Journal of Applied Sciences

ISSN 1812-5654

science alert

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OPEN ACCESS Journal of Applied Sciences

ISSN 1812-5654
DOI: 10.3923/jas.2019.690.700

Research Article
Relative Efficacy of Tsetse Traps and Live Cattle in Estimating the Real Abundance of Blood-Sucking Insects

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Abstract

Background and Objective: The rangelands of the Adamawa region of Cameroon were hyper-infested with boophilic hematophagous flies that caused production losses. For this reason, an entomological experiment was conducted to compare the effectiveness of tsetse traps and live cattle in estimating the real abundance of biting insects. **Materials and Methods:** A field experiment was carried out to compare biting fly intensities recorded from tsetse traps (TTs) and Live Cattle (LC) after 14 days of adult zebu Goudali cattle (n = 3: Black, brown and white) and odor-baited (Octenol) blue-black tsetse traps (n = 3: Nzi, Biconical and Vavoua) exposure time (8-20 h) in different micro-environments (gallery forest, river Vina borderline and open savanna grass) from October-November, 2016 and January, 2017 in Galim, Adamawa region of Cameroon. **Results:** In total, 27, 440 hematophagous flies were caught and identified with 26,779 of them observed on cattle and only 661 caught with TTs. Five genera were identified using the two methods in order of magnitude: *Stomoxys*, Culicids, *Simulium*, *Chrysops* and *Tabanus*. Only TTs permitted fly identification up to species level. Amongst all the fly-groups recorded, only the genus *Tabanus* did not show a statistically significant difference with the two exposed-trapping methods. Trap abundance only represented 2.49% of observed biting fly abundances on live animals throughout the study. **Conclusion:** Tsetse traps could show the species composition of some dipterans but were unable to give the real burden of such flies on live cattle.

Key words: Tsetse traps, biting insects, live cattle, haematophagous flies, diptera, bovine, Galim, boophilic hematophagous, biting fly

Citation: Sevidzem Silas Lendzele and Mavoungou Jacques François, 2019. Relative efficacy of tsetse traps and live cattle in estimating the real abundance of blood-sucking insects. J. Applied Sci., 19: 690-700.

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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

The last phase of the tsetse eradication campaign of 1994 in Cameroon consisted of the use of traps and barriers, a role that was carried out by the special mission for the eradication of glossines. Different traps have been designed as vital tools for fly control[1]. The most efficient TTs for mechanical vectors were: Nzi which caught tabanids and *Stomoxys* in large numbers[2] while Vavoua also caught tabanids and *Stomoxys* in large numbers but the Biconical traps were efficient for glossines[3]. In addition, trap-type, color and shape influenced the trapability of vectors[4], reason why traps have been reported to be species-specific. Artificial or natural odorants applied to traps improved their trapability and included octenol, acetone and cow urine[5]. The Esperanza window trap was efficient in the collection of *Simulium* black flies and was suggested to be a possible gear to replace human fly collectors[6] even though human silhouettes instead of live humans were used in such studies.

Live cattle and humans are host for most dipterans and possesses some morphological traits that attract them[7]. One of the key morphological trait was skin color[8]. The cattle breeds of the Adamawa region of Cameroon included Goudali, Bokolodji, Red Fulani, White Fulani and their cross-breeds (metis)[9]. The skin color of the indigenous breed (Goudali) ranged from black, white and brown as well as mixture of colors (red+brown, brown+white, black+white etc.). Movement of cattle also attracted biting flies[10]. The production of CO_2 by live cattle also attracted blood sucking insects[11]. Based on the attractive potential of live cattle for biting insects like glossines, a control option based on the spray of cattle using insecticide was used for tsetse control in infested range lands of Africa like the Adamawa plateau[12]. The use of live bait was an effective vector control barrier in the Adamawa plateau[12], but the method had a pitfall because flies were knocked down after biting and not before such that they were capable of introducing the pathogen[13,14]. Another method was developed to correct the short comings of the insecticide spray-method and this novel approach was pour-on application. The pour-on insecticide or repellant as well as their formulation (insecticide and repellant) application modes was designed to kill flies as well as prevented them from biting the host[15]. Pour-on insecticidal and repellant products are applied to the back flank of the animal and estimated that the product diffuses to all parts of the body. However, due to the influence of environmental factors, the product dried up and did not reach the targeted sites[15]. This method demanded the mastery of the predilection sites of potential biting insects so that the product was applied to the exact biting sites for an economical and effective control. Tsetse traps play a dual function notably for survey and control purposes[1]. They permit identification up to species level. The flies caught by the traps are killed due to stress caused by high temperatures of the Roubaud cage[1]. Tsetse traps gave an idea on the apparent abundance of trapable insects but such estimated values did not represent the real burden of the caught insects. Murchie *et al.*[16] reported that blue-black cloth traps caught mostly female gravid flies than their young nulliparous counterparts. Also, most vectors are more attracted to live host than physical traps[7]. To improve the efficacy of TTs, they most be pitched around areas that animals frequent around breeding sites of vectors, the attracting face must be pitched away from wind and must face the sun so as to reflect light within the U.V range, detected by flies[17]. If the mentioned conditions are not respected, TTs will not function well. However, observing and counting flies directly on cattle gave the real abundance of biting insects[18]. An observational study of biting vectors on cattle for instance gave the real injury threshold of an animal caused by biting vectors[18]. Despite the existing knowledge of tsetse traps and use of animals for vector control, there was no report on the use of live cattle to validate the estimated apparent abundance of vectors by tsetse traps.

