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ORIGINAL ARTICLE

Prevalence of cattle trypanosomosis in slaughterhouses and farms in Benin and impact on hematocrit in cattle

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ABSTRACT

Objective: The present study aimed to determine the prevalence of bovine trypanosomosis in Benin.
Materials and Methods: For this purpose, the blood samples were taken from 932 cattle: 573 from slaughterhouses and 359 from the farms of the targeted divisions. After the blood samples, thick drops and blood smears were made. The capillary tubes filled with blood made it possible to determine the hematocrit in cattle.
Results: The prevalence of trypanosomosis in farms (27.02%) was significantly higher ($p < 0.001$) than that of animals in slaughterhouses (16.75%). In farms, the prevalence was 38.55% during the rainy season against 16.67% in the dry season ($p < 0.001$). In slaughterhouses, prevalences were 10.99%, 17.58%, and 21.50%, respectively, in Bohicon, Cotonou/Porto-Novo, and Parakou. Hematocrit in slaughterhouses was 24.17% and 31.44%, respectively, in infested and non-infested animals. In farms, this rate was 22.85% in infested animals and 29.31% in non-infested animals ($p < 0.05$). Young cattle are more vulnerable to trypanosomosis than older cattle.
Conclusion: Given the endemic situation of bovine trypanosomosis and its impact on the economy, this knowledge of the health status of cattle will help out to seek ways and alternatives to reduce the damage.

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Benin; cattle trypanosomosis; prevalence; slaughterhouses; cattle farms

Introduction

In West Africa, the livestock sector occupies the first place in terms of trade and contributes to 44% of gross domestic product (GDP) within the countries of the Economic Community of African States West [1]. In 2012, the livestock sub-sector reported US $ 180.6 million being 2.4% of gross domestic product (GDP) [2]. In Benin, livestock contributed to 5% of the overall GDP and 18% of agricultural GDP in 2010 [1]. Cattle breeding is one of the main activities carried out by the populations to provide for their needs [3,4]. It represents the second field of activity of the Beninese agricultural sector with an annual growth of 2.6% [5–7]. It occupies an important place in the livestock sub-sector and represents 77% of total livestock GDP [8,9,10]. Cattle are reared in two systems; the traditional system and the semi-extensive system [11–14]. The animals of these two systems are confronted with several difficulties out of which pathologies constitute the main ones, considering their importance. Livestock diseases have adverse effects on animal productivity, and public health [15–19]. In both production systems, ticks that affect farm productivity have been identified and treatment by medicinal plants have been proposed [20–23] and the prevalence of tuberculosis and brucellosis was then evaluated [24–26]. In addition to

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these studies, the prevalence of trypanosomiasis has been evaluated in semi-extensive farms, particularly state farms, and means of trypanocidal control have been implemented [27,28]. On the other hand, in traditional farms, the prevalence of this pathology has been evaluated only in the Department of Atacora [29], whereas trypanosomosis has been classified as the second pathology that affects milk after foot-and-mouth disease [30,31]. It is important to limit the constraints of cattle breeding to endemic animal diseases such as African trypanosomosis to ensure food security [32]. To improve the productivity in farms, it is essential to extend the study on the prevalence of this disease to other departments of the country. In order to update the data on the disease and expand its study at the national level, the objective of this work is to determine the prevalence of cattle trypanosomosis in Benin and impact on hematocrit.

Materials and Methods

Study area

The present study was conducted in the three main slaughterhouses in Benin (Cotonou/Porto-Novo, Bohicon, and Parakou) (Fig. 1) and in eight departments of Bénin (Ouémé, Plateau, Zou, Collines, Borgou, Alibori, Atacora, and Donga) (Fig. 2). Benin is located between latitudes 6°10′N and 12°25′N and longitudes 0°45′E and 3°55′E. The rainy season and the dry season vary according to the latitudes. Below latitude 7°45′N, there are two rainy seasons (from April to July and from October to November) and two dry seasons (from December to March and from August to September). Above latitude 8°30′N, the rainy season extends from May to October while the dry season covers the period from November to May.

