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Frank d'Amico, Jp Gouteux, Florence Le Gall, D Cuisance. Are stable flies (Diptera: Stomoxyinae) vectors of *Trypanosoma vivax* in the Central African Republic?. *Veterinary Research*, 1996, 27 (2), pp.161-170. hal-00902409

HAL Id: hal-00902409

<https://hal.science/hal-00902409>

Submitted on 11 May 2020

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Are stable flies (Diptera: Stomoxyinae) vectors of *Trypanosoma vivax* in the Central African Republic?

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(Received 7 April 1995; accepted 21 November 1995)

Summary — The epidemiology of *Trypanosoma vivax* infections was studied at a riverside site in the Ouro-Djafoun livestock area situated in the Central African Republic during the period between July 1991 and July 1992. This paper examines the possibility that stable flies (Diptera: Stomoxyinae) were also vectors of this trypanosome species in a non-cyclic way. Previous studies have revealed that the usual cyclic transmission by the tsetse fly *Glossina fuscipes fuscipes* was probably not the only transmission route. At the study site, at least five species or subspecies of stable flies were encountered: *Stomoxys nigra nigra* (approximately 60% of the sample), *S taeniata*, *S sitiens*, *S omega omega* and *Haematobia* spp. The hypothesis that stable flies could be good vectors of *T vivax* in this country is supported by three main observations: i) stable flies were very abundant at the cattle resting site; ii) an estimation of the 'contact index' between the cattle and stable flies demonstrated close interactions between cattle and stable flies at this site, particularly during the rainy season, and iii) there was a good correlation ($P < 0.05$) between the apparent stable fly densities at the resting site and the frequency of *T vivax* in the cattle. The relevance of this phenomenon in terms of epidemiology and combatting *T vivax*-caused nagana is discussed.

***Trypanosoma vivax* / stable fly (Diptera: Stomoxyinae) / non-cyclical transmission / nagana epidemiology / Central African Republic**

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Résumé — Les stomoxes (*Diptera* : *Stomoxiinae*) sont-ils vecteurs de *Trypanosoma vivax* en République centrafricaine ? L'épidémiologie des trypanosomoses à *T vivax* a été étudiée le long d'une galerie forestière dans la zone d'élevage d'Ouro-Djafoun (République centrafricaine) entre juillet 1991 et juillet 1992. Des recherches antérieures indiquent que la transmission cyclique de ce trypanosome par la glossine *Glossina fuscipes fuscipes* n'est probablement pas la seule voie. Ainsi, le présent travail traite de la possibilité pour les stomoxes (*Diptera* : *Stomoxiinae*) d'être des vecteurs de *T vivax* par voie mécanique. Sur les sites étudiés, au moins cinq espèces ou sous-espèces de stomoxes ont été recensées : *Stomoxys nigra nigra* (approximativement 60 % de l'échantillon), *S taeniata*, *S sitiens*, *S omega omega* et *Haematobia spp.* L'hypothèse selon laquelle les stomoxes pourraient être de bons vecteurs de *T vivax* dans cette région est supportée par trois faits majeurs : i) les stomoxes étaient très abondants au niveau de l'aire de repos du bétail, ii) l'utilisation d'un «index de contact» entre le bétail et les stomoxes a permis d'établir l'existence d'interactions étroites entre ces animaux à l'aire de repos, en particulier en saison des pluies, iii) il existait une bonne corrélation ($p < 0,05$) entre les densités apparentes des stomoxes à l'aire de repos du bétail et les fréquences de *T vivax* chez les zébus. Les auteurs discutent finalement de l'importance de ce phénomène en termes d'épidémiologie et de lutte contre les trypanosomoses à *T vivax*.

***Trypanosoma vivax* / stomoxe (*Diptera* : *Stomoxiinae*) / transmission non-cyclique / épidémiologie du nagana / République centrafricaine**

INTRODUCTION

The Central African Republic (CAR) is a country that does not have a breeding tradition for cattle. Mbororo cattle arrived there around the 1920s (Boutrais, 1988). By 1928, the cattle number was estimated at 3 500 (Bertucat, 1965; Crouail, 1969). At present, there are two million head of cattle (99% of which are trypano-susceptible Mbororo zebu). Due to the presence of tsetse flies and ticks, the humid savannahs of the CAR remain somewhat unsuitable for livestock production. As Mbororo zebu cannot be kept without chemoprophylaxis, trypanosomosis is a major threat to productivity. We therefore undertook studies of the epidemiology of bovine trypanosomosis in the CAR. Previous studies have emphasized the role of *Glossina fusca congolensis* (Yvoré et al, 1965a, b) and *G fuscipes fuscipes* (Finelle, 1957; Cuisance et al, 1992). In the centre of the CAR, where *G fuscipes* is dominant, we found that this species appeared to be a poor vector of *Trypanosoma* species in cattle as no relationship was observed between the Berenil index (for definition, see Rogers, 1985, and

