



Research Article

Effect of a Natural Trypanosome Infection in Trypanotolerant Cattle Reared in a Tsetse-Infested Area in Southern Gabon and Monitored Under Field Conditions

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Abstract

Background: African trypanosomiasis is a major constraint to agricultural production in Sub-Saharan Africa. N'Dama has the ability to survive and be productive in tsetse-infested areas without the aid of treatment where other breeds quickly succumb to the disease. Although trypanotolerant, N'Dama can become vulnerable as a result of frequent infections.

Results: A comparative study on the effect of trypanosome infection on body weight, chest circumference and Packed Cell Volume (PCV) between naturally infected N'Dama, reared in a tsetse-infested area, versus uninfected N'Dama has been undertaken over 13 weeks. The study was conducted on 29 N'Dama bull calves, 3-year-old, with an average live weight of 242.8±27.25 kg and reared at similar tsetse challenge levels. Fourteen animals infected with trypanosomes belonged to the infected group (n=14), and 15 bull calves randomly selected and having received curative and preventive treatments against trypanosomiasis, composed the uninfected group (n=15). Trypanosomes have been detected using PCR. During the study, body weight, chest circumference and PCV of animals of both groups were monitored weekly. The body weight loss was much more pronounced in the infected animals (b = -1.0584) than in uninfected animals (b = -0.2609). The decrease of the average chest circumference was much more marked in the infected group (b = - 0.0052) compared with uninfected group (b = - 0.0025). However, trypanosome infection had no significant effect on PCV.

Conclusion: This study suggests that strict prophylactic programs should be conducted on N'Dama cattle to improve their production, in tsetse-infested areas.

Keywords: Body weight; Chest Circumference; N'Dama; PCV; Trypanosomes

Background

In sub-saharian Africa, African Animal Trypanosomiasis (AAT), also known as nagana, is caused mainly by *Trypanosoma congolense*, *Trypanosoma vivax* and *Trypanosoma brucei* (the most widespread pathogenic trypanosomes in Africa). According to the

Food and Agriculture Organization (FAO), animal trypanosomiasis is responsible for the death of 3 million cattle per year, with an annual economic loss estimated at \$1-1.2 billion [1,2]. These losses are due to stock mortality and depressed productivity, which may be of meat, milk, reproduction or traction [3]. The clinical signs of AAT in livestock include, anemia, intermittent fever, whimpering, lymphadenopathy, jaundice, edemas, progressive emaciation leading to cachexia, weakness and death, if not treated [4,5].

The prevalence of the AAT in tropical and equatorial Africa constitutes a major hurdle with the livestock productions. AAT control has mainly been directed towards eradicating or reducing the number of tsetse and towards the use of trypanocidal drugs. None of these two methods of control against this disease were fully satisfactory. As the attempts made against the parasite and the vector had not produced sustainable results, an alternative, potentially cost-effective and sustainable option to reduce the prevalence of trypanosomosis was the exploitation of the natural phenomenon known as trypanotolerance exhibited by certain livestock breeds, including the well-known N'Dama, that are indigenous to areas in which the disease is endemic [6].

Trypanotolerance was defined as 'the ability of some livestock breeds to survive, reproduce and produce in tsetse-trypanosome infested areas where others cannot, without recourse to use of chemical drug' [7]. In AAT, the criteria to define trypanotolerance are Packed Cell Volume (PCV), which is an indicator of anemia, parasitemia, and body weight [8]. Indeed, it has been consistently noted that trypanotolerant cattle when they become infected develop less severe anaemia [9]. Moreover, trypanotolerant cattle as West African Shorthorn and N'Dama have the ability to resist the effects of infection, i.e. not only to survive, but to gain weight and reproduce [7]. In Gabon, as in other countries of Central Africa, the use of animals naturally less susceptible to the disease, such as N'Dama cattle, has produced encouraging results. It has long been recognised that some breeds of trypanotolerant cattle such as N'Dama, has the ability to survive and be productive in tsetse-infested areas without the aid of treatment where other breeds quickly succumb to the disease [10].