Data collection

The study was conducted from August 2016 to July 2017. On the one hand, the data were collected in eight divisions of Benin (Ouémé, Plateau, Zou, Collines, Borgou, Alibori, Atacora, and Donga). Four farms were sampled per department being two farms per commune for each season (rainy and dry). The minimum distance between the two farms is 5 km. On the other hand, the same data were collected in each of the three central slaughterhouses (Cotonou/Porto-Novo, Bohicon, and Parakou). Data collected are blood sample, hematocrit determination, weight, breed, sex, age, geographical location of the sampling sites, and the date of the last treatment against bovine trypanosomosis.

Blood collection and determination of hematocrit

Blood was collected from the jugular vein using 21-gauge Venoject® needles and tubes containing anticoagulant (Ethylene Diamine Tetra Acetic Triphosphate). The hematocrit was determined from the works of Woo [28]. In fact, 0.6 ml of blood were directly pipetted into microhematocrit

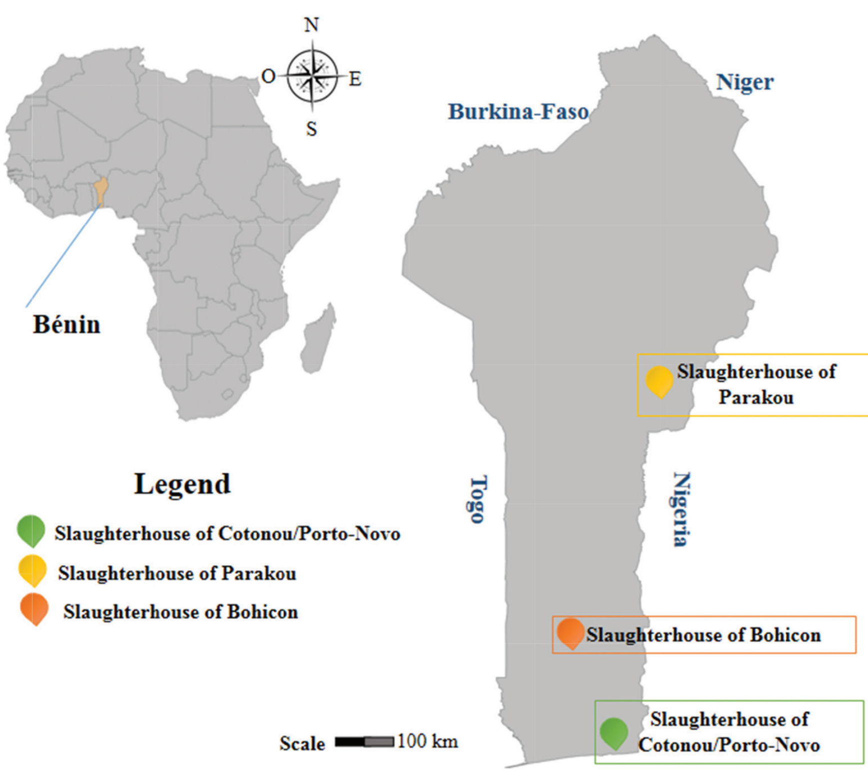

Figure 1. Slaughterhouses of Cotonou/Porto-Novo, Bohicon, and Parakou.