Claxton et al, 1989), trypanosome infections and challenges at three study sites (D'Amico, 1993). Moreover, a recent survey on the feeding behavior of this tsetse fly species demonstrated that the number of bloodmeals from cattle was rather low (12% on average) (Gouteux et al, 1994). Thus, we postulated that others vectors might play an important role in the transmission of trypanosomosis to the cattle, in particular *Trypanosoma vivax*.

The purpose of the present paper was to investigate stable flies as potential vectors of these trypanosomes.

MATERIALS AND METHODS

Area of study

The investigation was carried out in the centre of the CAR, in the Ouro-Djafoun breeding zone of the Ouaka district. A total of 19 Mbororo zebu cattle herds were studied (Le Gall et al, 1995). One of these, because of its epidemiological interest and numerous other facilities, was specifically chosen for this study. The study area lay 60 km east of the village of Bambari on the South

bank of the Mbonou river. In this zone, as elsewhere in the country, the climate is characterized by a short dry season from December to March and a longer rainy season from April to November (Franquin et al, 1988). The average rainfall recorded at Bambari from 1950 to 1981 was 1 500 mm per year. The landscape consists of wet savannahs with a large gallery forest network. The main tree species are *Burkea africana*, *Lophira lanceolata* and *Daniella olivieri*. The grasslands are characterized by the development of *Andropogon gayanus*, *Hyparrhenia welwitschii* and *H familiaris* (Boulvert, 1986).

Survey of stable flies

The apparent densities of stable flies were determined from catches in bipyramidal traps (Gouteux, 1991) and were expressed as the number of flies caught per trap per day. Specific identification of the flies was made according to Zumpt (1973). Their distribution was monitored along a 500 m transect using 11 traps set at regular intervals. The transect followed the usual path used by the herd daily to reach the drinking site and savannah grazing areas (fig 1). The flies were sampled for 9–12 days per month from July 1991 to July 1992, with the exception of January 1992.

Cattle surveys

The movements of the cattle were monitored in order to establish how their grazing range related to the distribution of the stable flies. This was achieved by observing the daily activity patterns for 5–7 days in the dry season (February), interseason (May) and rainy season (August 1992). The times of departure for the grazing and drinking site and the times of return from the savannah were noted.

The presence of trypanosomes in the blood of the cattle was determined by parasitological and serological techniques. Blood samples were collected from the cattle in October 1991 and February, June and August 1992. The parasitological techniques used were haematocrit centrifugation and examination of the buffy coat or direct microscopic examination of the thin and thick blood films stained with Giemsa (Molyneux, 1975; Murray et al, 1977). The serological technique used was antigen detection using monoclonal antibodies (ELISA), as described by Nantulya and Lindqvist (1989) and Nantulya et al (1992). Each animal that was found to be positive by buffy coat examination was immediately treated with Berenil®, at a dose of 3.5 mg/kg body weight. The frequency was assessed from parasitological and serological data and was calculated as the number of animals tested positive in the herd at one time.

