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Asian Journal of Animal and Veterinary Advances

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OPEN ACCESS Asian Journal of Animal and Veterinary Advances

ISSN 1683-9919
DOI: 10.3923/ajava.2022.111.117

Research Article
Bovine Trypanosomiasis Prevalence and Associated Risk Factors at the Ngaoundere Municipal Abattoir

[1]Mamoudou Abdoulmoumini, [1]Bouba Mohamadou and [2]Sevidzem Silas Lendzele

[1]Department of Parasitology and Parasitological Disease, School of Veterinary Medicine and Sciences, University of Ngaoundere, Ngaoundere, Cameroon
[2]Institute of Research in Tropical Ecology, Laboratory of Vector Ecology, BP 13354, Libreville, Gabon

Abstract

Background and Objective: African animal trypanosomiasis is an economically important cattle disease in Sub-Saharan Africa and Cameroon in particular. This study aimed to determine the prevalence of bovine trypanosomiasis and risk factors in the Ngaoundere Abattoir. **Materials and Methods:** A cross-sectional survey was conducted for the first time from January to December 2017, at the Ngaoundere Municipal Abattoir (NMA) to fill this gap. A total of 739 blood samples from cattle slaughtered at the abattoir were collected and examined parasitologically using the buffy coat technique (BCT). **Results:** Study showed the presence of trypanosomiasis in slaughtered cattle with an overall parasitological prevalence of 12.72% and significantly higher during the rainy season (15.60%) than in the dry season (10.44%). *Trypanosoma congolense* (43.62%) was the predominant trypanosome species infecting the animals and closely followed by *Trypanosoma brucei* (36.17%), *Trypanosoma vivax* (12.76%) and mixed infections (7.45%). A very low parasitaemia (10^2-10^3 trypanosomes mL^{-1}) was observed in 85% of the infected cattle. The mean packed cell volume (PCV) value of parasitaemic cattle (29.24%) was lower than that of aparasitaemic cattle (30.05%). Of the 182 cattle with poor body condition scores (BCS), 11% were infected. However, trypanosomiasis did not significantly affect PCV and BCS. **Conclusion:** Bovine trypanosomiasis occurs in cattle slaughtered in the NMA and the majority of these animals originate from the tsetse infested area of Mayo-Rey in North Cameroon.

Key words: Trypanosomiasis, cattle, prevalence, abattoir, season, Ngaoundere

Citation: Abdoulmoumini, M., B. Mohamadou and S.S. Lendzele, 2022. Bovine trypanosomiasis prevalence and associated risk factors at the Ngaoundere Municipal Abattoir. Asian J. Anim. Vet. Adv., 17: 111-117.

Corresponding Author: Sevidzem Silas Lendzele, Institute of Research in Tropical Ecology, Laboratory of Vector Ecology, BP 13354, Libreville, Gabon

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Trypanosomiasis is a disease caused by a parasite of the genus *Trypanosoma*. African Animal Trypanosomiasis (AAT) is biologically transmitted by tsetse flies of the genus *Glossina*[1] and mechanically transmitted by other blood-sucking dipterous insect groups (tabanids, stomoxyines, etc.)[2,3]. The members of the *Trypanosoma brucei*, *T. vivax* and *T. congolense* causes the disease in animals while *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* causes the disease in humans[4]. The AAT is considered the main health and production constraint in livestock production in Sub-Saharan Africa[5]. In Cameroon, AAT is ranked among the important cattle diseases that constitute a threat to its livestock industry[6]. Tsetse fly vectors are present in 8.5 million km^2 in 37 countries, where 46 million cattle are exposed to AAT that directly affect livestock health, agricultural capacity and land use[7].

The Adamawa Region of Cameroon is one of the important cattle rearing regions of the country[8]. After the 1994 tsetse eradication campaign, the Adamawa Plateau was segmented into three zones consisting of the infested, non-infested and the buffer by the Special Mission for Tsetse Eradication (MSEG) to ease control[9]. Recent reports of AAT in the region show that the disease still occurs in cattle in the tsetse infested zones with a prevalence of up to 30%[9,10]. However, there is no report on the AAT situation in the tsetse free rangeland of Ngaoundere of the Vina division in the region given that trade cattle from the infested Northern region especially Mayo-Rey are brought to cattle markets and abattoirs of such tsetse free areas. The North region harbours several tsetse pockets with AAT of 14.3 and 9% reported in the Division of Faro and Mayo-Rey, respectively[11]. Despite enormous resources deployed by the Cameroonian government and breeders to curb the disease, it remains prevalent in the Adamawa Plateau.