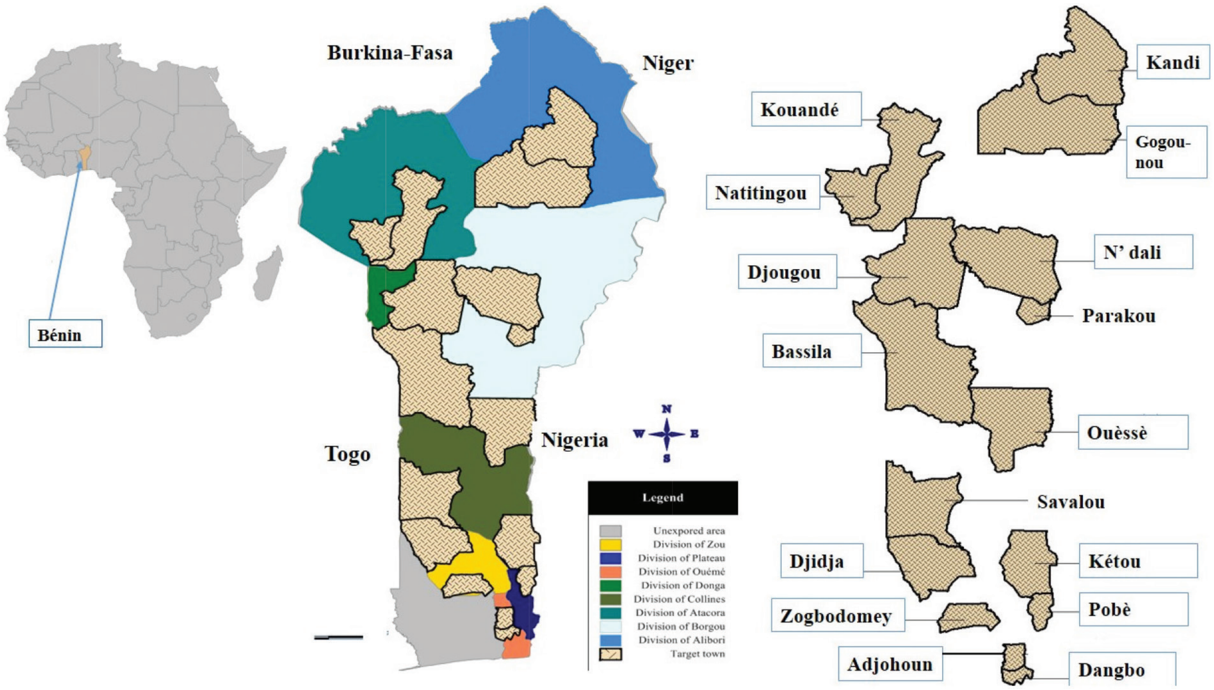

Figure 2. Cattle farms sampled in a specific town in eight departments of Bénin.

tubes which were centrifuged at 12,000 rpm for 4 min. The value of the hematocrit was determined using the Woo table [28].

The age of the cattle

The age of the cattle was obtained through the information given by the breeders, considering the dental formula and especially the tooth wear.

The weight of cattle

The methodology used for weight gain in cattle was adopted from previous studies by Naves [33] and Youssao et al. [22]. The graduated tape measure was the instrument used for measuring the thoracic perimeter. The chest perimeter was taken behind the shoulders and just behind the tip of the elbow. The weight was then estimated using the method of Youssao et al. [22].

Identification of trypanosomes

In order to identify the different trypanosome species, the parasitological analysis was carried out within 4–6 h after sampling, after extraction of the buffy coat [34]. The buffy coat was examined under an optical microscope after spreading between blade and coverslip at objective 40. The parasitemia was estimated from the correspondence sheet defined by Murray et al. [35]. In addition, the blood smears and thick drops were made and then examined by an optical microscope at objective 100 for identification of the trypanosome species based on the size and morphology criteria indicated by Hoare [24].

Statistical analysis

Data were analyzed with SAS [36] software. For the quantitative variable (hematocrit level), an analysis of variance was performed by the *Proc* Generalized Linear Model procedure. The Fisher F test was used to determine the significance of the seasonal effect, breed, age class, sex, and location on the variable and the comparisons between the means were made two by two using the Student *t*-test. For qualitative variables (prevalence, trypanosome species), observed frequencies were calculated by the FREQ procedure. The season effect, genetic type, age class, and sex were highlighted on the prevalence by a Chi-square test and the comparison of relative frequencies two by two was made by the bilateral test of Z. For each relative frequency, a 95% confidence interva was calculated using the formula:

$$IC = P \mp 1{,}96\sqrt{\frac{P(1-P)}{N}}$$

p is the relative frequency and N is the sample size.