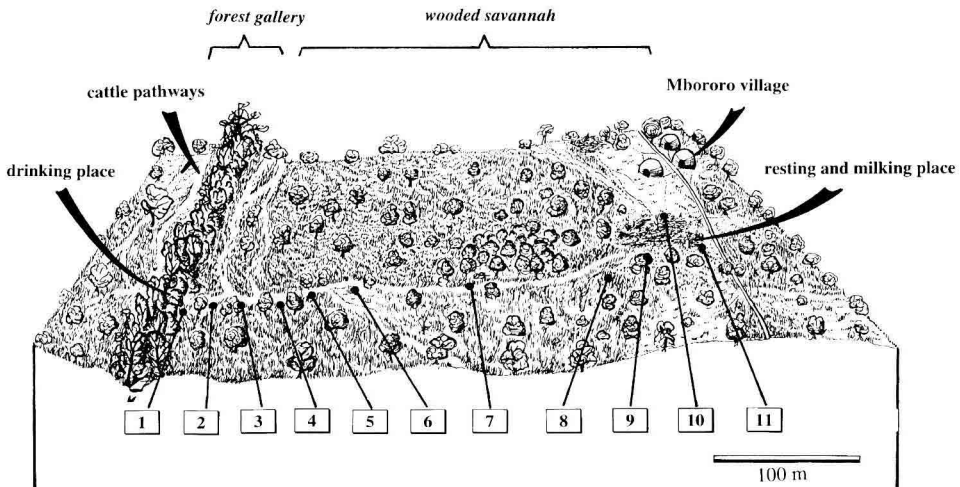


Fig 1. Location of the 11 bipyramidal traps set along the main cattle pathway connecting the resting and milking site (close to Mbororo village) to the drinking site (river under forest gallery). Grazing places are scattered throughout the wooded savannahs.

Data analysis

The data were analysed: i) in order to estimate the intensity of contact (K) between stable flies and cattle at the two points of presumed interaction, ie, the drinking site and the resting and milking site; K was determined from the product of the apparent density of flies and the time (h) spent daily by one zebu at these two sites (fig 1) divided by the number of zebu in each group (n); and ii) to examine the relationship between stable fly densities and trypanosome frequency. The latter relationship was measured after pooling monthly data for stable fly densities at the resting site; these values were then compared with the next month's trypanosome frequency in the cattle. Statistical analysis was performed using regression analysis (Foucher, 1992).

RESULTS

Stable fly species and relative densities

At the study site, at least five species belonging to two genera of Diptera were

encountered. Four species belonged to the genus *Stomoxys*: *Stomoxys nigra nigra* Macquart, 1851, *S taeniata* Bigot, 1888, *S sitiens* Rondani, 1873 and *S omega omega* Newstead, 1907. The individuals that belonged to the genus *Haematobia* have yet not been accurately identified. The most widespread species was *S nigra nigra* (approximately 60% of the whole sample) followed by *Haematobia* sp. In the following discussion, all these species are grouped under the name 'stable flies'. Their distribution and abundance, along the transect, are shown in figure 2. The stable flies were caught anywhere along the main cattle paths between the resting site and the drinking site as well as the grazing site further away. However, the maximum density was observed at the resting and milking site where it reached up to ten flies per day at trap 10, in June 1992. During the dry season, the number of flies caught greatly decreased, to less than 0.1 fly per trap per day.

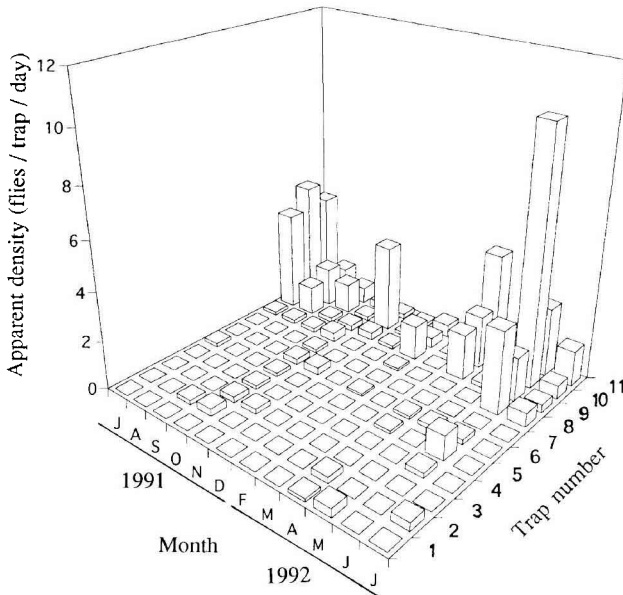


Fig 2. Apparent densities of stable flies along the transect (cf, fig 1), between July 1991 and July 1992 (no data available for the month of January 1992).

Daily activity pattern of cattle

Our field work showed that time spent at the resting site, as well as at pasture, varied according to the season. During the dry season, it was shorter than in the rainy season. The time spent at the drinking site was extremely short, averaging 5 to 10 min per day (see table I).

Contact index between stable flies and cattle

A simple index of contact (K) between one zebu and stable flies was calculated for calves and adults at two particular sites: i) the cattle resting and milking site; and ii) the cattle drinking site. K estimates for the months of February (dry season), May (interseason) and August (rainy season) are shown in table I. This index is extremely low or even zero at the drinking site. It ranges between 0.02 and 2.94 at the resting and milking place. K is always higher for calves than it is for adults. Thus, if K actually reflects the interactions existing between the cattle and the stable flies, we

can assume that only rare interactions occur between the cattle and the stable flies at the drinking site. In contrast, such interactions are frequent particularly during the rainy season (August) at the resting and milking place and they are higher for calves than for adults.