Although much work has been conducted in several African countries on the prevalence of bovine trypanosomiasis at the abattoir level, to the best of our knowledge, no such study has been conducted in Cameroon. It is in this context that this study was designed with the main objective to contribute to a better understanding of the epidemiology of bovine trypanosomiasis. Specifically to determine the parasitological prevalence of trypanosomiasis in cattle, to show the effect of bovine trypanosomiasis on the health status of animals, to study the seasonal variation of the infection rate of bovine trypanosomiasis and to trace the origin of the animals that are brought to the Ngaoundere abattoir to clearly define the potential source of infection.

MATERIALS AND METHODS

Study area: The study was conducted from January to December 2017 in the Abattoir of Ngaoundere located within the following geographical limits latitude 7°-8° North and longitude 13°-14° East where approximately 55 cattle are slaughtered daily and most of them originate from the Vina division (with records on *Trypanosoma* spp., infection rates and its mechanical vectors) in the Adamawa Plateau and Mayo-Rey division (with records on mechanical vectors and *Trypanosoma* spp., occurrence) in the North region of Cameroon[12]. The main breeds of cattle encountered in the abattoir include Red Fulani, Gudali×White Fulani, Charolais and Gudali[12].

Study population and sample size determination: The study population consisted of cattle slaughtered at the Ngaoundere Municipal Abattoir.

The sample size was determined using the following formula[9]:

$$N = \frac{1.96 \times 1.96\ p(1-p)}{d^2}$$

Where:
N = Estimated sample size
p = Known prevalence
d = Precision of estimation

An estimated prevalence of 50% was used with a precision level of 5% resulting in a minimum sample size of 384 cattle. The sample size was increased to 739 cattle far above the estimated value to avoid sampling errors.

Blood collection from cattle: The animals were randomly selected based on their availability and the consent of the owners. After restraining the animals, approximately 3 to 4 mL of blood was collected through the jugular vein into a tube containing Ethylene Diamine Tetra Acetic Acid (EDTA) anticoagulant. The tubes were labelled with the animal number, breed, sex, age, body condition score and origin.

Determination of breed, age and body condition score of animals: The cattle breed was determined using phenotypic characteristics. Age was determined by examining the dentition and horn rings. The animals were grouped into 3 age groups including young (<3 years), adults (3-8) and aged (>8 years). The BCS was established on a scale of 0-5,

after examining the animal by palpation of the lumbar region as well as by assessing its general appearance following the method proposed by Mamoudou *et al.*[13]. The animals were divided into 3 groups according to their BCS: Bad (0-2), average (3) and good (4-5). The origin of the animals was the village or division where they were purchased.

Parasitological testing of cattle blood: The collected blood samples were transported to the laboratory in a cooler containing ice packs. Parasitological analysis was conducted within 5 hrs of collection using the Murray or Buffy Coat Method[14]. Briefly, the anticoagulant-containing capillary tubes (EDTA) were filled with 4/5 blood. The tubes were sealed with plasticine at one end and placed in the rotor of the microcentrifuge (Hettich HAEMATOKRIT). The blood was centrifuged at 12,000 rpm for 5 min.

Determination of the Packed Red Blood Cell Volume (PCV): The PCV was measured using the hematocrit reader (Hawksley Microhematocrit Reader®). Animals with a PCV less than or equal to 24% were considered anaemic[11].

Microscopic identification of trypanosomes and determination of parasitaemia: The buffy coat was placed on a slide with a coverslip and examined microscopically (Micron optic®) using the ×40 magnification. The determination of parasitaemia was conducted using the score of +1 to +6, corresponding to the number of trypanosomes per mL of blood.

Data analysis: The prevalence was obtained by using the following formula[9]:

$$\text{Prevalence} = \frac{\text{Number of positive animals sampled}}{\text{Number of animals sampled}} \times 100$$

Statistical analysis was conducted using the XLSTAT version 19.4 statistical software. The Chi-square test and the Kruskal-Wallis test were used to compare prevalence rates with associated risk factors. The degrees of significance of all statistical tests were kept at $p<0.05$.