Tsetse traps due to their blue-black color material, attracted other day time dipterans even though their shape did not permit an effective trapping of other non-target groups. The blue-black cloth traps reflected light within the UV-range and equally mimicked the natural forest edges where most dipterans rested and digested their blood[17]. Lamberton *et al.*[19] revealed that human-fly collectors were most efficient in *Simulium* fly's collection than physical traps. The CDC trap was designed for the collection of adult culicids[20] and the application of chemical kairomone odorants on such traps suggested that they could be as effective as vertebrate animals in the collection of culicids. Information on the comparison of *Simulium* and culicids collection by human and trap collectors existed in literature but no information existed on the comparison of LC and TT fly-collectors for *Stomoxys*, *Chrysops* and *Tabanus* as well as culicids and *Simulium* that were important disease vectors of cattle in Galim. So in this study an entomological experiment was designed to compare the effectiveness of tsetse traps and live cattle in estimating the real abundance of biting insects.

MATERIALS AND METHODS

Study area: Galim where the experiment took place was a pasture area, located some 25 km south of Ngaoundere town along the Ngaoundere-Yaounde high way and 1.5 km away from the Ngaoundere modern abattoir. Cattle exposure sites were in an experimental DFG-COBE cattle paddock with 50 zebu goudali cattle breed and geographically situated within the following geographical coordinates: N 07°11, 887' and E 013°34, 919' as well as elevated at an altitude between 978-998 m a.s.l. There was no history of insecticide usage in this herd. The vegetation-type consisted of short and tall savanna grasses and a gallery forest (Fig. 1). The hydrographic network consisted of river Vina that was flowing towards the southern part of the country and emptied in river Sanaga. The study area consisted of a typical Soudano-Guinean climate and weather parameters during the study indicated that temperatures oscillated between 18.7-32°C, humidity (44.5-93.5%), air pressure (895.8-9023 hpa) and rainfall (7.5-132.8 mm).

Experimental design for fly collection: Three adult zebu Goudali cattle breed were used for the experiment i.e., animal type 1 (red color), animal type 2 (white color) and animal type 3 (black color) with color coverage of animals maintained at 80%. The ages of the animals were between 2 and 6 years with three sets of different cattle used throughout the exposure time. Three odor-baited (Octenol) blue-black tsetse traps [Nzi (L175×H90 cm, 100% polyester-100% polyethylene), Vavoua (W82×D 80×H 75, 100% polyester-100% polyethylene) and Biconical (W90×H120, 100% polyester)] (Vestergaard Frandsen Group S. A) (Fig. 2) were pitched in the same micro-environments that the animals were exposed and rotation of exposed cattle and traps was made daily following a 6×3 Latin square with rotation experimental design. The rotation was made across three micro-environments (corral, river border and gallery forest). The distance between experimental cattle block and traps was 10 m (Fig. 2). Biting fly observations as well as trapping was carried out from morning (8 h) till night (20 h) from October-November, 2016 and January, 2017 in Galim,