Trypanosome frequency in cattle

Table II shows the four monthly estimates of trypanosome frequency detected in Mbororo cattle using either parasitological techniques or a serological immunodiagnostic technique (ELISA). The ELISA technique revealed a high frequency of *T congolense*, the dominant species. *T vivax* frequency ranged from 0 to 14.5% using parasitological methods and from 8.2 to 19.4% using ELISA.

Relationship between stable flies densities and trypanosome frequency in cattle

No relationship was found between the four monthly estimates of trypanosome fre-

Table I. Estimation of contact index (K) between cattle and stable flies at two sites: cattle resting and milking place (rm) and cattle drinking site (d).

Month	Cattle	n	AD_{rm}	AD_d	t_{rm}	t_d	K_{rm}	K_d
February	Calves	11	0.40	0	12	0	0.44	0
	Adults	50	0.40	0	3	0.16	0.02	0
May	Calves	13	0.82	0	5	0.08	0.32	0
	Adults	45	0.82	0.36	4	0.08	0.07	6.4×10^{-4}
August	Calves	14	6.86	0.11	6	0.08	2.94	6.2×10^{-4}
	Adults	48	6.86	0	5	0.08	0.71	0

K is calculated from the product of apparent densities (AD) of the stable flies and the time (h) spent by one zebu at each site studied, divided by the number of zebu in the group (n). n : number of cattle in the herd. AD_{rm} : apparent density of stable flies at the resting and milking site. AD_d : apparent density of stable flies at the drinking site. t_{rm} : time (h) spent daily by one zebu cow (between 0600 and 1800 hours) at the resting and milking site. t_d : time (h) spent daily by one zebu cow at the drinking site. K_{rm} : contact index at the resting and milking site. K_d : contact index at the drinking site.

Table II. Quarterly estimates of trypanosome frequency in cattle (%).

Month	n	Parasitology				Serology				Both
		Tb	Tv	Tc	T	Tb	Tv	Tc	T	T
October 1991	52	1.9	9.6	0	9.6	13.7	15.7	43.1	47.1	52.9
February 1992	61	0	0	1.6	1.6	22.9	8.2	44.3	50.9	52.5
June 1992	58	0	0	0	0	32.8	10.3	58.6	68.9	68.9
August 1992	62	1.6	14.5	1.6	16.1	48.4	19.4	83.9	87.1	87.1

n: number of cattle sampled. Tb, Tv, Tc and T: *T. brucei*, *T. vivax*, *T. congolense* and the three species mixed; frequency revealed by a given technique (parasitology or serology). Both: trypanosome frequency (three species mixed) revealed by parasitological and serological techniques together.

quency of *T. congolense* and *T. brucei* using either parasitological techniques or the immunodiagnostic technique. Significant regressions occurred between the four monthly estimates of *T. vivax* frequency in cattle and the stable fly densities at the resting site. This relationship was highly significant ($P < 0.05$) with a correlation coefficient (r) of 0.968 for parasitological techniques and of 0.997 for the ELISA technique. The equations obtained for the regression analyses shown are $y = 0.207x - 0.062$ if the parasitological frequency was considered and $y = 0.149x + 0.046$ if ELISA data were used (fig 3).

DISCUSSION

The possibility of haematophagous arthropods transferring trypanosomes between hosts without any biological development of the agent has been under study for a long time (Bouet and Roubaud, 1912; Buxton, 1955). Many species of trypanosomatids are known to be transmitted through a mechanical mechanism (Wells, 1972; Foil, 1989). *T. evansi*, the first pathogenic trypanosome shown to cause disease in domestic livestock, is usually mechanically transmitted by tabanids and stable flies (Luckins, 1992). In South and Central Amer-