Results

Prevalence of bovine trypanosomosis in cattle farms in Benin

The overall annual prevalence of trypanosomosis was 27.02%. This prevalence was higher ($p < 0.05$) in the rainy season than in the dry season (38% *vs.* 16%). The prevalence of trypanosomosis recorded in males (27.96%) is not significantly different from that (26.69%) of females

(Table 1). Regardless of the season, bovine trypanosomosis is mainly caused by *T. vivax*; this species infested cattle in the rainy season more than in the dry season (88.24% *vs.* 68.96%) ($p < 0.05$). Other identified trypanosome species were *Trypanosoma congolense* and *Trypanosoma brucei* (Table 2). Bovine trypanosomosis affects the youngest cattle (0–2 years old) with a rate of 29.81%, followed by those aged 3–6 years with a prevalence of 28.46% and finally cattle aged at least 7 years with a prevalence of 23.39% (Table 1). No significant difference was recorded between the different prevalences obtained; however, the prevalence decreases with the increasing age of animals. The highest prevalences were obtained in the Departments of Alibori (47.5%), Atacora (31.37%), Borgou (27.27%), Donga (30.00%) and the lowest was determined in the Department of Collines (13.95%) (Table 1). From this study, it was found that the bull breed had a very high prevalence of 30.66% followed by crossbreed (17.39%) and zebu breed (16.88%). Bovine trypanosomosis affects all genetic types of cattle reared in Benin with a high infestation in bulls ($p < 0.05$) (Table 1).

Frequency of infestation of cattle by different species of trypanosomes according to the seasons in Benin farms

Trypanosoma vivax, T. congolense, and *T. brucei* were the three trypanosome species identified in this study. Of the positive samples, 70.1% came from samples taken during the rainy season and 29.89% from the dry season. The frequency of *T. vivax* infestation in the rainy season (88.24%) was significantly higher ($p < 0.05$) than in the dry season (68.96%). No significant variation was recorded for the proportions of *T. congolense* and *T. brucei*, neither during the rainy season nor in the dry season (Table 2).

Hematocrit variation factors in cattle reared in Benin

The average hematocrit of cattle in the different farms in Zou, Collines, Ouémé, Plateau, Borgou, Alibori, Atacora, and Donga was 27.63% ± 1.90 in the rainy season and 24.54% ± 1.88 in the dry season. Hematocrit in cattle with trypanosomosis in this study (22.85%) was significantly lower ($p < 0.05$) compared to apparently healthy cattle (29.31%). Genetic type has no influence on hematocrit expression (Table 3). Cattle in the department of Ouémé had a hematocrit 35.83% ± 3.27%, much higher than those recorded in the other departments, which varied between 23.70% and 27.03% ($p < 0.01$).

Hematocrit rate in relation to the season and the Division

In Ouémé, the average hematocrit rate in the rainy season was 44.10% ± 3.71% against 29.80% ± 3.71% in the dry season ($p < 0.01$). No significant variation was observed for mean hematocrit levels for cattle in other departments in both the rainy and dry seasons (Table 4).

Prevalence of bovine trypanosomosis in the three central slaughterhouses (Cotonou/Porto-Novo, Bohicon, and Parakou) of Benin

Overall prevalence of bovine trypanosomosis in central slaughterhouses of Benin was 16.75%. This prevalence was higher ($p < 0.05$) in animals slaughtered in Parakou (21.5%) than in those slaughtered at Bohicon (17.58%) and Cotonou (10.99%) slaughterhouses (Table 1). We recorded 16.2%, 19.56, and 14.79% for crossbreed, bull, and zebus, respectively. The lowest prevalence was observed in zebus and the highest was determined for bulls (Table 5). The prevalence of trypanosomosis by sex in the different slaughterhouses (Cotonou/Porto-Novo, Bohicon, and Parakou) revealed respective rates of 14.84% and 18.62% in females and males. Young cattle (aged 0–2 years) are more infested with trypanosomes with a prevalence of 19.57%; cattle aged between 3 and 6 years old was 16.96% and then old ones 15.97%, respectively (Table 5). Bovine trypanosomosis negatively affects the weight of cattle ($p < 0.05$).