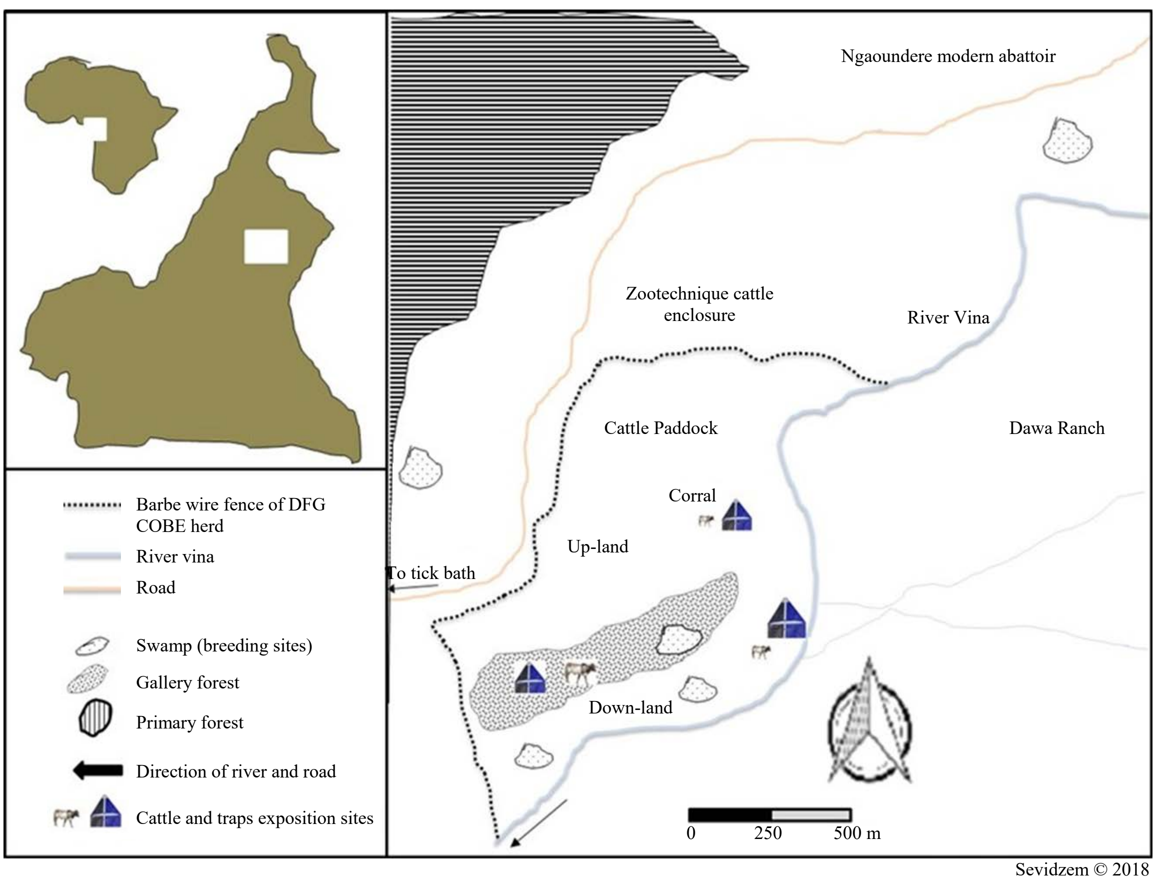

Fig. 1: Map of study area showing cattle and trap exposition sites
Source: Sevidzem *et al.*[3]

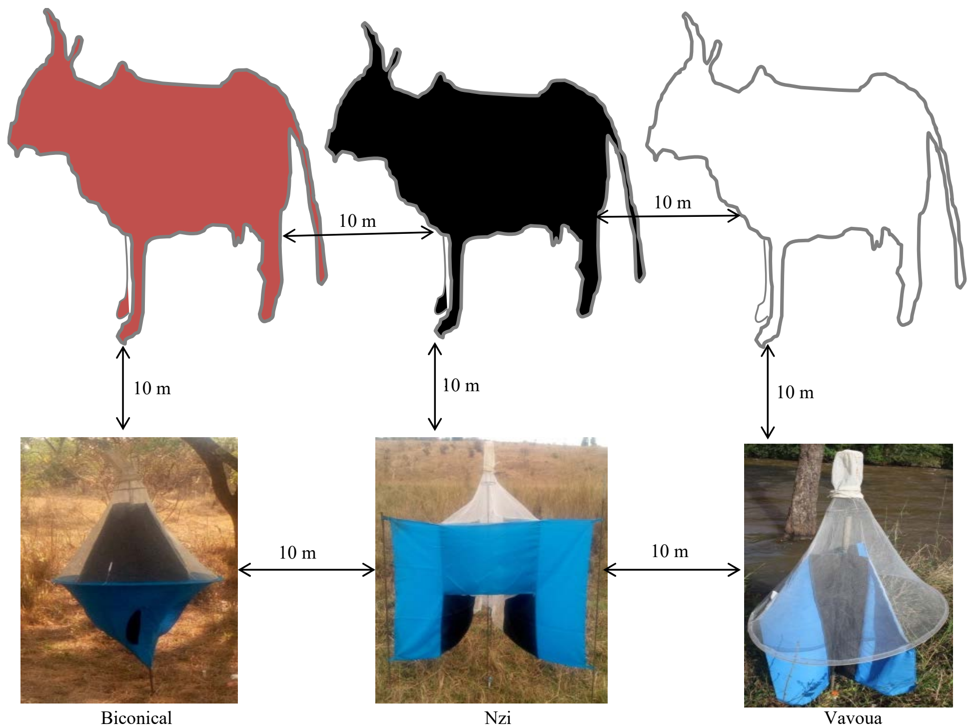

Fig. 2: Experimental design for comparing live cattle and tsetse trap catches
Source: Sevidzem *et al.*[3]

Adamawa region of Cameroon. Animals were restrained on fixed wood poles with ropes and kept at equi-distances[21] of 10 m and such spacing was same for traps. Three observers were used for this experiment and each was as close as 50 cm to the animal to identify and count the flies per lateral side of the animal. After sundown observation was realized with torches. The ambient temperature, humidity and air pressure of the micro-environments were measured using a portable weather tracker (Krestel® 4500, USA).

Fly identification: Observers were trained to identify *Tabanus* and *Chrysops* using the published taxonomic keys of Odroyd[22-24]. *Stomoxys* spp. were identified using the identification key of Zumpt[25]. For culicids, characteristic identification key for Anopheline species[26] and Culicinae[27] were used. Simuliidae were identified using the key of Freeman and De Meillon[28]. Fly identification was carried out at the Programme Onchocercoses field station laboratory of the University of Tübingen in Ngaoundere, Cameroon.

Data analysis: Data was analyzed using the R-statistical software. Fly numbers from the two methods were compared using the Kruskal Wallis rank sum test. The mean abundances from the different methods for each fly-group were compared using the student t-test. All statistical tests were kept at $p<0.05$ significant level. Abundance and attractivity of the collection methods were calculated:

$$\text{Tsetse traps fly abundance} = \frac{\text{No. of flies of each group}}{\text{No. of traps} \times \text{trapping days}}$$

$$\text{Live cattle fly abundance} = \frac{\text{No. of flies of each group}}{\text{No. of exposed cattle} \times \text{exposure days}}$$

$$\begin{array}{c}\text{Relative attractivity rate}\\ \text{of collection methods}\end{array} = \frac{\text{Total caught}}{\text{Total caught by the trapping tools}} \times 100$$