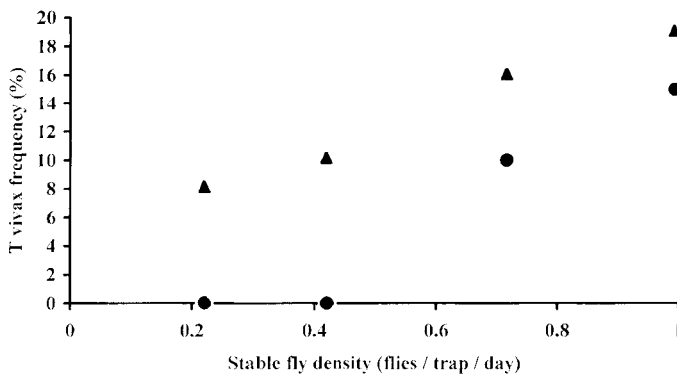


Fig 3. Relationship between the stable fly densities and *T. vivax* frequency in Mbororo cattle (triangles: trypanosome frequency obtained by parasitological technique; circles: trypanosome frequency revealed by the ELISA technique).

ica, *T vivax* is thought to be transmitted similarly (Gardiner, 1989). In these areas, devoid of tsetse, *S calcitrans* and horse flies such as *Cryptotylus unicolor* (Foil, 1989), *Tabanus importunus* (Raymond, 1990) and *T nebulosus* (Otte and Abuabara, 1991) are efficient transmission vectors. In tropical Africa, where tsetse flies, tabanids and stable flies coexist, the problem of determining the contribution of mechanical transmission is especially acute. The persistence of trypanosomiasis due to *T vivax* in endemic foci in tsetse-free areas emphasizes the role of other insects such as tabanids and stable flies (Bauer et al, 1988; Nawathe et al, 1988; Chollet, 1992; Van der Merwe, 1992). In Sudan (Buxton, 1955) and Zimbabwe (Boyt et al, 1970), the existence of mechanical transmission for *T congolense* has been suspected. It has been experimentally demonstrated with the tabanid *Tabanus thoracinus* (Foil, 1989). It has now been experimentally proven that *T brucei* can be transferred through non-cyclic propagation by tabanids (Foil, 1989), the stable fly *S calcitrans* (Straif et al, 1990) and by *G morsitans morsitans* (Roberts et al, 1989). Recently, Mihok et al (1995a) demonstrated that six taxa of African stomoxiinae biting flies (*S niger niger*, *S niger bilineatus*, *S varipes*, *S taeniatus*, *S pallidus* and *Haematobosca squalida*) were capable of transmitting *T brucei*, *T vivax* and *T evansi* mechanically to mice in laboratory. However, until now, little data was available to address the question of the relative importance of non-cyclic transmission of trypanosomes under natural conditions when the major route of transmission of these parasites is due to tsetse flies (Wells, 1972; D'Amico, 1993; D'Amico et al, 1992).

Since the beginning of studies of nagana in Africa, the group of Stomoxiinae flies has been the least studied among all proven or suspected vectors. Nevertheless, it is a well-distributed group in several countries and the recent study of Mihok (1993) in Kenya demonstrated 11 species and subspecies. In

the CAR, our preliminary investigations reveal the occurrence of at least five species. Further studies, together with a systematic revision of the stable fly group, will probably lead to an increase in this number. Independent of the abundance and ecology of each species, our work suggested that these arthropods were good candidates as vectors of *T vivax* trypanosomiasis in this country. This hypothesis, initially proposed to explain the apparent seasonal and uncertain aspects of cyclic trypanosome transmission due to *G f fuscipes* (D'Amico, 1993), was supported by the data presented above. The first observation was the abundance of stable flies at the cattle resting site. The second was the high value for the contact index between cattle and *Stomoxys* spp at this resting site. The concept of contact index, although imperfect and simply calculated, is of interest and deserves a more refined mathematical approach. The third correlation was the one existing between the apparent densities of the *Stomoxys* spp at the resting site and the *T vivax* frequency in cattle. Although it was based on a small sample, limited in space and time, and the ELISA immunodiagnostic technique needs further confirmation, the general association seemed valid. Moreover, this hypothesis was also supported by the fact that a control strategy using bipyramidal traps set at cattle drinking points had no effect upon the *T vivax* frequency in cattle (D'Amico, 1993). This observation suggested that this parasite species was probably not transmitted at this site by only the tsetse fly *G f fuscipes*, as populations of this tsetse species are seriously affected by this mode of control (Gouteux and Le Gall, 1992; Le Gall et al, 1995).

