Assessment of α-cypermethrin Pour-on Application and Diminazene Aceturate for the Treatment of Trypanosome-related Diseases Caused by Tsetse Flies in Cattle in Mô (Togo)

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Research

Keywords: African animal trypanosomosis, trypanocidal drugs, α-cypermethrin pour-on, Mô area, Togo

Posted Date: July 19th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-710560/v1

Abstract

Background

The effects of tsetse-transmitted trypanosomosis control in high tsetse challenge and trypanocidal drug resistance settings remain poorly understood in Togo, owing to the poor data underlying the current disease impact. This study was implemented in the framework of the PDRI-Mô project which is an African animal trypanosomosis control project using trypanocides and insecticides on cattle. This project, planned by the agricultural ministry, focused on all the sedentary cattle breeds in the 1000 km² area of the prefecture of Mô. Until 2013, the locality remained unconnected to outside areas, with long-term limited access to quality medicines, veterinary services, animal husbandry and new AAT control technologies.

Methods

From March 2014 to November 2017, a database of zoo-sanitary surveys integrating the evolution of the incidence of the disease and the coverage of interventions made it possible to quantify the apparent effect attributable to control effort in herds. The strategy involved an initial phase with cross-sectional entomological and parasitological surveys, including a rapid trypanocidal drug sensitivity testing. Treatment efficacy was assessed using parasitological status post-treatment of trypanosome-positive animals in each village, randomly assigned to three groups, one treated with 0.5 mg/kg b.w. Isometamidium chloride, the second with 3.5 mg/kg b.w. Diminazene diaceturate and the third with distillated water as control. Using parasitological status of blood sample collected on day 0, day 14 and day 28 post-treatment as the outcome result, trypanosome phenotype resistance of drugs treatment was determined if relapse occur. Then, three times a year, 20% of the herd received α-cypermethrin pour-on, and parasitaemic cattle with poor health were individually given diminazene aceturate at 7 mg/kg of body weight (b.w.).

Results

The tsetse density in the area decreased significantly (P-value 0.001) from 1.78 ± 0.37 in March 2014 prior to the α-cypermethrin application to 0.48 ± 0.07 in February 2017. Prior to the trypanocidal treatment, relapse of trypanosome infections in the cattle was approximately 12.5% for diminazene at 3.5 mg/kg of b.w., 35% for isometamidium at 0.5 mg/kg of b.w. and null for diminazeen at 7 mg/kg of b.w. Target deployment led to the largest reduction in disease incidence from 28.1% in 2014 to 7.8% in 2017, an improvement in haematocrit from 24.27±4.9% to 27.5±4.6% and a reduction in calf mortality from 15.9±11% to 5.9%.

Conclusions

Improving the access to these interventions for different types of livestock and maintaining their effectiveness in the face of high tsetse challenges should be the primary focus of control strategies in areas of Togo.

Background

Tsetse-transmitted animal trypanosomosis is caused by a blood parasite, and from a symptomological point of view, it has a close affinity to malaria disease, whose impact on the efficiency of livestock production in sub-Saharan Africa is considerable [1]. The economic costs of this disease include direct and indirect effects and the costs associated with preventive measures implemented by farmers and the transmission control policies of governments [2], [3].

Togo is among the trypanosomosis endemic countries with the highest impact of this disease, and up to 90% of cattle and goat populations occur in substantially tsetse-infested villages [4]–[6]. Traditionally, the control of tsetse-transmitted animal diseases during livestock production in Togo is mainly based on local trypanotolerant cattle populations and the use of trypanocidal drugs [7], [8]. Before Togo's political crisis of the 1990s, a number of interventions against African animal trypanosomosis (AAT) were carried out to improve the areas with incidences of onchocerciasis that occurred over a long period of time based on the promotion of agriculture and livestock development [9]. Interventions have significantly reduced disease incidence in some areas [10]. Currently, although trypanosomosis is on the priority zoonosis list for West Africa, animal trypanosomosis in Togo is not listed as a priority for technical service intervention since the human form of this disease, named sleeping sickness, is declared eradicated (WHO, 2020). The rapid privatization of veterinary medicine in Togo has also led to a decline in animal health coverage and monitoring of cattle herds. Recent studies confirmed trypanocidal drug resistance in Togo [5], while the impact of this additional problem on the effectiveness of AAT control measures is poorly understood.

The Mô Plain is an area of the Guinean savanna in the central region of Togo with high agricultural potential over 1000 km^2, adjacent to the Fazao-Malfackassa National Reserve [10]. This area is severely affected by the negative impact of AAT, despite its predominantly dwarf taurine-phenotype cattle herd [6], [10], [12]. In fact, the farmers in this locality improve herd productivity through practices characterized by various levels crossing between the local trypanotolerant dwarf somba populations and the large bovine breeds, in particular the zebu, which are trypanosensitive compared to taurine cattle.