RESULTS

Prevalence with the origin of slaughtered cattle in Ngaoundere Abattoir: The 739 cattle were sampled, including 412 in the dry season (January to March) and 327 in the rainy season (May to June). These animals originated from 14 cattle markets notably Dang, Dibi, Djalingo, Galdi, Likok, Mbe, Ngaoundere, Tchabal and Tello located in the Vina division and others from Gop Rey, Mbaka, Touboro, Vogzom and Yoko, found in the Mayo-Rey division. About 55% of these animals originated from the Mayo-Rey Division (n = 406) and 45% from the Vina division (n = 333). The prevalence concerning the divisions of origin indicated that higher cases were encountered in animals from Mayo-Rey than those from the Vina division.

Prevalence of bovine trypanosomiasis at the Ngaoundere Abattoir: Of the 739 cattle sampled, parasitological examination revealed 94 positive samples with parasitological prevalence rate of 12.72% (95% CI: [10.32-15.12%]).

***Trypanosoma* species microscopically typed:** The trypanosome species encountered included *Trypanosoma congolense*, *Trypanosoma brucei*, *Trypanosoma vivax* and their mixed infections. A predominance of *Trypanosoma congolense* (43.62%:41/94) was observed, followed by *Trypanosoma brucei* (36.17%:34/94) and finally *Trypanosoma vivax* (12.76%:12/94). In addition to these monospecific infections, mixed infections were recorded, including *Trypanosoma congolense* + *Trypanosoma brucei* (5.32%:5/94), *Trypanosoma congolense* + *Trypanosoma vivax* (1.06%:1/94) and *Trypanosoma congolense* + *Trypanosoma brucei* + *Trypanosoma vivax* (1.06%:1/94). This difference between trypanosome species infection rates was significant ($p<0.05$).

Association of *Trypanosoma* spp. and parasitaemia on number of cases: The number of infected animals depended on the *Trypanosoma* infection states (monospecies and polyspecies) in Table 1. For the trypanosome species frequency, *Trypanosoma brucei* recorded high number

Table 1: Association of *Trypanosoma* spp. and parasitaemia on the number of cases

Factors	Numbers	p-value
***Trypanosoma* spp.**		
Tb	34	
Tc	41	
Tv	12	
Tb+Tc	5	
Tc+Tv	1	
Tb+Tc+Tv	1	0.458
Parasitaemia		
10^2-10^4	80	
10^3-10^4	12	
5×10^3 to 5×10^4	2	0.245

Tc: *Trypanosoma congolense*, Tv: *Trypanosoma vivax* and Tb: *Trypanosoma brucei*

of cases than other species with a non-statistically significant difference (p = 0.458). The highest number of cases was recorded in animals with the lowest parasitaemia (Table 1). Concerning parasitaemia of animals, a low trypanosome load (10^2-10^4) was recorded for most of the animals (80 in total).

Prevalence concerning season: The parasitological prevalence of bovine trypanosomiasis with season showed that high cases were diagnosed in the rainy season (15.60%) than in the dry (10.44%) with a statistically significant difference (p<0.05) in Table 2.

Prevalence with breed, sex, age and season: In the dry season, it was noticed that the bokolo breed recorded the highest prevalence rate (33.33%) with a non-statistically significant difference (p = 0.487). In this same period, male (13.67%) cattle recorded a high prevalence rate with no statistically significant difference (p = 0.463). The age cohort with high prevalence was 3-8 years (11.11%) with no statistically significant difference (p = 0.701). For the rainy season, the Gudali cattle breed recorded a high prevalence rate (19.28%) with no statistically significant difference (p = 0.496). Male (21.43%) cattle during the rainy season had high infection rate than their female (15.05%) counterparts with no statistically significant difference (p = 0.374) and the age cohort with a high prevalence rate consisted of those <3 years old (19.05%) with no statistically significant difference (p = 0.821).

Influence of bovine trypanosomiasis on hematocrit: Of the 96 animals with hematocrit less than or equal to 24, only 11 (11.46%) had bovine trypanosomiasis. In the dry season, 20.93% (9/43) of trypanosomiasis-positive animals had haematocrit lower or equal to 24 with no statistically significant difference (p = 0.690), while in the rainy season only 3.92% (2/51) of positive animals had haematocrit less than or equal to 24 with no statistically significant difference (p = 0.530) in Table 4.