However, to confirm the proposed hypothesis that stable flies were efficient vectors of *T vivax* we must initially extend our observations to larger epidemiological foci in the CAR and secondly we need to acquire direct evidence. The presence of trypanosomes in stable fly proboscids

should be conclusively proven by PCR techniques (Masiga *et al*, 1992; Bromidge *et al*, 1993). However, the detection of the parasite does not unequivocally prove its transmission to cattle. Similarly, the existence of the pathology in the cattle is not proof of transmission. This knowledge is, nevertheless, essential as it permits the estimation of the proportion of stable flies bearing trypanosomes in natural populations and is of particular value for quantifying the importance of mechanical transmission under natural conditions. The actual demonstration of the mechanical transmission of *T vivax* by *Stomoxys* spp requires *in situ* experimental transmission trials. Eventually, the information obtained from control campaigns against haematophagous insects using cattle 'pour-on' insecticides will be important in demonstrating the existence of mechanical transmission. At the moment, we are testing the effects of pour-on impregnations (Deltamethrin Spot-on® and Flumethrin Bayticol®) on natural populations of stable flies and tsetse flies in the Ouro-Djafoun livestock area of the CAR.

In conclusion, in tsetse-infested areas, the possible occurrence of mechanical transmission of *T vivax* to cattle by haematophagous insects continues to be an unresolved problem. Although mechanical transmission by stable flies and particularly by horse flies has already been experimentally proven, the epidemiological importance of this phenomenon in the field is still poorly understood. In the CAR, our initial investigations suggest that mechanical transmission does take place and we have presented evidence that stable flies were significant vectors of *T vivax* and perhaps *T congolense* or *T brucei*. This study provides new data concerning the modes of transmission of bovine trypanosomiasis in the CAR. Until now, in most epidemiological foci, the transmission of nagana was chiefly ascribed to *G f fuscipes* and the cattle drinking point was considered to be the

privileged site of transmission. In future, it must be considered that modes of trypanosomiasis transmission are more complex than previously thought.

G f fuscipes is the main vector of *T congolense*, *T vivax* and *T brucei*, because it transmits these parasites cyclically and perhaps mechanically and also because it constitutes an important reservoir for these trypanosomes (D'Amico *et al*, 1992). Although intervention by stable flies is highly probable, the possible role of other haematophagous diptera such as horse flies and *G fusca* must be kept in mind (Finelle, 1957; Finelle *et al*, 1963). Finally, it is very likely that this epidemiological system is based on a mixed transmission, combining mechanical and cyclical methods, and involving at least two groups of vectors. According to this theory, cattle resting and milking sites are at risk from trypanosome challenge as well as drinking points. The epidemiological implications of movement patterns of cattle are detailed elsewhere (D'Amico *et al*, 1995).

These facts are important for nagana control. At present, the campaign against this pest in CAR is based on a combination of chemoprophylaxis and trapping against *G f fuscipes* using bipyramidal traps (Gouteux, 1991) set at drinking points (Cuisance *et al*, 1992; Gouteux and Le Gall, 1992). Strategies should now include techniques for reducing the number of stable flies which are, in any case, serious livestock pests, causing irritation to the cattle which results in weight loss and reduction in milk yield. Following the strategy chosen in this country, we advocate the use of bipyramidal traps at both the drinking points and the resting sites. The traps designed for collecting tsetse flies have also proved effective against *Stomoxys* (Rugg, 1982; Mihok *et al*, 1995b). There is also scope for improving the traps and controlling stable fly populations by using colour blue (Holloway and Phelps, 1991). In the future, there is a need for studies to assess the

efficacy of bipyrimal traps in catching stable flies in the CAR. We also look forward to the results of the programme presently being initiated in the same areas to attempt to control the populations of tsetse flies and stable flies using 'pour-on' and 'spot-on' formulations of insecticides placed directly on the cattle.

ACKNOWLEDGMENTS

This study received financial support from the Government of the CAR, the World Bank, the European Development Fund and the government of France (Fonds d'aide et de coopération). F D'Amico was financially sponsored by the government of France (Ministère de la Recherche et de l'Espace). We thank the director of CIRAD-EMVT (France), the director of the Département Santé of ORSTOM (France), the director and the staff of the Agence nationale pour le développement de l'élevage (CAR), VM Nantulya and PAO Majiwa of ILRAD (Kenya), JL Frezil and C Bellec of ORSTOM (France), C Mouchès of Université de Pau et des Pays de l'Adour (France), M Bouchier of INRA (France), C Foucher of CIRAD (France) and especially P Gardiner of ILRAD (Kenya) and G Uilenberg of CIRAD-EMVT (France) for their help and valuable comments on the manuscript.

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