RESULTS

The overall fly number recorded by the two methods was 27,440 with cattle counts of 26,779 (97.59%) and only 661 (2.41%) trapped with tsetse traps (Fig. 3a). This showed the low attractivity of tsetse-baited traps as compared to live cattle baits. The 2 methods led to the identification of 5 genera of boophilic blood sucking flies of medical and veterinary importance in order of magnitude: *Stomoxys*, Culicids (*Anopheles* and *Culex*), *Simulium*, *Chrysops* and *Tabanus* (Fig. 3b-f). Tsetse traps enabled the collection of flies for identification up to species level. For the genus

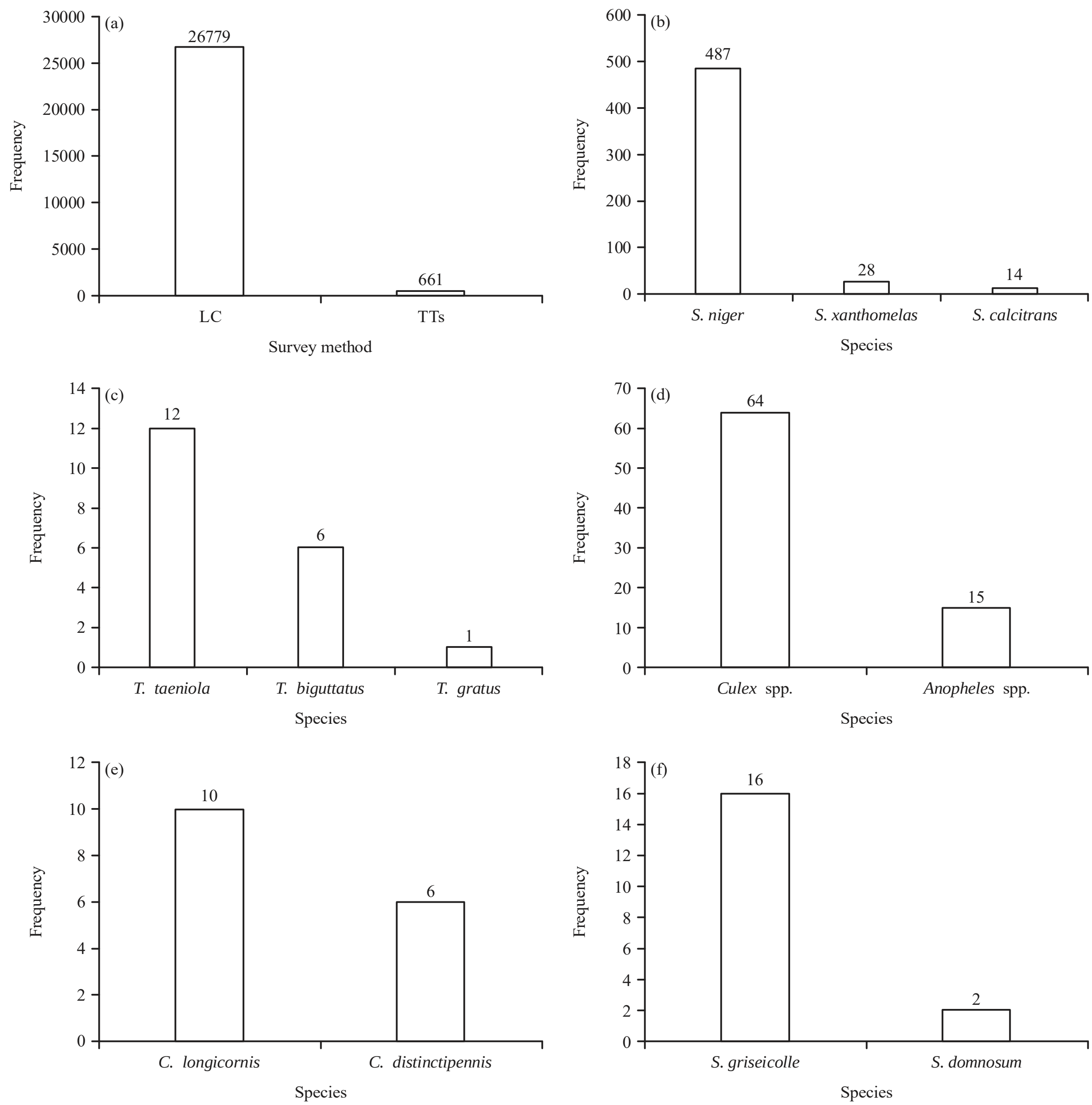

Fig. 3(a-f): Biting fly counts based on entomological prospection methods and species, (a) Number of biting fly-counts with respect to survey method, (b) Fly-count with respect to *Stomoxys* spp., (c) Fly-count with respect to *Tabanus* spp., (d) Fly-count with respect to genus of culicids, (e) Fly-count with respect to *Chrysops* spp. and (f) Fly-count with respect to *Simulium* spp.

Stomoxys, 3 species were identified notably *S. niger* (487), *S. xanthomelas* (28) and *S. calcitrans* (14) (Fig. 3b). For the genus *Tabanus*, 3 species were identified notably *T. taeniola* (12), *T. biguttatus* (6) and *T. gratus* (1) (Fig. 3c). For culicids, 2 genera were identified notably *Anopheles* spp. (15) and *Culex* spp. (64) (Fig. 3d). For *Chrysops*, 2 species were identified notably *C. longicornis* (10) and *C. distinctipennis* (6) (Fig. 3e). For *Simulium*, 2 species were identified notably *S. griseicolle* (16) and *S. damnosum* (2) (Fig. 3f).