Factors of variation of hematocrit in cattle slaughtered in slaughterhouses of Benin

The hematocrit of cattle infested with trypanosomes (24.17%) was significantly lower ($p < 0.001$) compared to the rate obtained in cattle apparently healthy (31.44%), (Table 6). The hematocrit rate in cattle aged between 3 years and 6 years was 29.67 ± 1.14, compared to 25.98 ± 1.26 for cattle aged ≥ 7 years ($p > 0.05$). The hematocrit level obtained in the youngest animals (0–2 years) was 27.76% ± 2.19%. The consideration of the three age classes shows a variation ($p < 0.05$) (Table 6).

Frequencies and species of trypanosomes present in the animals slaughtered in the different slaughterhouses of Benin

Out of the 96 cattle infested by the various species of *Trypanosoma*, three species were identified. These are *T. vivax, T. congolense*, and *Trypanosoma brucei*. *Trypanosoma vivax* was abundantly observed with a prevalence of 79.17% compared to 8.33% for *T. congolense* and 5.21% for *T. brucei*. A mixed infestation rate of 7.29% was obtained in the animals reported positive in this study (Fig. 3). The highest infestations were obtained during the period from July to December (Fig. 4).

Discussion

The annual prevalence of trypanosomosis in farms was 27.02%. Lower prevalence of 0%–20% have been reported in bulls at the Okpara breeding farm in Benin [27]. The difference between our value and that of these authors is explained by the breeding method. In fact, the samples

Table 1. Predominance of bovine trypanosomosis in cattle farms in Benin according to the seasons.

Variables		Number	Prevalence (%)	Confidence interval
Season	Rainy season	179	38.55a	7.13
	Dry season	180	16.67b	5.44
	Total	359	27.58	4.62
	Threshold of significance		***	
Sex	Female	266	26.69[a]	5.32
	Male	93	27.96[a]	9.20
	Threshold of significance		NS	
Genetic type	Bull	259	31.66a	5.66
	Zebu	77	16.88b	8.37
	Crossbred	23	17.39ab	15.49
	Threshold of significance		*	
Age class	[0 – 3 year]	104	29.81a	8.79
	[3 – 7 year]	130	28.46a	7.76
	≥7 years	125	23.39a	7.42
	Threshold of significance		NS	
Departments	Alibori	40	47.50a	15.48
	Atacora	51	31.37ab	12.73
	Borgou	44	27.27b	13.16
	Collines	43	13.95b	10.36
	Donga	50	30.00ab	12.70
	Ouémé	40	22.50b	12.94
	Plateau	49	20.41b	11.29
	Zou	42	23.81b	12.88
	Threshold of significance		*	

The inter-column percentages of the same column followed by different letters differ significantly at the 5% threshold. NS = not significant, * = $p < 0.05$, *** = $p < 0.001$.

analyzed in this study were taken from cattle produced in the traditional system whereas the animals on which Allou et al. [37] worked, are reared in the semi-improved system. In this semi-improved breeding, the animals benefit from a regular preventive treatment against trypanosomosis contrary to the traditional system where the animals are treated rarely, most often symptomatically [38]. The increase in the prevalence of trypanosomosis in extensive production compared to intensive breeding has been reported in Nigeria by Majekodunmi et al. [25]. Higher prevalence (33%–44%) than those in our study were reported in Burkina Faso [39] and Togo [40]. However, this pathology remains a concern in the Okpara farm because the cumulative prevalence over a year is about 80% and control measures must go beyond trypanocidal treatments by including means of combating the vector that is the tse-tse fly [41,42]. This is the fundamental reason why [43] advocates the use of neem oil in tsetse control.