However, trypanotolerant animals tolerate infections, but this does not mean that the infection has no impact on their health. Indeed, study done in Senegal have shown that, even though it is trypanotolerant, N'Dama cattle sometimes becomes vulnerable, which affects its work performance (i.e. speed of work, distance covered) [11] because trypanotolerance is relative and its role in the immune defenses of the animal can be considerably reduced, in particular during frequent infections. Similarly, [12] showed that N'Dama cattle can suffer for trypanosomosis under high tsetse challenge. The objective of this study is to evaluate the effect of a trypanosome infection on N'Dama naturally infected, reared in a tsetse-infested area, by comparing the evolution of body weight, chest circumference and Packed Cell Volume (PCV) in infected and free from trypanosomes N'Dama.

Materials and Methods

Study Site

The study was conducted from June to September in the Nyanga ranch, located in the largest savannah zone of the country in the Mongo County, located about 65 km from Tchibanga (the

main town of Nyanga province, southwest Gabon) and extended over 100,000 hectares. The Nyanga ranch includes three divisions (Nyanga, Bibora, and Voungou) divided into sections. The ranch represents a hilly landscape covered with herbaceous vegetation type and dotted with vegetation (*Brachiaria*, *Hyparrhenia*, *Panicum*, *Andropogon* and *Digitaria* species). Forest galleries are present along the gullies and rivers. Climate is equatorial with two dry seasons and two wet seasons. The presence of *Glossina haningtoni*, *Glossina palpalis palpalis* and *Glossina tabaniformis* has been confirmed in forests and forest galleries of the Nyanga valley [13,14]. In the Gala section of the Nyanga ranch, *T. vivax* and *T. congolense* have been detected in cattle.

Animals

Initially, a total of 57 N'Dama bulls calves, reared at similar tsetse challenge levels, were screened for trypanosomosis using PCR [15]. These animals live outdoors, feed through natural grazing, composed predominantly of *Hyparrhenia diplandra* and *Panicum maximum*, and drink from natural water courses or water troughs, for low irrigated areas. A mineral supplement based on a mixture of sodium chloride, copper sulfate, zinc carbonate and cobalt sulfate is provided *ad libitum*. Every two weeks, animals were dipped into a flumethrin bath to protect them from blood-sucking arthropods.

PCR assay was performed from DNA extracted from whole blood samples, collected by puncture of the caudal vein, using QIAamp DNA Blood Mini Kit (Qiagen, Germany). Blood samples from each animal was subjected to parasitological and hematological analyses, using the buffy coat method to estimate the parasitaemia according to the scoring system based on the darkground/phase contrast buffy coat technique [16,17], and the Packed Cell Volume (PCV), respectively. Animal was considered infected when PCR was found positive and at least one trypanosome was observed. Uninfected animals were those in which PCR was found negative and no parasite was observed.

Firstly, the animals of both groups (infected and uninfected) were subjected to different treatments: deworming using Levamisole (Alfamisol); every 2 weeks, animals were dipped into a Cypermethrin (Cypertop) bath to kill and repel ticks and tsetse flies (the transition to dipping tank was done every two weeks for all animals throughout the duration of the experiment); preventive antibiotic therapy based on oxytetracycline (Tenaline 20% L.A.); vaccinations against contagious bovine pleuropneumonia and bovine pasteurellosis. Moreover, uninfected animals received in addition to these treatments, a trypanocidal (Diminazene) (Veriben). Then, the animals were put to rest for one week before the start of the experiment. Two weeks after the start of the experiment, the uninfected animals received a second trypanocide based on Isometamidium chloride (Trypamidium-Samorin).

Monitoring of Animals and Data Collection

Once a week, the animals were brought back to the health center for blood sampling, weighing and taking chest circumference. So, during this weekly passage, for each animal approximately 5 mL of whole blood was collected in EDTA vacuum tubes VENOJECT[®], by puncture of the caudal vein. The buffy coat zone, prepared in a micro-hematocrit capillary tube heparinized (Drummond[®], USA) filled with 75 µL of blood and centrifuged for 6 min was examined for trypanosomes and packed cell volume value was determined. Animals were weighed using weighing bars (Avery-Weigh Tronix PC 820, UK), chest circumference were taken using a ribbon. At the end of the experiment, blood samples from uninfected animals were again screened by PCR to exclude any possibility of infection during the experiment.