Until 2013, the locality remained unconnected to outside areas, with long-term limited access to quality medicines, veterinary services, animal husbandry and new AAT control with pour-on technology [13]. This study was designed to assess the effect of α-cypermethrin pour-on application and diminazene aceturate (DA) for the treatment of trypanosome-related diseases on Mô traditional cattle herd health. The study was not designed to assess the effectiveness of interventions in relation to the absence of Tsetse fly, so data were collected exclusively in the project area.

Methods

Study area

The study was carried out in Mô prefecture that has an area of approximately 1000 km^2 in the western part of the central region of Togo, bordering Ghana. The coordinates of the points of the survey are shown in Figure 1.

The prefecture has a subhumid climate typical of the Guinean zone with two main seasons: a dry season from November to March and a rainy season from April to October. The annual rainfall is in the range of 1074-1649 mm, and the number of annual rain days is 108-115 days [14]. The main vegetation is wooded Guinean savanna with surrounding gallery forests bordering the Mô River. The variation in rainfall greatly influences livestock production. According to the information supplied by the technical staff of the project, the sedentary cattle population is estimated to be 40 cattle herds with 1437 heads [14]. Cattle in this area are the phenotypically taurine dwarf Somba (trypanotolerant) and their crosses with zebu that are maintained under a traditional extensive system (7). Cross-sectional analyses of several studies suggest animal trypanosomosis as a risk factor for local cattle production in this area [6], [10].

Study design

This study was implemented in the framework of the PDRI-Mô project. PDRI-Mô is an AAT control project using trypanocides and insecticides on cattle. This project, planned by the agricultural ministry, focused on all the sedentary cattle breeds in the area of the prefecture of Mô. To characterize the tsetse challenge across the prefecture, the grazing area, including the 40 cattle herds of the villages, was taken into account. The prefecture was divided into four strata of villages representing different administrative and cattle breeding areas: Bolohou, Djarkpanga, Tindjasse and Saiboude (Figure 1). The strategy implemented for trypanocidal and α-cypermethrin pour-on treatment effectiveness involved cross-sectional (for baseline data collection) and longitudinal monitoring surveys. Thus, for the first processing operation (March 2014), all 40 herds of the prefecture (counting an average of 2000 cattle) were treated. Following this, periodical (four month) treatment operations (trypanocidal and cypermethrin pour-on) were conducted from March 2014 to November 2017 according to a subjective assessment (report of the cattle owners and the technicians) of the level of challenge. The day before each intervention, per village, owners were encouraged to cooperate by preventing their cattle from going to pasture in the morning for free deworming and insecticide treatments against tsetse flies and ticks. The sick animals identified by owners were examined for AAT symptoms, and parasitological analysis of blood samples was conducted [16]. The monitoring consisted of recording calf mortality, tsetse abundance and new AAT cases, including their parasitological and anaemia status.

Entomological data collection

The collection of entomological baseline data is a prerequisite for the development of an appropriate control strategy [17], [18]. We conducted a cross-sectional monitoring survey of tsetse abundance in March 2014, March 2015, December 2016 and February 2017 using 20 biconical traps [19]. To estimate the abundance of the tsetse population, traps were deployed for 24 hours per day in each of the four villages. The selection of the trapping points considered accessibility (road) and hotspots of tsetse flies: small patches of remnant vegetation, gallery forests, cattle crossing points, etc.

Rapid trypanocidal drug sensitivity testing

The DA and Isometamidium chloride (IMC) was used for treatment of infected cattle in the treatment group (Table 1).

Table 1: Composition of the experimental batches for the treatments

Herd	Batch	Treatment
12 animal/experimental herd	10 Positive	40 young male treated with diminazen
		40 young male treated with isometamidium
		20* young male treated with distilled water
	2 Negative	20* young male treated with distilled water

*: Animal was used as control.

A comparative assessment of the sensitivity of the trypanosome parasite population to the two drugs was performed according to the rapid test [20]. This was a parallel study designed, blinded, randomized and negatively controlled. Ten random cattle herds (n = 120 cows) were selected based on the results of a parasitological survey performed in March 2016. The 120 experimental animals were all young males with an average age of 12 to 24 months. The detailed group design is presented in Table 1. The inclusion criteria were (i) phenotypic dwarf taurine cattle, (ii) trypanosome-related prevalence in herds of at least 10% and (iii) at least 12 trypanosome-positive cows in each sample of the herds. For each random herd, consent was obtained from the owner for inclusion in the study. Cattle tags with a unique identifying number were placed in the ear at the time of inclusion. The cattle received a randomized treatment (DA, IMC or distilled water as a placebo) using simple randomization that was concealed from the technician and the owner enrolling the animals in the test. The positive and negative control cattle per batch were added for the eventual natural clearing of parasites to provide trypanotolerance to the cattle population in the area. All cattle in each bioassay batch were sprayed with α- cypermethrin at a dose of 1 ml/10 kg of b.w. The aim of this insecticide treatment was to protect tested animals from new bites of trypanosome vectors, regardless of whether they became infected after drug treatment. Additionally, all the animals were treated with Ivermectin (1 ml/50 kg) one week before the drug treatment bioassay.