Number of *Stomoxys* spp. caught with respect to tsetse trap types: Based on the trapability of *Stomoxys* with the various tsetse-traps, it was realized that *S. niger*, *S. xanthomelas* and *Stomoxys* spp. were most frequently caught using the Vavoua trap than with the other TTs with a statistically significant difference ($p<0.05$) (Fig. 4a, b, d). However, *S. calcitrans* was slightly highly caught by Nzi as compared to other TTs with no statistically significantly difference ($p>0.05$) (Fig. 4c).

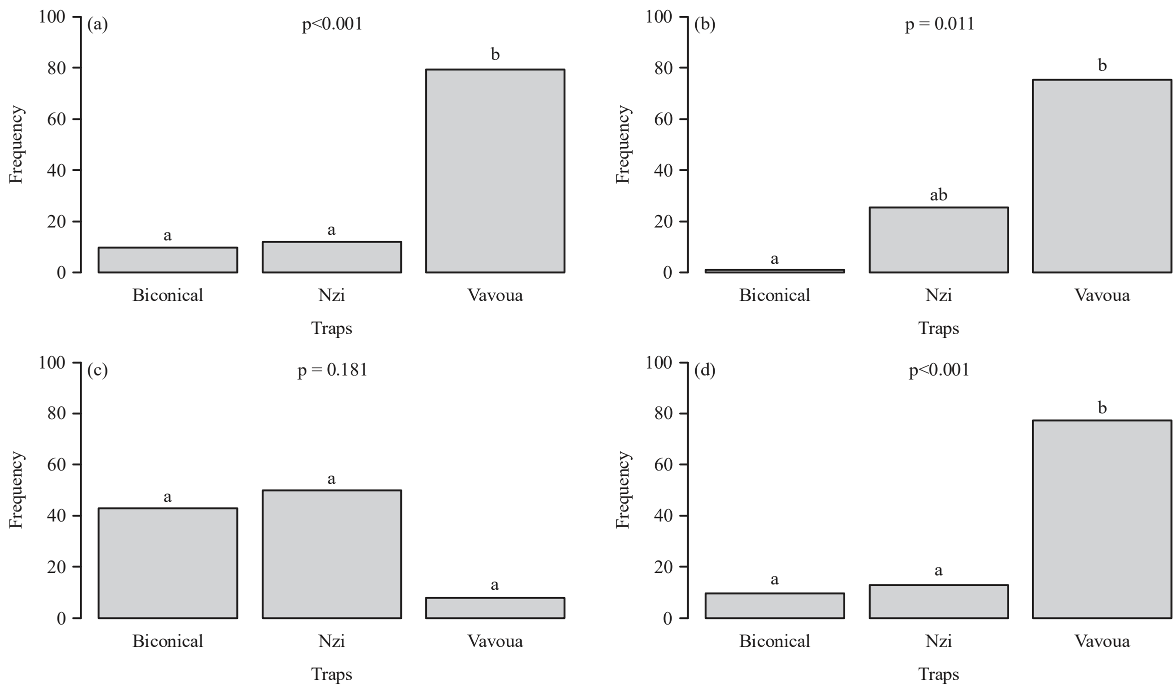

Fig. 4(a-d): *Stomoxys* spp. frequencies based on tsetse trap-types, (a) *S. niger*, (b) *S. xanthomelas*, (c) *S. calcitrans* and (d) General frequency graph for *Stomoxys* spp.

Number of culicids caught with respect to tsetse trap types: *Anopheles* spp., *Culex* spp. and culicids in general were frequently caught using the Nzi trap as compared to other TTs even though there was no statistical difference ($p > 0.05$) (Fig. 5a-c).

Number of *Simulium* spp. caught with respect to tsetse trap types: The *S. damnosum* was highly trapped with Vavoua as compared to other traps with no statistically significant difference ($p > 0.05$) (Fig. 6a). However, *S. griseicolle* and the genus *Simulium* were highly trapped with the Vavoua trap with a statistically significant difference ($p < 0.05$) (Fig. 6b-c).

Number of Tabanidae caught with respect to tsetse trap types: For tabanids, species of the genus *Tabanus* like *T. taeniola*, *T. biguttatus* and *T. gratus* were highly caught with the Nzi trap than with other TTs with no statistically significant difference ($p > 0.05$) (Fig. 7a-c). However, *C. distinctipennis*, *C. longicornis* and tabanids in general were highly caught using the Nzi traps as compared to other TTs with a statistically significant difference ($p < 0.05$) (Fig. 7d-f).

Table 1: Number, attractivity rate and abundance of the different fly-groups based on trial methods

Methods	*Stomoxys*	*Tabanus*	*Chrysops*	Culicids	*Simulium*
Tsetse traps					
Number	529	19	16	79	18
RARCM (%)	2.94	35.85	17.78	0.88	5.79
F/T/D	12.6	0.5	0.4	1.9	0.4
Live cattle					
Number	17453	34	74	8925	293
RARCM (%)	97.06	64.15	82.22	99.12	94.21
F/T/D	410.3	0.8	1.8	212.5	6.7

F/T/D: Number for each fly-group per entomological method and exposure days
RARCM: Relative attractivity rate of collection methods

Abundance with respect to fly collection methods: The calculated abundance was survey-method and fly-group dependent with *Stomoxys* being the most abundant fly-group (Table 1). Based on the relative attractivity rate (RARCM) of the collection methods, all the blood-sucking insects were most attractive to live cattle (with RARCM >60%) as compared to TTs (Table 1).