Statistical Analysis

The data obtained was previously analyzed as recommended by [18]. The effect of trypanosome infection on body weight, chest circumference and PCV was studied in two ways. First, by analyzing the weekly average gain of the parameter considered. A “gain” is a positive variation (or increase) of the parameter over a given period. The weekly gain (GP) was calculated as the ratio of the variation of the parameter on the time variation:

$$GP = \frac{\delta P}{\delta T} = \frac{P_{it+k} - P_{it}}{T_{t+k} - T_t}$$

P_{it} is the measured value of the parameter P on the individual i at time t ; P_{it+k} ($P_{it+k} > P_{it}$) is the value of the same parameter, measured on the same individual at time $t + k$, where k is the number of weeks between the two measurements.

This variation was studied using a linear mixed effects model. Indeed, with data from multiple measurements on the same individuals, these observations are dependent and correlated. An appropriate procedure for analyzing this type of data is the use of mixed models, which allow the inclusion of random effects, to account for individual [18-20] and temporal variability. A logarithmic transformation has been applied to reduce the large disparity between observations [20]. This transformation concerns only the body weight and chest circumference variables; the added constant allows small values to be taken into account (Equation 1).

Regarding the PCV, it is an arcsinus transformation ($\text{Arcsinus}(\sqrt{p})$, p is in proportion) that was performed (Equation 2 and Equation 4), as recommended by [21] for the analysis of percentage data.

$$\ln(GP_{ist} + 1) = \alpha + \beta \times S + \alpha_t + \alpha_{is} + \varepsilon_{ist} \quad (\text{Equation 1})$$

$$GH_{ist} = \alpha + \beta \times S + \alpha_t + \alpha_{is} + \varepsilon_{ist} \quad (\text{Equation 2})$$

\ln = logarithm base “e”; GP_{ist} = gain or increase in body weight or chest circumference of animal i of status s in period t ; GH_{ist} = gain or increase in hematocrit or PCV of animal i of status s in period t ; S = infectious status of the animal with respect to trypanosomosis (S is 0 if the animal is uninfected and 1 if it is infected); α, β = fixed parameters to be estimated; α_t, α_{is} = random parameters related to the period and the subject, respectively. The individual random effect is fitted into the effect period; $\varepsilon_{ist} \sim N\{0, \sigma^2\}$.

Then, by analyzing the temporal evolution of the average of the parameter in each group. The average body weight and the average chest circumference were analyzed as a simple linear function of time (Equation 3). However, the average PCV was adjusted as a quadratic function of time (Equation 4).

$$\bar{P}_s = a_s + b_s \times T + \varepsilon_{is} \quad (\text{Equation 3})$$

$$\bar{H}_s = a_s + b_s \times T + c_s \times T^2 + \varepsilon_{is} \quad (\text{Equation 4})$$

\bar{P}_s = average body weight or chest circumference calculated for individuals of the same infectious status; \bar{H}_s = average hematocrit or PCV calculated for individuals of the same infectious status; T = time (in weeks); a_s, b_s, c_s = fixed parameters to estimate varying according to infectious status; $\varepsilon_{is} \sim N\{0, \sigma^2\}$. All statistical analyzes were performed using the R software version 3.4.1.

Results

Experimental Animals

From the 57 N'Dama bull calves, 29 animals, 3-year-old, with an average live weight of 242.8±27.25 kg, were selected and divided into 2 groups: 14 bull calves composing the group of infected animals, among which 5 were infected by *Trypanosoma vivax* et 9 by *Trypanosoma congolense*, and 15 bull calves randomly selected (random sampling double raw) among the 43 non-infected animals, which were the uninfected group. PCV values and parasitemia of all the infected animals are shown in (Table 1). The average PCV of the infected animals was 23.8% before the start of the experiment. All animals of both groups appeared healthy.