Post treatment (14 and 28 days) counts of the treated and control animals were used to evaluate the efficacy of the treatments [20]. Presence of trypanosomes in the Buffy coat smear was considered as indication of drug resistance. For all groups, the number of positive cows in each group was expressed as a percentage of the number of initial positive cattle before the treatment. At 14 days post treatment, for the cattle that were administered trypanocidal for apparent infestation, a second dose of DA was administered at 7 mg/kg of b.w., and the animals were monitored for two weeks thereafter.

Cattle mass treatment

Owners were encouraged to cooperate by identifying sick animals for free drug and insecticide treatments. The animals selected conventionally for treatment included any animal considered to present trypanosome-related symptoms or to be in poor condition [21]. Parasitological analysis of the blood samples [16] of the selected cattle was performed to confirm their health status before treatment. The packed cell volume (PCV) was read in a microhaematocrit tube after centrifugation for 5 min at 12 500 rpm [22]. Only positive and/or anaemic cattle (haematocrit or PCV lower than 25%) were administered trypanocidal and cypermethrin pour-on treatments [23]. DA was administered deeply intramuscularly at a dose of 7 mg/kg of body weight (b.w.). The dose of commonly used insecticide is 10 ml/100 kg of b.w. The treated adult cattle and all calves up to three months old were enrolled for deworming with ivermectin (1 ml/10 kg of body weight) [24], [25]. The purpose of deworming was not to systematically eliminate all internal parasites but to prevent anaemia.

Data analysis

Tsetse fly apparent densities were evaluated as the number of tsetse flies per trap per day (TAD), calculated by dividing the total number of tsetse flies captured by the product of the number of functioning traps used to catch them and the number of days for which the traps were operational [26]. By herd, mean annual birth-to-weaning calf mortality was determined by dividing the number of deaths by the number of alive births. For each processing operation period, the numbers of new sick cattle were expressed as a percentage of the anterior hull cattle count in the 40 herds. The data was recorded into a Microsoft excel spread sheet to create a database and transferred to the statistical package for social sciences (SPSS) version 20 software programs of the computer for the statistical analysis. The Chi-square test was used to compare the frequency of trypanosome related species and frequency of the disease cases with respect to absence or presence of infection) at a precision level of 5%. Student-Newman-Keuls was used to compare between year mean calf mortality and mean TAD. ANOVA multiple comparisons test was applied by comparing the PCV values with respect to year and absence/presence of infection. The trypanosome population of the study area was considered to be phenotypically resistant to the given trypanocidal drug dose when relapse occurred in more than 20% of the batch [20].

Results

Entomology and parasitology

In total, 178 samples of five *Glossina* species were captured. The five species of *Glossina* in the area were determined on the basis of morphological identification. By species of *Glossina*, two riverine species, *Glossina tachinoides* (*G.t*) and *Glossina palpalis palpalis* (*G.pp*), one savanna species (*Glossina morsitans submorsitans* (*G.ms*)) and two forest species *Glossina fusc*a (*G.f*) and *Glossina longipalpis* (*G.l*). The savanna and forest species were detected at a low frequency. The apparent density of the tsetse flies captured in the first (2014) cross-sectional survey was higher, at approximately 1.78 ± 0.37, than that at other sites but did not significantly differ from one collection site to another in a given area. *G.t* and *G.pp* were the most abundant species, with TADs varying from 0.25 ± 0.1 to 0.8 ± 0.32 for *G.t* and from 0.8 ± 0.32 to 1.3 ± 0.57 for *G.pp*. The average TADs of the other species were approximately 0.05. Insecticide pour-on application led to a significant decrease in the tsetse fly density in the area to 73.03% of the initial TAD. In fact, the average tsetse flies collected per day of trapping was significantly lower in the two last years of the study, when the TAD for the month of March 2014 (TAD: 1.78 ± 0.37) was compared with that in March 2015 (TAD: 1.14 ± 0.76; P-value □ 0.021), December 2016 (TAD: 0.63 ± 0.32; P-value □ 0.001) and February 2017 (TAD: 0.48 ± 0.07; P-value □ 0.001) (Figure 2).

A blood sample analysis of 798 animals with poor health during the first cross-sectional survey revealed 224 (28.1%) positive cases. The frequency of *Trypanosoma vivax* (*T.v*) infection (129 positives/798 given 16.1%) was significantly higher than that of *Trypanosoma congolense* (*T.c*) infections (52 positives/798 given 6.5%) and mixed (*T.v/T.c*: 40 positives/798 given 5%) infections (Chi-square: 19,29; df: 3).

