	0.83390	1	0.01101	1	1

**Table 5** Pairwise comparison of proportions CATT- $P \geq 1/4$ 

	Ceria_E1	Ceria_E2	NDrikro	Tagoura	Tagoura_E	Toroguhe	Farm_1
Ceria_E2	0.9199						
NDrikro	0.4582	1					
Tagoura	0.3071	1	1				
Tagoura_E	0.0019	1	-	1			
Toroguhe	0.6043	1	1	1	1		
Farm_1	0.3071	1	1	1	1	1	
Farm_2	1	1	1	1	0.0443	1	1

#### 4. Discussion

This study, based on purposive sampling, used the CATT serological test (CATT on blood and CATT on plasma) to assess the seroprevalence of trypanosomes in cattle from 8 localities in the departments of Daloa and Zoukougbeu. The CATT serological test has already been used to detect trypanosome signature in livestock in Côte d'Ivoire [2], as well as in Nigeria and Sudan [7, 8].

The overall seroprevalence obtained with CATT indicates that the cattle tested in this study carry antibodies against trypanosomes. This does not indicate an active infection. In fact, the CATT test is based on the detection of antibodies and cannot distinguish between past and active infections [9]. The importance of bovine/trypanosome contacts revealed by the CATT serological reveal the burden of disease although the test might have overestimated current infection due to the fact that cattle are sometime treated with trypanocides [2].

The CATT-B+ ( $p$ -value = 0.8472) and CATT- $P \geq 1/4$  ( $p$ -value = 0.1114) results showed that the sex of the cattle (male or female) does not have a statistically significant impact on the proportions of infections although prevalence related to sex were higher in females, as reported in previous studies [10, 7]. Indeed, the chronic nature of trypanosome infections makes females more likely to be detected because they stay longer in the herds for reproduction [11].

The seroprevalence with CATT-B+ in cattle from the Zoukougbeu department was significantly higher ( $p$ -value = 0.0207) than that obtained in Daloa. This result suggests that the animals in Zoukougbeu are more exposed to trypanosome infection. In contrast to CATT-B+, the seroprevalence obtained with CATT- $P \geq 1/4$  was higher in cattle from Daloa (62.07%) than in those from Zoukougbeu (50%), although no significant difference was observed ( $p$ -value = 0.5336). However, these CATT results suggest significant contact between cattle and trypanosomes in both departments.

The binomial test conducted to evaluate the difference between the proportions obtained with CATT-B+ showed that the proportion of cattle testing positive in serology was significantly higher ( $p$ -value = 0.0362) in the Daloa department (64.44%) compared to Zoukougbeu (35.56%). This significant difference suggests that there is a difference in the presence of trypanosomes between the two departments. However, this relative difference could be explained by the sample size. Indeed, 127 cattle were sampled in Daloa compared to 37 in Zoukougbeu, resulting in ratio greater than 3. This uneven distribution could bias the analysis results in this study.

The results of the binomial test suggest that the department has a significant effect ( $p$ -value = 0.0090) on the proportions of CATT-B+. This confirms previous analyses of a higher prevalence in Zoukougbeu compared to Daloa. Similarly, the sites have a significant effect ( $p$ -value < 0.001) on the proportions of CATT-B+. Additionally, the results of the binomial test suggest that only the site has a significant effect ( $p$ -value < 0.001) on the proportions of CATT- $P \geq 1/4$ . These observations indicate that the prevalence of CATT-B+ varies considerably between departments on one hand and between sites on the other hand. Also, the prevalence of cattle with a CATT- $P \geq 1/4$  titre varies significantly between the visited sites. These different observations could be due to environmental factors, as well as local conditions influencing exposure to trypanosomes.

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## 5. Conclusion

In this study, the serological tests performed on blood and plasma samples showed a significant burden of trypanosomes in cattle, with a predominance in females. The prevalence recorded in the departments of Daloa and Zoukougbeu indicate significant contact between cattle and trypanosomes. This significant trypanosome burden remains a concern for livestock farming in the region despite the region's pasture advantages.

These new data should be considered in the development of effective control strategies against Animal African trypanosomes in the region.

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## Compliance with ethical standards

### *Acknowledgments*

The authors are grateful to M. Manga for their support in sample collection

### *Disclosure of conflict of interest*

The authors have not declared any conflict of interest.

### *Statement of ethical approval*

No ethical statement is required by local authorities for domestic animal sampling. Any veterinarian may carry out blood sampling on domestic animals, with the authorization of the owner, as it is performed during prophylaxis or diagnostic campaign. No samples other than those for routine screening and diagnostic procedures were collected. Breeders gave their consent for animal sampling after being informed of the objectives of the study. For cattle care, venous sampling was performed by a veterinary.

### *Statement of informed consent*

Informed consent was obtained from all individual participants included in the study.

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