Animal ID	PCV (%)	Trypanosome/field	Trypanosome identified by PCR
265	30.5	1	<i>T. vivax</i>
1402	23,5	1	<i>T. congolense</i>
1618	22	2	<i>T. congolense</i>
1709	30.5	1	<i>T. vivax</i>
2606	24	1	<i>T. vivax</i>
2656	25	1	<i>T. congolense</i>

2662	29	1	<i>T. vivax</i>
2896	20	1	<i>T. congolense</i>
2881	26	2	<i>T. congolense</i>
2884	20	1	<i>T. congolense</i>
1146	20	3	<i>T. congolense</i>
2852	21	1	<i>T. vivax</i>
2878	21	2	<i>T. congolense</i>
2895	22	2	<i>T. congolense</i>

Table 1: Characteristics of the infected animals selected for the study before different treatments applied.

Effect of Trypanosome Infection on Body Weight and Chest Circumference

The study of the correlation between variables using principal component analysis, showed a strong association between body weight and chest circumference (Figure 1). A possible link between the infectious status of animals and changes in their weight and chest circumference was observed in (Figure 1).

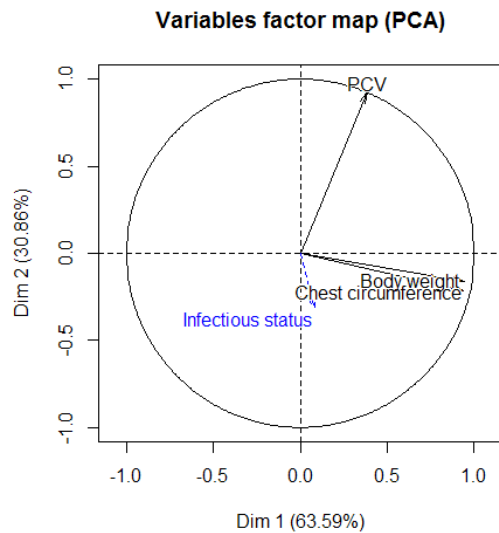


Figure 1: Correlation between the infectious status of animals and the body weight, chest circumference and packed cell volume (PCV).

Analysis of weight gain in both groups of animals showed that over the same period, the average body weight of infected N'Dama bull calves would increase more slowly (1.501 ± 0.268 kg per week) than that of uninfected animals (1.769 ± 0.259 kg per week). This weekly difference of 0.238 ± 0.251 kg would be significant at 10% threshold (Table 2). Thus, considering two individuals, one infected and the other uninfected, and of the same body weight at time t_0 , if their mass increases steadily during the period t_{0+k} (k number of weeks passed), the body weight of the uninfected is expected to be $k \times 0.238 \pm 0.251$ higher than that of the infected animal. The results of the regression of the average body

weight according to time in both groups of animal are summarized in Figure 2 and (Table 2).

Parameters	Values	<i>p</i> values
α (\pm CI)	1.739 (\pm 0.259)	<0.0001
β (\pm IC)	- 0.238 (\pm 0.251)	0.0617
Standard deviation of time-related random effects	0.399	
Standard deviation of the random effects related to animal	0.549	
Residual standard deviation	0.222	
α, β : fixed parameters to be estimated		

Table 2: Summary of the weight gain model.

We noted a decrease of the average body weight over the experimentation period regardless of the group (Figure 2). While this decrease was not statistically different between the both groups ($p = 0.2463$) (Table 3), it does not occur nevertheless at the same pace. However, the average body weight of the infected animals was significantly higher in early experimentation ($p = 0.0222$) (Table 3) and the body weight loss was much more pronounced in the infected animals ($b = -1.0584$) than in uninfected animals ($b = -0.2609$). In addition, in the infected group, the average body weight decreased so much throughout the observation period that it was almost equal to the average weight, which fell slightly, in the uninfected group, at the end of the study (Figure 2).

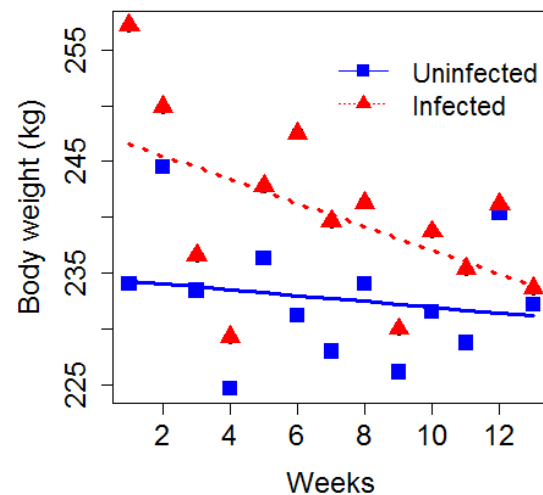


Figure 2: Regression of average body weight in both groups of animals. Blue square and black triangle represent the average for animals of each group.