Drug sensitivity

In March 2016, persistent clinical signs were reported by owners approximately one month after anterior trypanocidal treatment, but there was no clinical evidence to support the inefficacy of the trypanocidal drugs for the treated animals compared to the untreated animals. The results obtained for the qualitative assay on cattle indicated the proportion of DA phenotype resistance at a dose of 3.5 mg/kg of b.w. was low, at approximately 12.5% (4 cattle of 40), compared to the average of 35% (14 given cattle upon 40) of IMC phenotype resistance at a dose of 0.5 mg/kg of b.w. In fact, for the 3.5 mg/kg of b.w. DA-administered cow, relapses were observed for two cases of *T.v* and two cases of *T.c*. Cattle administered IMC at 0.5 mg/kg of b.w. had relapses with 10 cases of *T.v* and 4 cases of *T.c*. No parasite showed phenotypic resistance to 7 mg/kg of b.w. of DA; thus, this dose was recommended for mass treatment.

Efficacy of the treatments

The number of new cases decreased significantly (Khi-Square: 65,36; df: 3), from 28.1% (224/798 cases) in 2014 to 7.8% (28/358 cases) in 2017 (20.3 points from the baseline) (Figure 3). From 2016 (60/307 cases) to 2017, the occurrence of the new cases remained almost stable, with a slight upturn in 2017. Nevertheless, whether in 2014 or in 2017, there was a significant disparity in new cases at the village level (Figure 4).

Generally, PCV was significantly reduced in positive animals (23.56 ± 5.3%) compared to in the other animals (24.54 ± 4.7%). The proportion of animals that had anaemia in the first year was approximately 57.39% (458/798 animals), and the average PCV value was approximately 24.2 ± 4.9%. The values measured three years later were significantly higher than the baseline average recorded in the first year, when PCVs for 2014 (PCV: 24.27 ± 4.9%) and 2017 (PCV: 27.5 ± 4.6%) were compared. In 2015 and 2016, the reordered values were 26.6 ± 4.9% and 25.2 ± 4.7%, respectively. The analysis of the model corresponding to differences in PCV year values suggests that the treatment improved cattle health in the area of study (Table 2).

Table 2: Mean PCV values following the period of trypanosomose control (Tukey test multiple comparisons)

(I) Year	(J) Year	Difference (I-J)	SE	p	Confidence interval (95%)	
					Terminal <	Limit >
2014 (To)	2015	-2.334*	0.263	0.000	-3.01	-1.66
	2016	-0.994*	0.326	0.012	-1.83	-0.16
	2017	-3.280*	0.308	0.000	-4.07	-2.49
2015 (T_1)	2016	1.340*	0.341	0.001	0.46	2.22
	2017	-0.946*	0.325	0.019	-1.78	-0.11
2016 (T_2)	2017	-2.286*	0.377	0.000	-3.26	-1.32
2017 (T_3)	2014	3.280*	0.308	0.000	2.49	4.07
	2015	0.946*	0.325	0.019	0.11	1.78
	2016	2.286*	0.377	0.000	1.32	3.26

T: period (12 month); SE: standard error; p: p-values; IC: confidence interval; * p-value < 0.05.

A calf mortality mean rate of 15,9 ± 11% was recorded in 2014. This mean value was significantly higher than the baseline value of 5,9% in 2017 (P-value 0.001), showing a significant reduction in mortality (Figure 5). Nevertheless, by village, there was a significant difference in the herd (village) at the beginning and end of the follow-up.

Discussion

This study provided a unique opportunity to quantify the attributable effect of trypanosome and tsetse control efforts on animal trypanosomiasis in high transmission settings of the Guinea savanna in Mô. Our data are important within the context of AAT control planning locally in endemic areas with crossbred trypanotolerant-trypanosensitive cattle breeding and important elsewhere on ranches where AATs a similar epidemiologic pattern.

Our results confirm previous observations that salivary AAT vectors in this area are *G.t* and *G.pp*, *G.ms*, *G.f* [6] and *G.l* [10]. The baseline entomological survey in the present study was conducted by collecting data and monitoring at tsetse-cattle herd contact points. Therefore, the tsetse TAD evaluated in the Mô area is likely to be higher than that reported in this study. In contrast, species diversity and TAD values were higher than those previously reported in the same area [6]. This implies specific concentration points and a temporary rise in forest tsetse species from the wildlife in the Mazao-Malfakassa forest including in Mô area.