Mean catches based on the trapping method: Based on mean blood-sucking insect groups count with respect to the different methods of vector study, it occurred that *Stomoxys* and culicids mean counts were higher with the LC than with TTs with a statistically significant difference

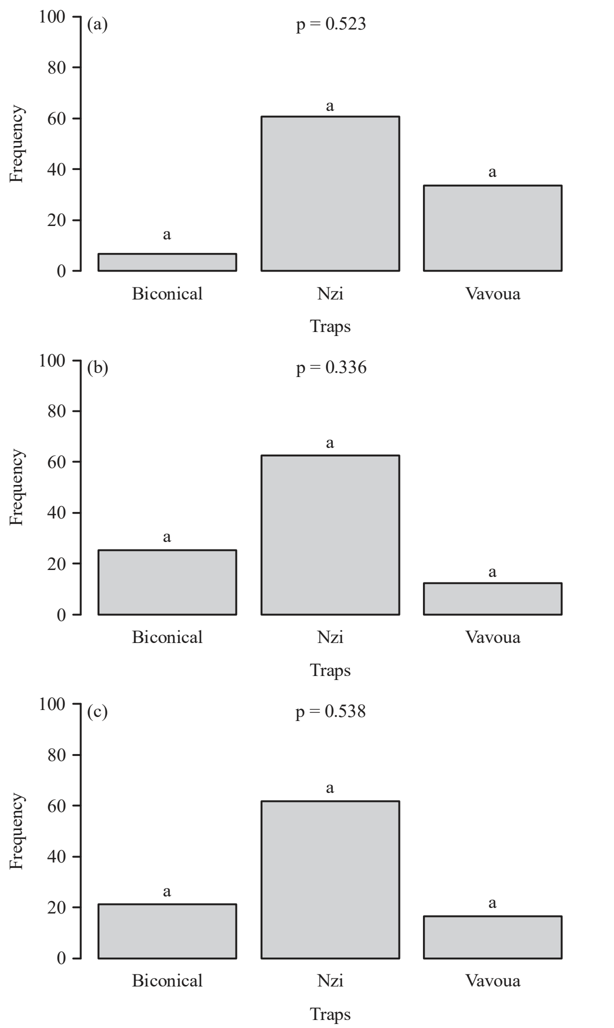

Fig. 5(a-c): Culicids frequencies based on tsetse-trap types, (a) *Anopheles* spp., (b) *Culex* spp. and (c) General frequency graph for culicids

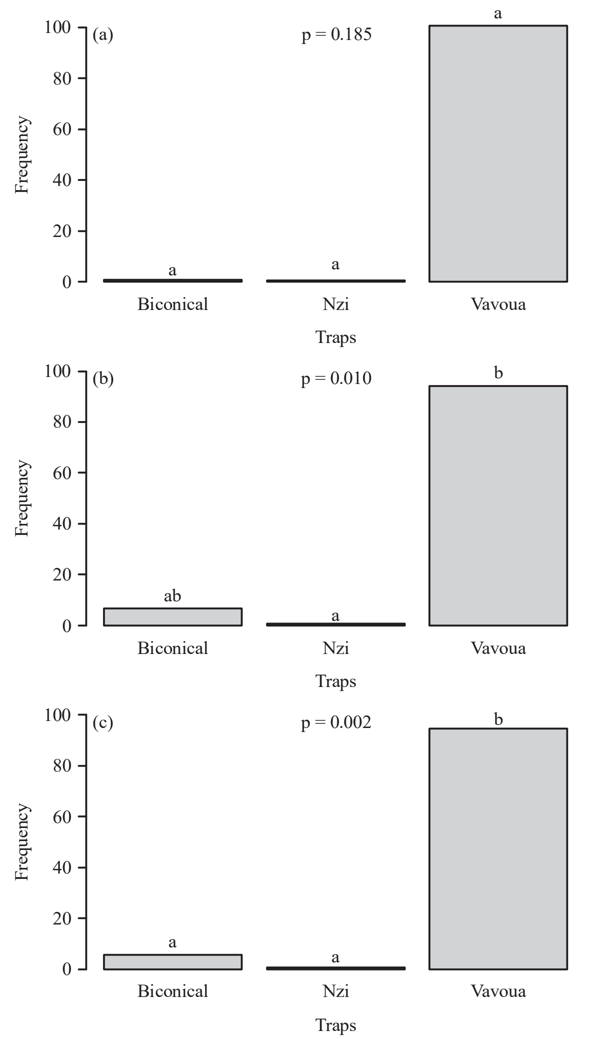

Fig. 6(a-c): *Simulium* spp. based on tsetse-trap types, (a) *S. damnosum*, (b) *S. griseicolle* and (c) General frequency graph for *Simulium*

($p<0.05$) (Fig. 8a). The mean counts of *Simulium* and *Chrysops* were higher with LC than with TTs with a statistically significant difference ($p<0.05$) (Fig. 8b) but the mean counts of *Tabanus* was slightly higher with LC than with TTs with no statistically significant difference ($p>0.005$) (Fig. 8b).