A strong proliferation of tsé-tsé fly in the rainy season, a period more conducive to its proliferation [27], justifies the increase in prevalence in the rainy season compared to the dry season. This same seasonal variation in the prevalence of trypanosomosis has been reported by Talaki et al. [2] in Mali, Ohaeri [19] in Nigeria, Abdoulmoumini et al. [30] in Cameroon and Degneh et al. [32] in Ethiopia and confirms the finding of Dicko et al. [44] who observe a high risk of trypanosomosis prevalent in the rainy season and around rivers in the dry season. Trypanosomosis

Table 2. Frequency of cattle infestation by trypanosome species by season.

Variables	Rainy season (*N* = 68)		Dry season (*N* = 29)	
	Rate (%)	Confidence interval	Rate (%)	Confidence interval
T. vivax	88.24[a]	7.66	68.96[b]	16.84
T. congolense	7.35[a]	6.20	10.34[a]	11.08
T. brucei	4.41[a]	4.88	3.45[a]	6.64
Mixed infestations	0.00	0.00	17.24	13.75

The percentages of the same line followed by different letters differ significantly at the 5% threshold. N = effective.

Table 3. Factors of changes in hematocrit in cattle.

Variables		Numbers	Mean	Standard error
Status with trypanosomosis	Positive	97	22.85a	1.63
	Negative	262	29.31b	2.23
	Threshold of significance		**	
Genetic type	Bull	259	26.63[a]	1.36
	Zebu	77	28.12[a]	1.91
	Crossbred	23	26.49[a]	3.71
	Threshold of significance		NS	
Age class	[0 – 3 year]	104	25.84[a]	3.03
	[3 – 7 year]	130	28.01[a]	1.70
	≥7 year	125	24.39[a]	1.95
	Threshold of significance		NS	
Season	Rainy season	179	27.63[a]	1.90
	Dry season	180	24.54[a]	1.88
	Threshold of significance		NS	
Departments	Alibori	40	24.83[b]	2.91
	Atacora	51	24.26[b]	2.61
	Borgou	44	24.58[b]	3.01
	Collines	43	25.09[b]	2.75
	Donga	50	23.36[b]	2.70
	Ouémé	40	35.83[a]	3.27
	Plateau	49	23.70[b]	2.92
	Zou	42	27.03[b]	2.93
	Threshold of significance		*	

The inter-column percentages of the same column followed by different letters differ significantly at the 5% threshold. NS = not significant, * = $p < 0.05$, ** = $p < 0.01$.

is strongly associated with the presence of tsetse flies [45]. The three species of trypanosomes present in different farms and slaughterhouses were *T. vivax*, *T. congolense*, and *T. brucei*. These species have already been reported in Benin as responsible for animal trypanosomosis [27–29,35,46]. Among these species, *T. vivax* is responsible for most diseases as reported by Allou et al. [37] in Benin, Sow et al. [47] and Dayo et al. [48] in Burkina Faso, Mamoudou et al. [49] in Cameroon, and Nnko et al. [34] in Tanzania and Boulangé et al. [50] in Sub-Saharan Africa.

The prevalence of trypanosomosis in slaughterhouses has been lower than that recorded on farms because slaughterhouses receive animals from other countries besides those raised on the national territory. This difference can also be explained by the antemortem inspections carried out in slaughterhouses, which make it possible to isolate sick animals before slaughter. The prevalence of trypanosomosis at the Parakou slaughterhouse was higher than that at the slaughterhouses of Bohicon and Cotonou/Porto-Novo because this slaughterhouse is located in northern Benin and receives more animals from northern farms

Table 4. Hematocrit rate in relation to the season and the division.