Tsetse density as a whole decreased significantly; however, it remained relatively high, at slightly over the maximum of 0.26 measured in the north of Togo [10]. Several studies have shown that the insecticide pour-on control strategy has a limited effect on tsetse fly density. A survey carried out as part of pilot projects with the selected insecticide method showed a significant impact on tsetse fly density, with a reduction of 99,55% [27]. Two case factors accounted for these lapses, apart from a small tsetse density, which was between 0.2 and 0.7, the frequency of treatment processing operations (every two months) and the high insecticide-treated cattle coverage rate, which was 100%. In the agropastoral area of Yale in Burkina Faso, [24] obtained a good result of a 90% reduction of *Glossina* density. The technique combined the use of insecticide (deltamethrin 1%) in pour-on and insecticide-impregnated screens against tsetse (the frequency of treatment processing operation was twice a month). The relative low efficacy recorded the present study can be partly explained by the absence of intervention in the third treatment, which was meant to ensure health coverage from November 2016 to February 2017. In addition, in the present study, treatments were limited to three times a year, and for each intervention, only 10–20% of the cattle were treated in a herd. This time frame of up to 4 months between two consecutive operations provides a sufficient window of time for the rise of the new tsetse population from buried pupae [28], [29].

The main categories of local cattle breeds in Togo are dwarf trypanotolerant cattle and their crossbreeds (dwarf x zebu) [7]. The use of insecticide cattle treatments in such heavily tsetse-infested areas has not been shown to be particularly effective against tsetse flies [23]. However, it is possible to prevent other insect bites, such as ticks, that can cause illness and thus contribute to the poor health of the animals. In the present study, although insecticide application was carried out on tick-infested cattle, we did not evaluate the impact of this operation. In addition, limiting insecticidal interventions makes it possible to maintain minimum contact between cattle and tsetse flies, which is essential to allow multiplication of parasites prior to drug treatment to induce immunity in the host [30].

The high incidence of AAT disease in Mô is in agreement with previous observations [6]. With such a high incidence, previous routines and substantial use of trypanocidal drugs by farmers can lead to the development of trypanosome resistance (*T.v* and *T.c* are the two strains identified in the area) to the only available DA/IMC drugs. However, in the present study, no baseline data were established. This testing of operation effectiveness by monitoring staff aimed to validate the approach and previous findings concerning trypanocidal drug resistance elsewhere in West Africa [31].

Evidence of a relapse of parasites, i.e., presence of trypanosomes in the blood after trypanocide treatment, can occur due to the appearance of trypanosome populations from other sites, such as cerebrospinal fluid [32]. This might have also been observed due to new vector contamination in the field when treatments failed to protect animals against bites from tsetse flies after certain days. Given the cattle production system in this locality, a failure of the immune system to control the multiplication of chemically resistant parasites in trypanotolerant cattle may lead to a higher impact of trypanosomosis in this rearing system.

The mean PCV after three years of treatment was 1.4 absolute % higher than the value recorded by Talaki et al. (2014) and 3.2 absolute % higher than the initial value before processing operations. Consequently, as an overall health impact of control, the incidence of the disease decreased to 7.4% in 2017 (reduced by 20.3 absolute %) but remained almost stable and higher than the value of prevalence (2%) recorded by [27] in the North of the area in the dry savanna. There is no evidence to date to indicate that AAT transmission can be interrupted with existing tools in the study area of high tsetse challenge. Some important insights into the feasibility of reducing and maintaining tsetse TADs at a low level in areas of high transmission were obtained on the Adamawa Plateau, Cameroon [33].

The average mortality rate in 2017 was slightly above average at 3.37% in somba herds (north of Togo) based on Ibrahim et al. [34] but lower than 15.9 ± 11% in 2014 and the average (mortality rate of 10.98 ± 3.41% for crossbreed and 11.85 ± 4.19% for dwarf taurine) between Mô and Sokode recorded by Soudah et al. [12]. This result can partly be explained by interference with other diseases while not ignoring a high vulnerability to the reinvasion of tsetse from the Fazao-Malfakassa wildlife forest reserve in Mô area. This situation suggests that taking into account all health conditions can lead to more effective results in the herds.

Conclusions

The AAT processing operation in the Mô area was not sufficient to avoid trypanosome parasite transmission in Mô prefecture, as tsetse density was still relatively high. The present study was conducted in a highly endemic trypanosomosis area where trypanocides and insecticide-treated cattle were introduced for the first time. The low haematocrit levels initially recorded clearly reflected the severity of the disease in the animals. The impact of animal health control has thus resulted in a reduction in anaemia and the mortality of calves. As a result, we recommend continuing this activity and extending it to other production areas targeted by the “International Fund for Agricultural Development: IFAD-Livestock Togo” by integrating other technological packages to improve herd productivity.