DISCUSSION

The present study revealed the high occurrence and biting intensity of the genus *Stomoxys* as compared to other groups. This finding was like that of Llyod and Dipeolu[29] and Mihok and Clausen[30] who found out that muscids especially *Stomoxys* were most abundant in their collection. This might be linked to the favorable environmental conditions for this group, availability of breeding sites and the presence of their most preferred cattle vertebrate host during the study as well as the efficiency of TTs in their capture as compared to other fly-groups whose trap ability with those gears was least. For culicid vectors, the present results could not claim their real intensity with the 2 methods because traps and cattle were exposed from 8-20 h but culicids activity started at 18 h, so the present trial for this group was partial and was not fully conclusive as compared to a fully diurnal group like *Stomoxys*. According to Muenworn *et al.*[31], the peak abundance of culicids occurred between 18-23 h. From the sensitivity of the various fly-groups to the different traps, it was deduced that the Vavoua trap was very sensitive to *Stomoxys* spp. and *Simulium* spp. The Nzi trap was sensitive to *Tabanus* spp., *Chrysops* spp., *Anopheles* spp. and *Culex* spp. But the

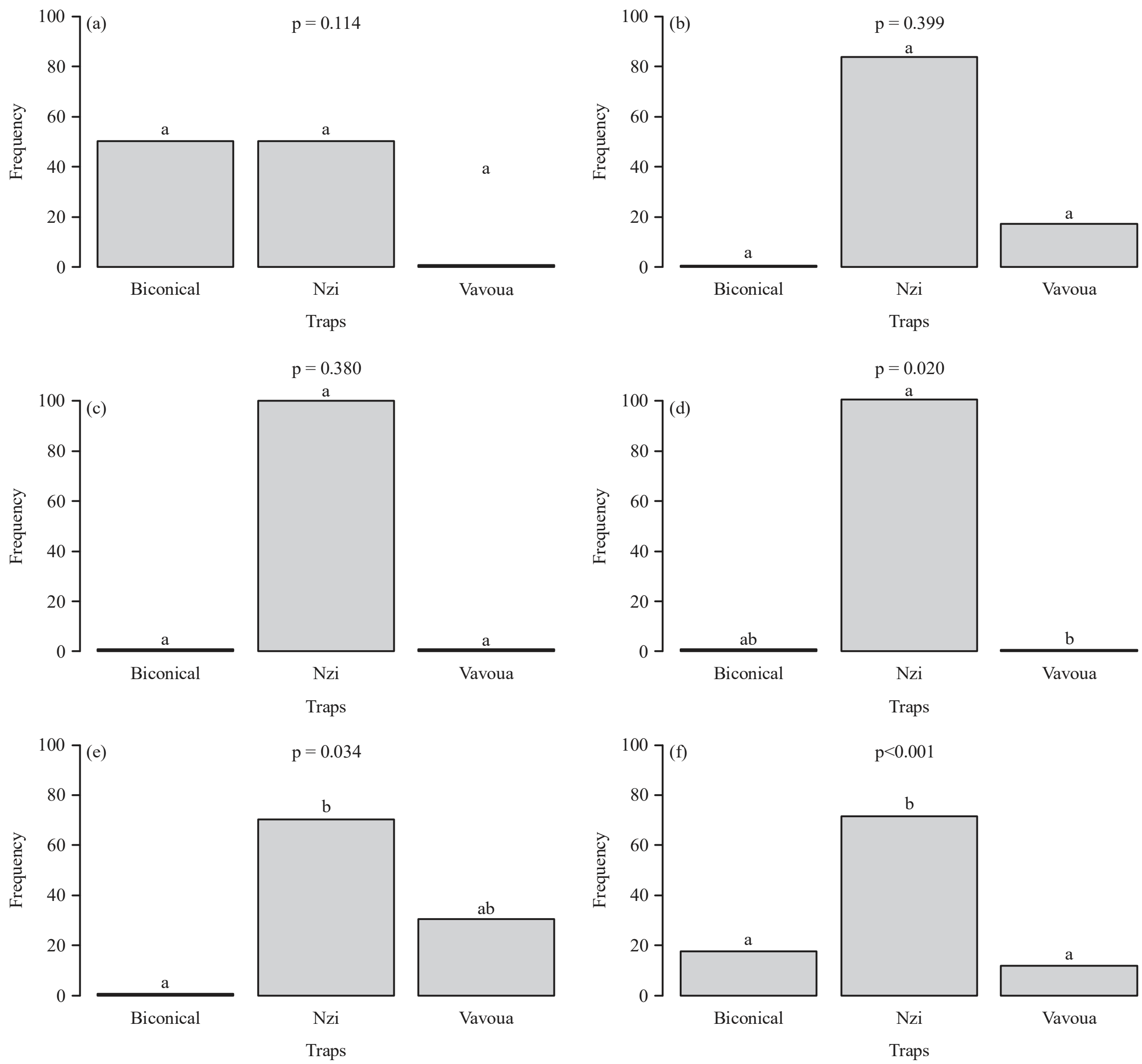

Fig. 7(a-f): Tabanid frequencies with respect to the different tsetse-trap types, (a) *T. taeniola*, (b) *T. biguttatus*, (c) *T. gratus*, (d) *C. distinctipennis*, (e) *C. longicornis* and (f) General frequency graph for tabanids