Table 2: Prevalence of trypanosomes concerning season

Seasons	Numbers	Positive	Prevalence (%)	CI (%)
Dry (January to March)	412	43	10.44[a]	7.49-13.39
Rainy (May to June)	327	51	15.60[b]	11.18-18.94

Different letters of the alphabet indicate a statistically significant difference (p<0.05) at the 95% confidence interval (CI)

Table 3: Prevalence with breed, sex, age and season

Seasons	Risk factors	Numbers	Positive	Prevalence (%)	p-value
Dry (January to March)					
Breed	Akou	150	13	8.67	
	Bokolo	3	1	33.33	
	Djafoun	118	14	11.86	
	Goudali	131	14	10.69	
	Metis	10	1	10	0.487
Sex	Female	368	37	10.05	
	Male	28	6	13.64	0.463
Age	<3	14	1	7.14	
	[3-8]	351	39	11.11	
	≥8	47	3	6.38	0.701
Rainy (May to June)					
Breed	Akou	81	11	13.58	
	Bokolo	18	2	11.11	
	Djafoun	45	5	11.11	
	Goudali	166	32	19.28	
	Metis	17	1	5.88	0.496
Sex	Female	299	45	15.05	
	Male	28	6	21.43	0.374
Age	<3	21	4	19.05	
	[3-8]	212	32	15.09	
	≥8	94	15	15.96	0.821

Table 4: Bovine trypanosomiasis with haematocrit and sampling seasons

Seasons	PCV	Numbers	Positive	Negative	p-value
Dry (January to March)	≤24	77	9	68	0.690
	>24	335	34	301	
Rainy (May to June)	≤24	19	2	17	0.530
	>24	308	49	259	

Table 5: Influence of BCS on prevalence in the sampling seasons

Seasons	BCS	Numbers	Positive	Negative	Prevalence (%)	p-value
Dry (January to March)	Poor (0 to 2)	80	2	78	2.5	0.028
	Medium (3)	329	41	288	12.46	
	Good (4 to 5)	3	0	3	0	
Rainy (May to June)	Poor (0 to 2)	102	18	84	17.56	0.782
	Medium (3)	219	32	187	14.61	
	Good (4 to 5)	6	1	5	16.67	

Identification of mechanical vectors of trypanosomes in Galim

Influence of bovine trypanosomiasis on the body condition score: Based on the body condition score (BCS) of the animals, only 21.28% (20/94) of trypanosomiasis-positive cases were identified to exhibit a poor body condition. These 20 (10.99%) trypanosomiasis-positive poor condition animals were inferior to animals with medium and good body conditions and infected with trypanosomes (74: 13.28%). This difference was not statistically significant ($p = 0.420$). In the dry season in Table 5, the medium BCS animals were the most infected (12.5%) compared to poor BCS animals (2.5%) and this difference was significant ($p = 0.028$). In the rainy season, the most infected animals were those with a poor (17.65%) BCS while those with medium (14.61%) BCS were the least infected with a non-statistically significant difference ($p = 0.782$) (Table 5).

DISCUSSION

The present study that was conducted at the Ngaoundere Municipal Abattoir confirmed the presence of trypanosomiasis in cattle with a parasitological prevalence of 12.72%. This result was superior to that reported by Mamoudou *et al.*,[11] in Mayo-Rey with prevalence rates of 11.5 and 9%, respectively. This difference may be the animals slaughtered at the Ngaoundere Municipal Abattoir originated from bovine trypanosomiasis endemic areas such as Mayo-Rey with the highest number of cattle recorded in the Ngaoundere Abattoir. Conversely, this result was less than 29.4%, obtained in Almé in the Faro and Déo division[9]. This difference may be due to the nature of study areas in terms of tsetse fly infestation, where Almé is a tsetse infested area compared to Vina with continuous and intensive ongoing vector control. It was interesting to know that those animals coming from the Mayo-Rey Division were more highly infected with trypanosomiasis than those from the Vina Division. This finding was not surprising because Mayo-Rey-harbours dense tsetse and tabanids pockets as already reported that could be implicated in transmission as compared to Vina division with no reports on tsetse[1,2,11,15]. Regarding season, the prevalence is significantly higher in the rainy season (15.60%) compared to the dry season (10.44%), probably due to the highest tsetse and other fly vectors densities occurring during this season in the Plateau of Adamawa[16]. This increase in prevalence during the rainy season corroborates the results obtained in Mayo-Rey[10]. In the present study, *Trypanosoma congolense* was the predominant species. The predominance of this species has been previously reported in Ngaoundere[17]. This indicates that there is contact between the host animals and tsetse flies and other biting dipterous insects that could lead to high transmission in those areas[11]. The very low parasitaemia observed in this present study was similar to that reported by Mpouam *et al.*[18]. This result reflects the regular use of trypanocides by farmers in their breeding areas. The highest parasitaemia levels (5×10^3 to 5×10^4) were observed in infections caused by *Trypanosoma congolense*. This observation was consistent with that of Mpouam *et al.*[18] and could be explained by the fact that *Trypanosoma congolense* is the most pathogenic species for cattle.