Abbreviations

ITRA
Institut Togolais de Recherche Agronomique
PDRI-Mô
Projet de Développement Rural Intégré de la plaine de Mô
b.w.
body weight
AAT
African Animal Trypanosomiasis

TAD
trap per day
WAAP
West Africa Agricultural Productivity Program
PCV
packed cell volume
DA
Diminazene diaceturate
IMC
Isometamidium chloride
G.t
Glossina tachinoides
G.pp
Glossina palpalis palpalis
G.ms
Glossina morsitans submorsitans
G.f
Glossina fusca
G.l
Glossina longipalpis
IFAD
International Fund for Agricultural Development

Declarations

Ethics approval and consent to participate

Consent was obtained from the owner for cattle inclusion in the study.

Consent for publication

'Not applicable' for that section

Availability of data and materials

Any data generated in this study is included in the published article.

Competing interests

"The authors declare that they have no competing interests"

Funding

The corresponding author received Ph.D research grants from WAAP-Togo (IDA N°5955-TG) for field data collection and personal contribution from authors for language editing service.

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Contributions

Dr Essodina Talaki and M. N'Féidé Toï were involved in the data collection protocol, analysis and interpretation of the results, and they revised the content of the article. They approved the final version to be published. Dr. Balabadi Dao was involved in the field data collection, analysis of the results and revision of the content of the article. He approved the final version to be published. Doctors Tchamdja Eyaba, Somda M. Bienvenu, Salou Ernest and Lombo Yao were involved in the analysis and interpretation of the results, revising the scientific content and language style of the article. They approved the final version to be published.

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Acknowledgements

We thank Dr. Bonfoh Bédibétè and Dr. Lattah Appère for technical assistance. Thank to Dr. Dayo Guiguigbaza-Kossigan for scientific assistance and Dr OKE Oyeggunle Emmanuel for writing assitance. The work was supported by the Mô Rural Development Project in Togo (PDRI-Mô). The authors would like to thank and acknowledge the initiators of this project, which agreed to make their extension agents available to us for data collection.