Biconical trap was weak in collecting all the five-biting fly-groups identified in this study. This finding on the specificity of trap-types with respect to the various fly-groups as well as the scanty catches with the Biconical trap as compared to Vavoua was also reported by Sevidzem *et al.*[3]. The collection of some hematophagous vector groups like *Stomoxys*, tabanids and *Simulium* by TTs was not surprising because Eteme *et al.*[32] caught them in their prospection in east Cameroon. The entomological prospection of Lehane[8] revealed that most dipterans were attracted to blue, black and white surfaces. The weak collections of TTs in relation to fly-groups like culicids and *Simulium* was because these traps were designed to target tsetse flies (which were apparently absent in study area) and later modified to get other mechanical vectors like *Stomoxys* and tabanids[33]. Direct animal skin observation to know the actual fly burden was laborious but gave a real image of the situation. This hectic experience was opposite with TTs which were easy to manipulate. For the purpose of fly-species screening in ecological surveys, TTs were highly recommended[3]. In the case of live bait technology, the use of LC to know the intensity of biting flies before the spray of insecticides and their mixtures on live cattle was deemed essential[15]. The stated recommendation for live bait technology was based on the present finding that biting insects were more attractive to LC than TTs. The percentage attractivity of the different fly-groups to LC was 97.59% as compared to 2.41% to TTs. This showed how weak TTs gave a virtual impression of the

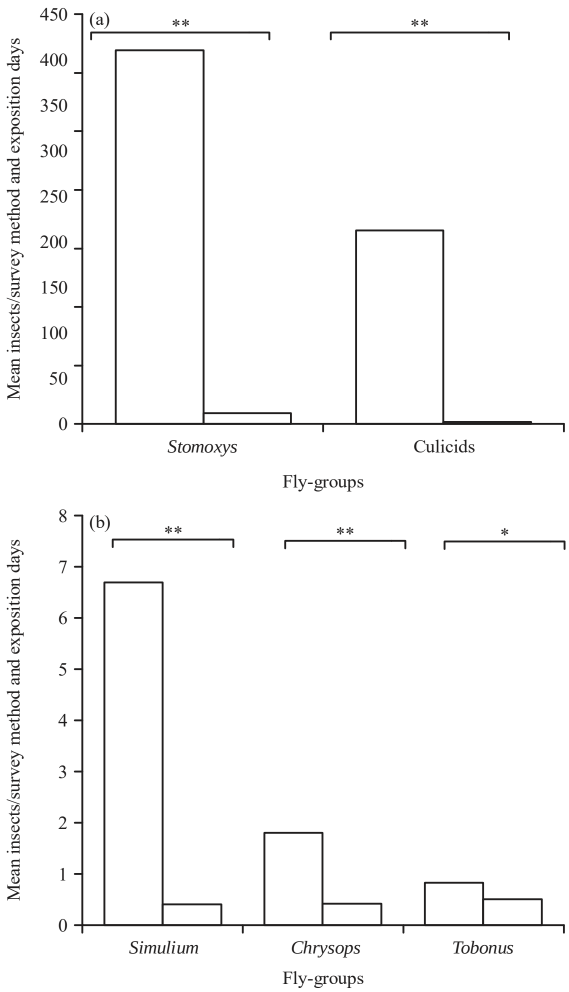

Fig. 8(a-b): Comparison of the abundance of each fly-group with the two methods, (a) Mean *Stomoxys* and culicids per method and exposure days and (b) Mean *Simulium*, *Chrysops* and *Tabanus* per method and exposure days

**Statistically significant difference at p<0.05 level, *No statistically significant difference p>0.005

biting intensity of the identified fly-groups on cattle. This finding was like that of Hendy *et al.*[7] who reported that *Simulium* was highly caught with human fly catcher as compared to physical traps. The attractivity index of *Tabanus* in terms of their abundance to the blue-black TTs and LC was not statistically significant. This finding was like the finding of Mihok[33] that tabanids and other dipterans were attractive to blue-black cloth Nzi trap. This showed that high odor-baited Zero Fly® tsetse trap densities could replace LC baits in the control of *Tabanus* populations in given areas and not the other fly-groups. The incorporation of the tsetse fly trap ideas in the construction of Zero Fly® screens proved to be effective in the control of tsetse flies and other biting flies in Tanzania[34]. However, the use of live cattle as baits in the control of ectoparasites like tsetse flies and ticks was very efficient in the Adamawa region of Cameroon[12,18]. It was realized that knowing the real fly biting intensities on cattle was a prerequisite for the application of live baits (i.e., use of insecticide treated cattle for fly management) and this cannot be deduced from trap apparent abundances as noticed from the results obtained in the present prospection on most of the biting fly-groups.

CONCLUSION

From the present study, the Nzi trap was sensitive to tabanids and culicids while the Vavoua was very sensitive to *Simulium* blackflies and *Stomoxys* but the Biconical trap did not show any striking sensitivity to all the five fly-groups identified. Although the use of LC as a tool to determine the real biting fly intensities in animal farms is very cumbersome, it is still useful if live bait is a control option for most fly-groups but such real intensities for *Tabanus* can simply be gotten from odor-baited TTs. This study recommended the validation of TTs results using LC observation before applying insecticides on cattle in Galim.

SIGNIFICANCE STATEMENT

This study focuses on determining the relative efficacy of live cattle baits and tsetse traps in estimating the biting intensities of boophilic dipterans in a fly hyper-infested rangelands of the Adamawa plateau of Cameroon. This is to optimize the use of live cattle in the mobile live bait technology as an alternative to physical traps in determining the real fly burden as well as in control. The present study will better inform decision makers on the best approach to evaluate fly apparent abundance before implementing an antivectorial fight program in the region.

ACKNOWLEDGMENTS

Authors are grateful to the DFG-COBE project and the MSEG for sponsoring part of this work. We also thank the herdsmen for their assistance throughout the study.

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