Parameters	Average body weight		p value of equality test parameters between the two groups
	Uninfected	Infected	
α (\pm CI)	234.5612 (\pm 7.3330) ***	247.631 (\pm 9.1123) ***	0.0222
b (\pm CI)	- 0.2609 (\pm 0.9238)	- 1.0584 (\pm 1.1480) ^a	0.2463
Residual standard deviation	5.663	7.037	

α = Intercept; b = regression coefficient; ***: Highly significant at the 5% significance level ; ^a : significant at the 10% significance level.

Table 3: Summary of the model of evolution of the average body weight over the study period for both groups.

The observation of the evolution of the chest circumference in both groups during the same period showed that the average thoracic perimeter of the infected animals would increase significantly less rapidly ($p = 0.0422$) than the uninfected animals; with a difference of about 0.013 ± 0.012 m per week (Table 4). For a trypanosome-infected animal a weekly chest circumference increase would be approximately 0.026 ± 0.010 m, whereas it would be around 0.039 ± 0.007 m for an uninfected animal.

Parameters	Values	p values
α (\pm CI)	0.0386 (\pm 0.0074)***	<0.001
β (\pm CI)	- 0.0125 (\pm 0.0120)*	0.0422
Standard deviation of time-related random effects	0.0014	
Standard deviation of the random effects related to animal	0.0283	
Residual standard deviation	0.0047	

*: significant at the 5% significance level.

Table 4: Summary of the chest circumference variation model.

The results of the regression of the average chest circumference according to time in both groups of animal are summarized in (Figure 3 and Table 5). We noted a decrease of the average chest circumference over the experimentation period regardless of the group (Figure 3). Although the gradients of the

two regressions are not statistically different ($p = 0.1472$), that of the infected group is nevertheless more accentuated. The decrease is much more marked in the infected group ($b = - 0.0052$) compared with uninfected group ($b = - 0.00256$). Moreover, the average chest circumference of the infected group was significantly higher ($p = 0.0101$) at the start of the study (Table 5), and the regression coefficient of the infected group was significant ($p = 0.000864$). The Figure 3 showed that the average chest circumference of the trypanosome-infected animals was significantly higher at the start of the experimentation, before decreasing and getting closer to the value of uninfected group, which weakly regressed, at the end of the experiment.

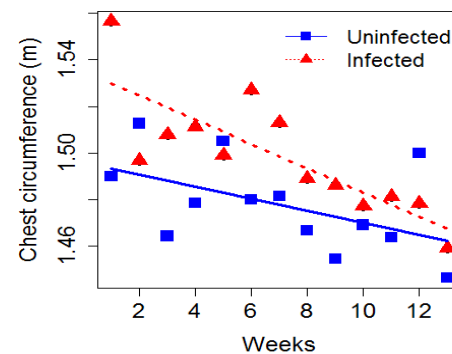


Figure 3: Regression of average chest circumference in both groups of animals. Blue square and black triangle represent the average for animals of each group.

Parameters	Average chest circumference		p value of equality test parameters between the two groups
	Uninfected	Infected	
α (\pm CI)	1.4959 (\pm 0.0231) ***	1.535 (\pm 0.02007) ***	0.0101
b (\pm CI)	- 0.00256 (\pm 0.0029) ^a	- 0.0052 (\pm 0.00253) ***	0.1472
Residual standard deviation	0.01782	0.0155	

α =Intercept; b = regression coefficient; ***: Highly significant at the 5% significance level ; ^a : significant at the 10% significance level

Table 5: Summary of the model of evolution of the average chest circumference over the study period for both groups.