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Figures

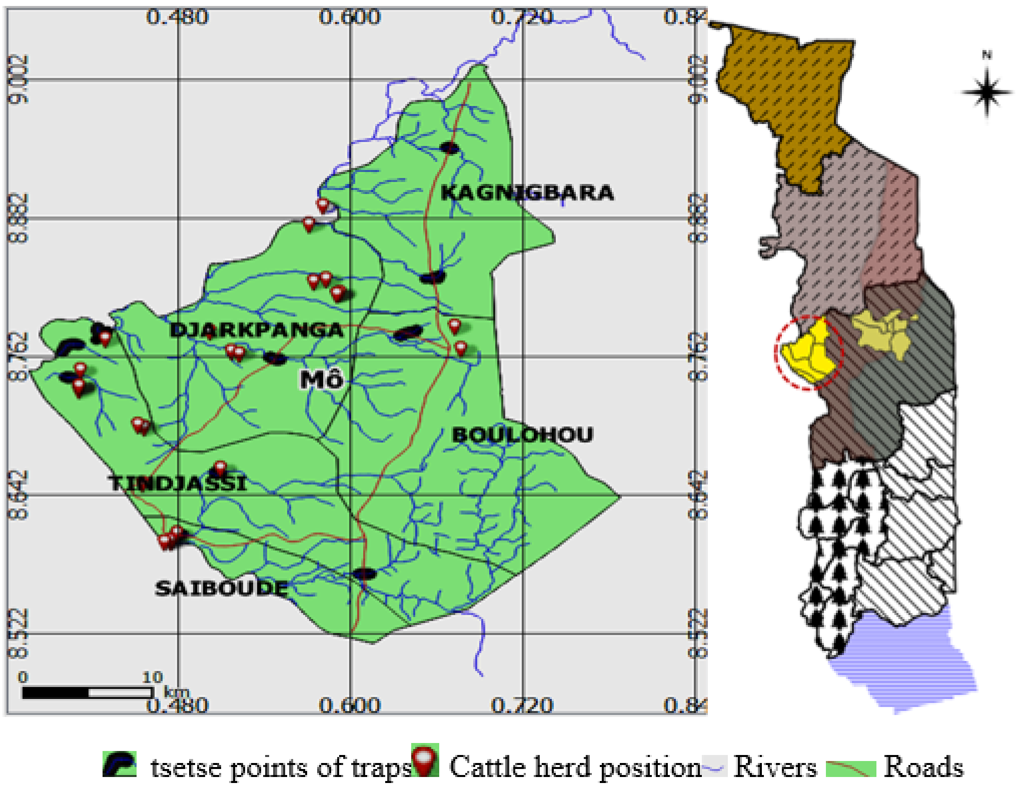


Figure 1

Sites of the entomological survey and cattle herd positions

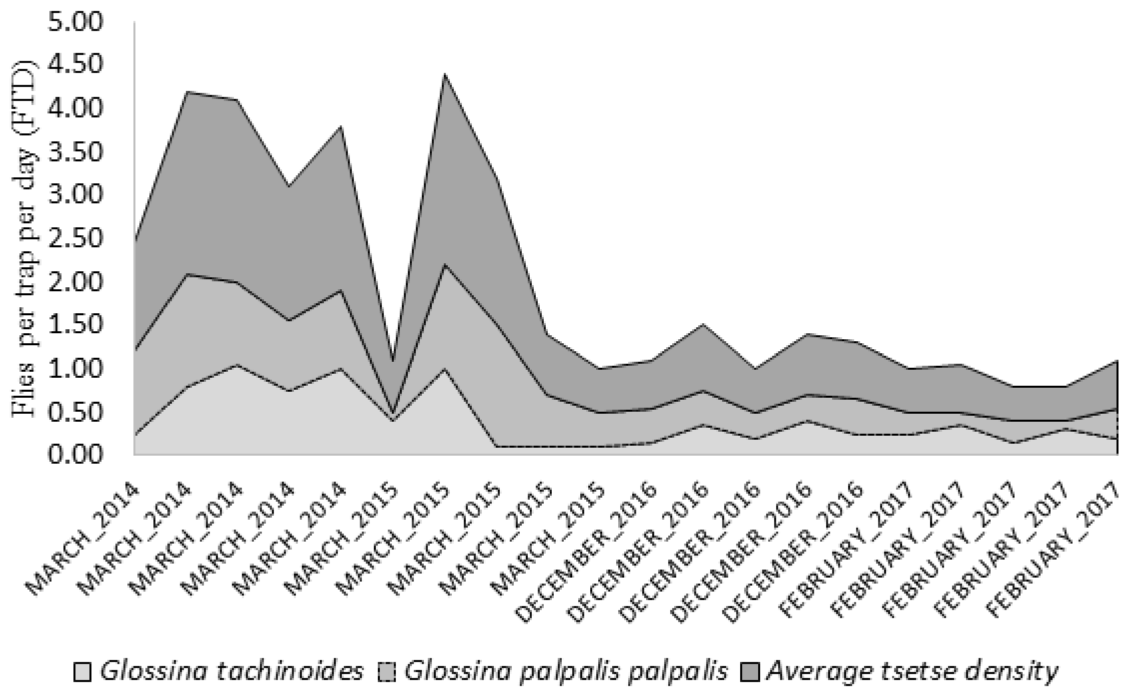


Figure 2

Periodical tsetse density variation in separate species

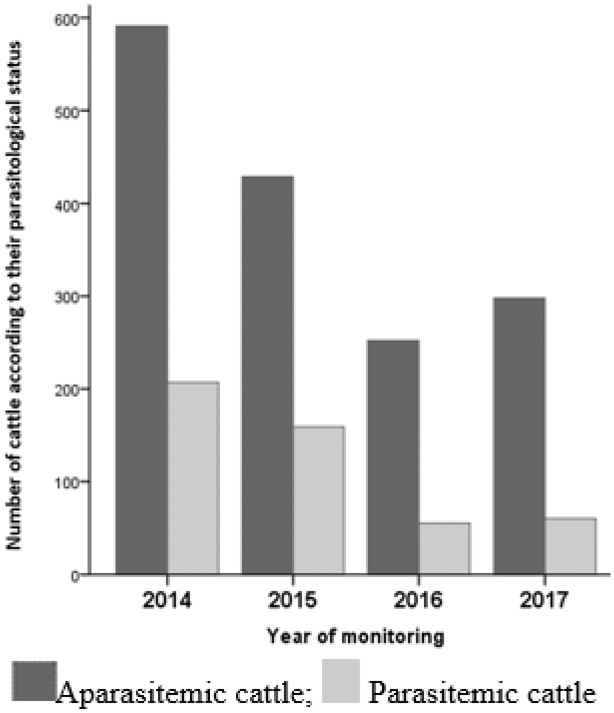
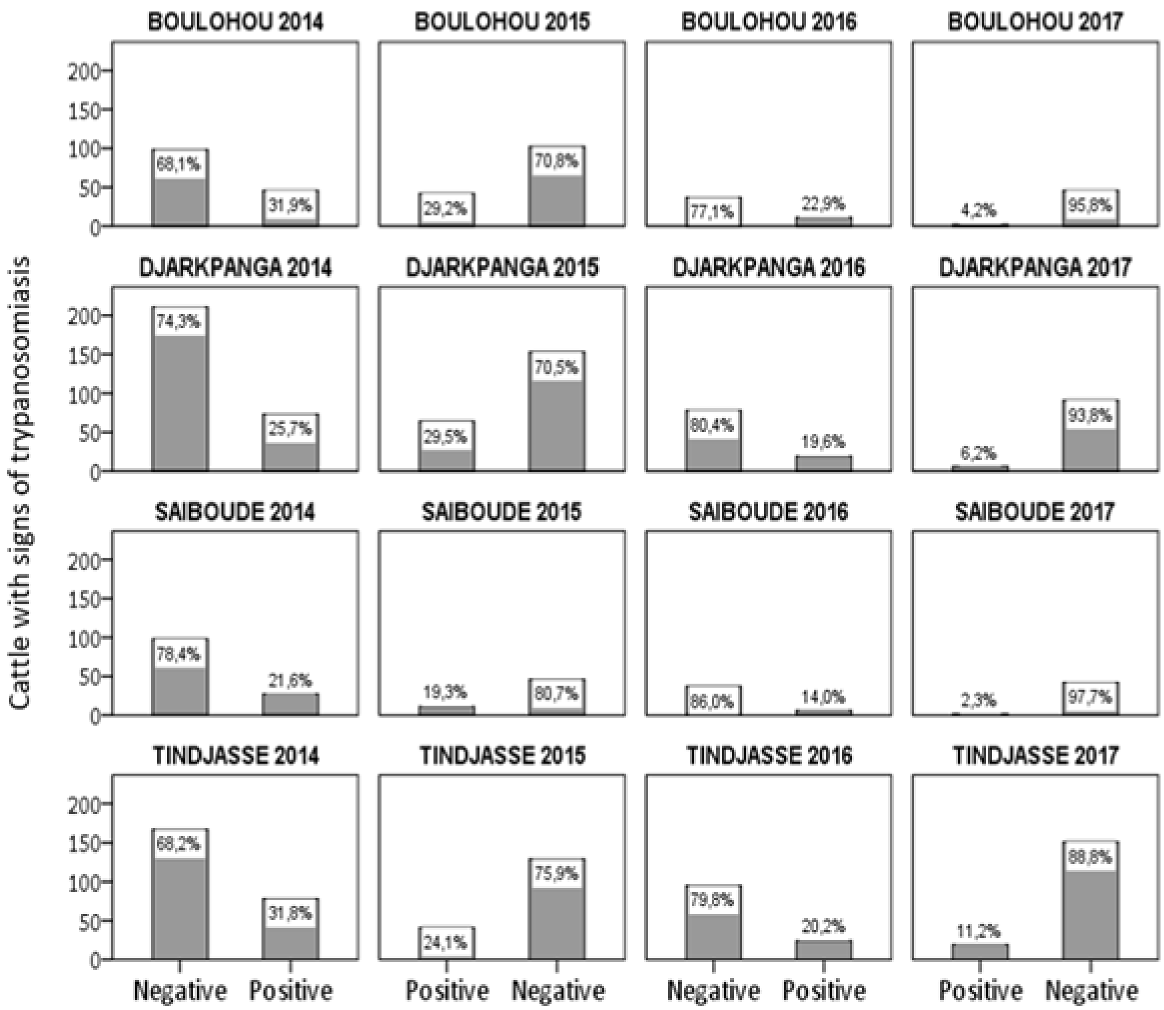


Figure 3