Departments	Hematocrit in rainy season (%)			Hematocrit in dry season (%)			Threshold significance
	N	Mean	ES	*N*	Mean	ES	
Alibori	20	25.10	3.71	20	25.50	3.71	NS
Atacora	24	25.71	3.38	27	25.52	3.19	NS
Borgou	22	26.77	3.54	22	24.68	3.54	NS
Collines	23	27.39	3.46	20	28.20	3.71	NS
Donga	25	26.56	3.32	25	24.16	3.32	NS
Ouémé	20	44.10	3.71	20	29.80	3.71	***
Plateau	23	24.39	3.46	26	27.00	3.25	NS
Zou	22	30.45	3.54	20	28.85	3.71	NS

NS = not significant, *** = $p < 0.001$.

where high prevalences were registered compared to the other two slaughterhouses in the south of the country. This recorded the spatial variation of the trypanosomosis prevalence has already been reported in several countries such as Benin, Togo, Côte d'Ivoire, Mali [29,36,40,51,52,53]. The

Table 5. Overall prevalence of bovine trypanosomosis according to central slaughterhouses, genetic type, age, weight, and sex of cattle.

Variables		Numbers	Rate (%)	Confidence interval
Slaughterhouses	Bohicon	191	10.99[b]	4.44
	Cotonou/Porto-Novo	182	17.58[ab]	5.53
	Parakou	200	21.50[a]	5.69
	Threshold significance		*	
	Total	573	16.75	3.05
Genetic type	Bull	225	19.56[a]	5.18
	Zebu	311	14.79[a]	3.95
	Crossbreed	37	16.22[a]	11.88
	Threshold significance		NS	
Age class	[0 – 3 year]	46	19.57[a]	11.47
	[3 – 7 year]	289	16.96[a]	4.33
	≥7 year	238	15.97[a]	4.65
	Threshold significance		NS	
Sex	Female	283	14.84[a]	4.14
	Male	290	18.62[a]	4.48
	Threshold significance		NS	
Weight	Positive to trypanosomosis	96	221.67	4.90[1]
	Negative to trypanosomosis	477	239.98	2.20[1]
	Threshold significance		*	

The inter-column percentages of the same column followed by different letters differ significantly at the 5% threshold. [1] = standard deviation, NS = not significant, * = $p < 0.05$.

Table 6. Hematocrit variation factors in cattle slaughtered in central slaughterhouses in Benin.

Variables		Numbers	Mean	Standard error
Status for trypanosomosis	Positive	96	24.17b	1.64
	Negative	477	31.44a	1.05
	Threshold significance		***	
Genetic type	Bull	225	26.09a	1.18
	Zebu	311	28.44a	1.20
	Crossbreed	37	28.88a	2.40
	Seuil de significativité		NS	
Age class	[0 – 3 year]	46	27.76ab	2.19
	[3 – 7 year]	289	29.67a	1.14
	≥7 year	238	25.98b	1.26
	Threshold significance		*	
Sex	Female	283	28.09a	1.31
	Male	290	27.51a	1.31
	Threshold significance		NS	

The interclass averages of the same column followed by different letters differ significantly at the 5% threshold. NS = not significant, * = $p < 0.05$; *** = $p < 0.001$.

prevalence of trypanosomosis in Donga (30%) was significantly higher than that (6.7%) reported by Farougou et al. [51]. This difference can be explained by breeding mode, sample size, and genetic type.