Effect of Trypanosome Infection on Packed Cell Volume

The increase of PCV in trypanotolerant N'Dama bull's calves would appear to be more attenuated ($\alpha = 0.22 > 0$ and $\beta = -0.03 < 0$) in individuals infected with trypanosomes (Table 6). We would expect a weekly increase of approximately 3.58% in an affected animal against 4.78% for a trypanosome free animal. However, we found that the average PCV did not differ significantly between the two groups, from the start ($p = 0.2481$) to the end of the experiment ($p = 0.9761$) (Figure 4 and Table 7). The figure 1 already showed a lack of association between PCV and infectious status of animals. In addition, the difference observed at the start of the study between the two groups is maintained throughout the experiment period (see regression curve in Figure 4).

Parameters	Values	p values
α (\pm CI)	0.2204 (\pm 0.0316)***	<0.0001
β (\pm CI)	- 0.0300 (\pm 0.0355) ^a	0.0972
Standard deviation of time-related random effects	0.0403	
Standard deviation of the random effects related to animal	0.0863	
Residual standard deviation	0.0327	
***: Highly significant at the 5% significance level; ^a : significant at the 10% significance level.		

Table 6: Summary of the PCV variation model.

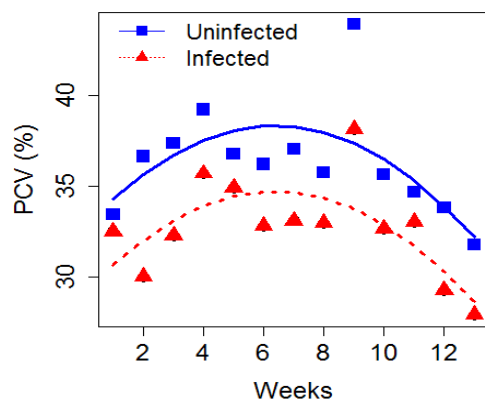


Figure 4: Regression of average PCV in both groups of animals. Blue square and black triangle represent the average for animals of each group.

Parameters	Average PCV		p value of equality test parameters between the two groups
	Uninfected	Infected	
a (\pm CI)	0.608 (\pm 0.055)***	0.569 (\pm 0,047)***	0.248
b (\pm CI)	0.0187 (\pm 0.018)*	0.019 (\pm 0,015)*	0.977
c (\pm CI)	- 0.0014 (\pm 0.0013)*	- 0.0015 (\pm 0.0011)*	0.976
Residual standard deviation	0.0254	0.0216	
α =Intercept; b , c = regression coefficient; *: significant at the 5% significance level.			

Table 7: Summary of the model of evolution of the average PCV over the study period for both groups.

Discussion

One of the most important factors which influences the level of trypanotolerance is the severity of the tsetse challenge to which the animals are exposed. In fact, as the level of challenge rises, productivity falls, and in high-risk situations even N'Dama cattle can be severely affected by trypanosomiasis [10]. Very little studies [22] have been conducted on the effect of trypanosome infection on N'Dama trypanotolerant cattle by comparing naturally infected N'Damas with a control group of uninfected N'Dama under field conditions.

Our study was conducted on N'Dama bulls aged 3 years; they are growing animals which allows to appreciate the evolution of the morphological parameters. Indeed, the complete development of N'Dama is reached at 7 years for males and 6 years for females [23]. Previous study carried out in one section of the Nyanga ranch showed that the prevalence of trypanosomiasis was high (57.3%), with the co-circulation of two pathogenic species of trypanosomes, *T. congolense* (47.3%) and *T. vivax* (10%) [24]. The study animals were found naturally infected either by *T. vivax* or *T. congolense* [25,22], major pathogenic trypanosome species in Africa. Thus, the 14 animals in the infected group were predominantly found infected with *T. congolense* (9 individuals). The 5 *T. vivax*-infected individuals were included in the study because of the virulent nature of this species in cattle and to increase the number of animals infected with trypanosomes. Although *T. congolense* is often considered to be the most virulent of the trypanosomes

in cattle, *T. vivax* is often found to be responsible for the first infections in cattle [26].