Trypanosome apparent incidence during trypanocide and insecticide intervention at Mô



Positive and negative are the status of cattle infection.

Figure 4

Change in the incidence of AAT in the control villages

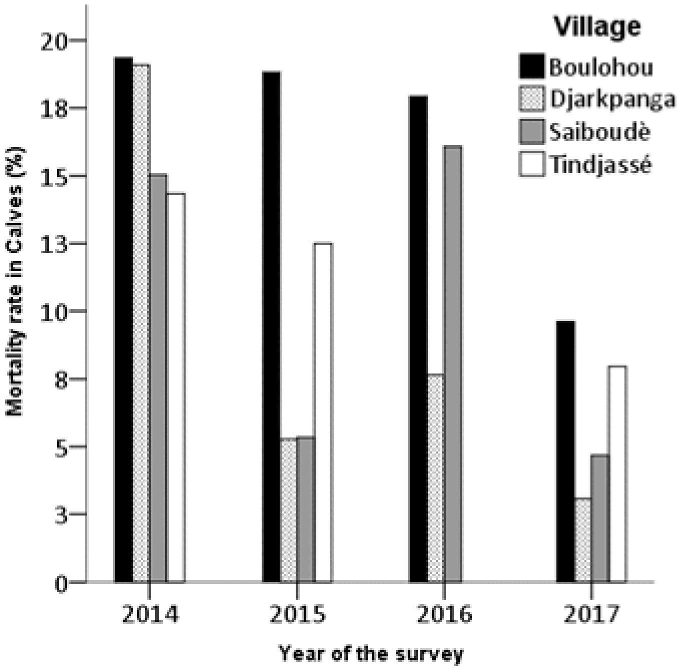


Figure 5

Yearly mortality occurring of calves during the trypanocide and insecticide intervention

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