Bovine trypanosomosis increases the vulnerability of cattle to anemic disorders by a drop of hematocrit. The results of this study are similar to those found by Van den Bossche and Rowlands [42] in Zambia, Abebe et al. [55]

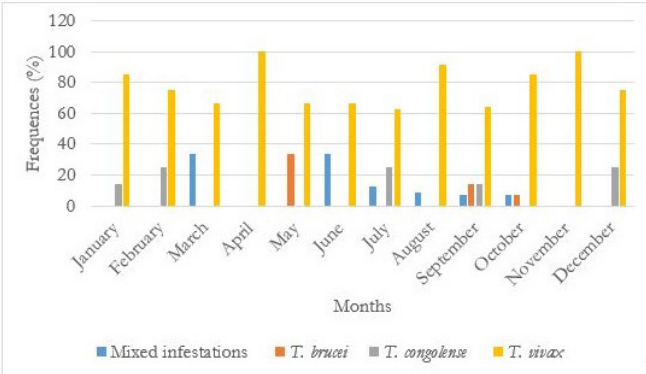

Figure 3. Monthly frequency of different species of trypanosomes.

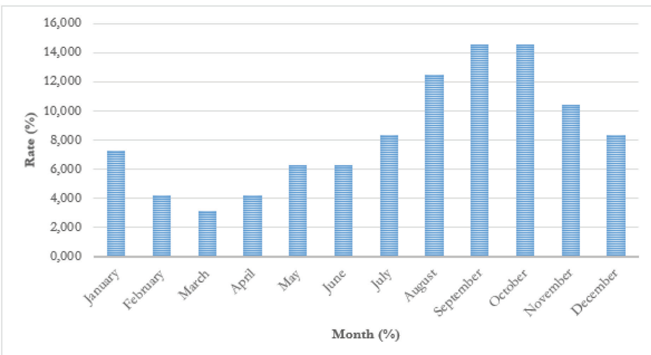

Figure 4. Monthly frequency of trypanosome infestations.

and Moti et al. [3] in Ethiopia, Sheferaw et al. [56] and Ngomtcho et al. [57] in Cameroon who concluded that hematocrit and red blood cell counts are declining in cattle with trypanosomosis. This is linked to the hematophagous nature of trypanosomes.

The absence of the effect of sex on the prevalence of trypanosomosis recorded in this study was also reported by Achukwi and Musongong [43] in Cameroon and Isamah and Otesile [6] in Nigeria. Animals aged 0–2 years had a prevalence above animals over 7 years old. However, this difference was not significantly different, unlike Farougou et al. [51] who report significant variation in the prevalence of trypanosomosis by age group with higher frequencies in calves and juveniles. The absence of this effect in this study is due to the difference observed in the age categories at the level of the two works. This age effect was reported by Magona et al. [20] in Uganda. The effect of the genetic type recorded in this study was also reported by Farougou et al. [51] in Benin. The high prevalence recorded in bulls is due to the trypanotolerance of this genetic type which makes it less treated by trypanocidal by breeders.

Hematocrit rate was not influenced by the age of farmed cattle, contrary to that of slaughtered animals. This difference in variation is related to the sample size, which was low for farm animals than those in slaughterhouses. These results obtained in farms go in the same direction as those reported by Tanenbe et al. [21] who point out that the age of animals has little influence on hematocrit levels. It is the same for the genetic type.

Conclusion

Bovine trypanosomosis is a parasitic disease caused by *T. vivax, T. congolense*, and *T. brucei* with a predominance of *T. vivax* in Bénin. This prevalence varies from one slaughterhouse to another and from one department to another. The disease has a high prevalence in the rainy season compared to the dry season. It also causes a decrease of the hematocrit in cattle with trypanosomosis because of the hematophagous nature of *Trypanosoma* spp., which exposes them to anemic disorders responsible for their vulnerability. The results of the present study show that the hematocrit rate does not vary regardless of the genetic type and sex of the cattle, but decreases due to trypanosome infestation.

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Conflict of interests

There is no conflict of interest to declare.

Authors' contribution

SSAS and ID have participated in developing the protocol, the sample blood, and in drafting the manuscript. SSAS and AG participated in the identification of *Trypanosoma* and the development of the database. PS contributed to the translation of the manuscript. DTJ; YAKI; FS, and KTTM supervised the analysis of the statistical results and the correction of the manuscript. All authors have read and approved the content.

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