The results of this study showed a link between the infectious status of N'Dama naturally infected in field and changes in their body weight and chest circumference. The principal component analysis already showed a strong association between body weight and chest circumference as previously reported by [27]. The effect of trypanosome infection on the body weight of N'Dama bulls was significant. The body weight loss was much more pronounced in the infected animals than in uninfected animals, as shown by [22] who studied N'Dama from Mushie Ranch, in Democratic Republic of Congo. However, this finding was contrary to the result obtained by [11] who, working over a period of 4 weeks on N'Dama experimentally infected with *T. congolense*, had not observed a significant effect of the infection on weight probably due to the relatively short period of his study. Moreover, authors argue that if the experiment had been prolonged for a few more weeks, a significant difference in weight gain would probably have been observed in the event that the animals did not feed well. These conditions could correspond to those of our study, insofar as this one was carried out over a period 3 times longer than that of their study and in the dry season, where the food in the pastures of the ranch was scarce. In general, compared to other cattle breeds such as Boran, trypanosome infection does not affect liveweight gains in the N'Dama [7].

The chest circumference of infected animals was significantly influenced by trypanosome infection. Indeed, the average chest circumference of infected animals decreased significantly faster than that of uninfected animals, with a difference of about 0.013 ± 0.012 m per week ($p = 0.0422$). The uninfected animals have a more significant weight gain than the infected animals, and therefore would grow larger, resulting in widening of the chest circumference. The very significant regression coefficient of the infected group reflects a proven effect of the infection on the chest circumference, i.e. the trypanosome infection has a negative effect on the evolution of the chest circumference of the animals. Besides we noted a decrease of the average chest circumference over the experimentation period regardless of the group which would result of the scarcity of forage on pastures linked to the dry season. Our results contrast with the results obtained by [28], who could not conclude from the significant effects of trypanosome infection on the chest circumference. According to them, in their study, this parameter was influenced by the feeding problem and by the effects of gastrointestinal parasitism. In our study, the animals of both groups (infected and uninfected) have been de-wormed. The feeding pattern of animals was the same for all of them. They fed in the park based on natural forage.

We found that the average PCV did not differ significantly between the two groups, from the start to the end of the experiment,

although the increase of PCV in trypanotolerant N'Dama bull's calves would appear to be more attenuated in individuals infected with trypanosomes, as shown by [22], suggesting the existence of a weak possible effect of the infectious status on the PCV level, but it would be difficult to better quantify in the field conditions. In their studies, [29] and [11] found that the PCV of infected N'Dama was lower than that of healthy N'Dama. A lower PCV is synonymous with anemia, and the latter is one of the hallmarks of trypanosomiasis [11]. The N'Dama's ability to control parasitaemia, a capacity conferred by trypanotolerance [23,30], was confirmed by the low parasitaemias observed in infected animals. In addition, the detection of trypanosomes in infected animals, despite the very low parasitaemia noted, confirms the very good sensitivity provided by PCR of the order of 1 to 50 trypanosomes/ml of blood [31,32].

Conclusion

This is a recent study that describes the effect of trypanosome infection on naturally infected trypanotolerant cattle and monitored under field conditions, raised in a country where cattle breeding is struggling to develop. Bovine trypanosomiasis is a limiting factor in cattle rearing. The disease affects the growth and reproduction performance of these animals. The breeding of trypanotolerant cattle is one of the main solutions to this problem. This study showed that N'Dama cattle would therefore not be simply reservoirs of trypanosomes but could be affected by these parasites in the absence of prophylactic programs. This study, however, would need to be carried out over a longer period of time and in the rainy season when pastures are rich in fodder, in order to better appreciate the real impact of this disease on trypanotolerant cattle breeds reared in their natural environment, which according to some authors, would not suffer from this infection. In parallel, this study could also be reproduced under experimental conditions, in order to better control some parameters which could interfere with the observed effects; such as the level of infection of the animals, trypanosome species involved and availability of food resources.

Declarations

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Authors' Contribution

Maganga GD has initiated the study and drafted the manuscript. Dibanganga GL performed analyses and drafted

the manuscript. Mbeang Beyeme AM, Mikala Okouyi CS and Adjahoutonon B performed analyses. All authors read and approved the final.

Competing Interests

The authors declare that they have no competing interests.

Consent for Publication

Not applicable.

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