The average hematocrit of the animals was 30.09 and did not differ from that (30.2%) obtained in Vina[18]. The proportion of individuals with hematocrit less than 24 was higher in the dry season (18.69%) than in the rainy season (5.81%). This increase in mean hematocrit from the dry season to the rainy season was also recorded by Mamoudou *et al.*[11]. This may be as result of increased pasture availability associated with increased nutrient values during the rainy season. No endogenous factor (sex, age and breed) considered had a significant influence on the prevalence of bovine trypanosomiasis at the Ngaoundere Municipal Abattoir and this observation was similar to that made by other authors[9,19].

The mean hematocrit of positive animals for trypanosomiasis was lower than that of negative animals. This decrease in mean hematocrit in positive animals was significant during the dry season. Several authors have reported a decrease in mean hematocrit in animals infected with trypanosomiasis[10,11]. This result can be explained by the fact that bovine trypanosomiasis causes a sharp decrease in the number of red blood cells, resulting in a drop in the hematocrit. The scarcity of pasture and stress related to the search for food during the dry season, this has contributed to

the poor body condition of the animals during this period. The influence of trypanosome species and degree of parasitaemia were not significant in trypanosomiasis-positive animals. However, mean hematocrit was lower in cattle infected with *Trypanosoma congolense* and especially in cattle with mixed infections due to *Trypanosoma congolense* + *Trypanosoma vivax* and *Trypanosoma congolense* + *Trypanosoma brucei*. These results were similar to those obtained by Mamoudou *et al*.[13] and could be explained by the fact that *Trypanosoma congolense* is the most pathogenic species of cattle and its presence in the host system greatly reduces its red blood cell count. Only 11.46% of animals with hematocrit less than or equal to 24 had trypanosomiasis, hence the existence of other factors or anaemia-causing diseases such as gastrointestinal helminthiasis[20], haemoparasites, ectoparasites (ticks)[21] and nutritional deficiencies[11] cannot be rolled-out. Several studies have shown that animals parasitized by both trypanosomes and helminths have lower hematocrit compared to animals with a single infection[20].

Of a total of 182 poor BCS animals, only 20 were trypanosomiasis positive. These 20 trypanosomiasis-positive poor BCS animals were inferior to animals with medium and good BCS infected with trypanosome (74). This result was different from that obtained by Samdi *et al*.[22] in a study conducted at the Kaduna Abattoir in Nigeria. The decrease in the BCS could be partly linked to *Trypanosoma* spp., infection as well as other factors including gastrointestinal helminthiasis, ectoparasitosis, haemoparasites is transmitted by ticks, stress and nutritional deficiencies during the dry season[11].

CONCLUSION

From the abattoir survey, the parasitological prevalence was 12.72%. This prevalence was significantly higher in the rainy season than in the dry season and *Trypanosoma congolense* was the most frequent species. Infected cattle had lower hematocrit values than uninfected cattle. Poor body condition animals were highly infected with this parasite. This study confirmed the presence of trypanosomiasis in cattle slaughtered in Ngaoundere and indicates that a high risk could be from cattle coming from the tsetse infested Mayo-Rey Division in the North Region.

SIGNIFICANCE STATEMENT

Three pathogenic *Trypanosoma* spp. and their mixed infections occurred in cattle slaughtered at the Ngaoundere Municipal Abattoir. High cases of bovine trypanosomiasis were identified in cattle originating from the tsetse infested Mayo-Rey Division in the North Region which could be a possible source of introduction of the disease in the tsetse free Ngaoundere of Cameroon.

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