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First insights into the cattle serological response to tsetse salivary antigens: A promising direct biomarker of exposure to tsetse bites

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ABSTRACT

In the context of the Pan African Tsetse and Trypanosomiasis Eradication Campaign, the value of tsetse saliva antibodies as a biomarker of cattle exposure to tsetse flies was evaluated, as this could provide an alternative and complementary tool to conventional entomological methods. Serum immune reactivity to *Glossina* (*G.*) *palpalis* (*p.*) *gambiensis*, *G. tachinoides* and *G. morsitans* (*m.*) *submorsitans* whole saliva extracts (WSE) were monitored in cattle from both tsetse free and tsetse infested areas, and in cows experimentally exposed to tsetse flies and other hematophagous arthropods. In the tsetse infested area, cattle IgG responses to *Glossina* WSE were significantly higher during the dry season ($p < 0.0001$) when herds are most exposed to tsetse flies and in infected animals ($p = 0.01$) as expected in the case of a biomarker of exposure. Experimental studies further confirmed this as a quick rise of specific IgGs was observed in animals exposed to tsetse flies (within weeks), followed by a rapid clearance after exposure was stopped. In contrast to the two other tsetse species, *G. m. submorsitans* WSE enabled to detect exposure to all tsetse species and were associated with low level of cross-reactivity to other blood sucking arthropods. Finally, IgG responses to *G. m. submorsitans* salivary antigens enabled to distinguish different groups of cows according to exposure levels, thus indicating that tsetse saliva antibodies are not only indicators of tsetse exposure but also are correlated to the intensity of tsetse contacts ($p = 0.0031$). Implementation of this new sero-epidemiological marker of cattle exposure to tsetse flies in the framework of tsetse elimination campaigns is discussed.

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1. Introduction

Tsetse flies (*Diptera: Glossinidae*) are found in most of the sub-Saharan Africa area (Itard et al., 2003). Human welfare is affected by trypanosomes transmitted by tsetse flies because of the chronic and acute forms of sleeping sickness (due to *Trypanosoma (T.) brucei gambiense* and *T. brucei rhodesiense* respectively) and of the wasting diseases of livestock, grouped under the name African Animal Trypanosomiasis (AAT) and essentially caused by *T. congolense*, *T. vivax* and *T. brucei brucei* (Desquesnes and Dia, 2003). Tsetse flies are the only known cyclical vectors for these trypanosome species although they can sometimes be transmitted mechanically by tsetse flies or other biting insects such as tabanids and stable flies (Moloo et al., 2000). In tsetse infested areas, AAT constitutes a major obstacle to efficient and sustainable livestock production systems and mixed crop-livestock farming (Shaw, 2004) and has a major impact on the socio-economic development of these areas (Kabayo, 2002). Agriculture and livestock production annual loss due to AAT has been estimated to range between 4 and 4.5 billion US\$ per year (Budd, 1999). *Glossina* species from the *palpalis* (subgenus *Nemorhina*) and *morsitans* group (subgenus *Glossina*) are the main vectors of AAT and HAT. Species from the *palpalis* group are usually more associated with coastal (including mangrove) habitats, degraded forests of West Africa and riverine vegetation, nonetheless, some can be found in savannah regions along river systems (Solano et al., 2009). All species of the *morsitans* group are restricted to savannah woodlands where their density is highly dependent on the presence of wild fauna. Species of this group are major vectors of AAT in Eastern and Southern Africa whereas they are decreasing rapidly in West Africa as the result of increased human density and disappearance of wild animals (Van den Bossche et al., 2010).

As no vaccine is yet available to prevent infection by trypanosomes, the control of AAT relies on a variety of strategies including (i) the prophylactic or curative treatment of animals with trypanocidal drugs, (ii) the promotion of West African trypanotolerant taurine breeds able to limit parasitaemia and anemia and remain productive in enzootic areas and (iii) the control of tsetse populations by diverse means such as ground and aerial spraying of insecticides, live-bait technologies, insecticide-treated targets/traps and use of the sterile insect technique (Vreysen et al., 2013). Nevertheless the first strategy is largely hampered because of few prospects for developing new drugs and because trypanocidal drugs are often used indiscriminately and unsupervised resulting in increased resistance of the parasite (Delespaulx et al., 2008). Maintaining trypanotolerant cattle has given promising results, nevertheless these animals are not very popular with livestock owners regarding both their low productivity and draught power (Holmes, 1997). In this regard controlling tsetse fly populations remains theoretically the best way of controlling AAT. The African Union has recently launched the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC), a continental initiative that focuses on the progressive elimination of discrete tsetse-infested areas. Uganda, Ethiopia, Kenya in East Africa and Mali,

Ghana, Burkina Faso in West Africa, have started to implement area-wide tsetse eradication campaigns (Schofield and Kabayo, 2008). In order to reduce tsetse densities efficiently, vector control strategies have to lean on accurate entomological evaluations to define adapted and targeted vector control measures (Solano et al., 2009). Currently, *Glossina* densities are evaluated by the use of tsetse traps but this reference method presents several limits: (i) the deployment of traps is labor intensive, expensive, requires a complex logistic in certain environmental settings and is thus poorly adapted to large scale interventions; (ii) traps are becoming poorly efficient when tsetse densities become low possibly due to density-dependant dispersal (Bouyer et al., 2010); (iii) trapping represents an indirect method to assess host exposure to tsetse bites. There is therefore an urgent need to develop new alternative tools to better target populations exposed to tsetse bites and to facilitate the monitoring of entomological interventions especially for the declaration of a pest free status (Barclay and Hargrove, 2005).

During the probing and ingestion phase of the feeding process, blood sucking arthropods inoculate a complex mixture of pharmacologically active components into the host skin. The main functions of saliva molecules are to antagonize the vertebrate's mechanism of blood clotting, platelet aggregation, vasoconstriction, pain and itching which are triggered by tissue destruction, and immune reaction to insect products (Ribeiro et al., 2010). Importantly, salivary proteins are also recognized as foreign antigens and elicit the production of specific antibodies making them potential biomarkers of exposure. Indeed, antibody responses directed against total arthropod saliva, specific recombinant proteins or specific peptides, were shown to correlate with host exposure to several arthropods such as ticks, sand flies, triatomines, *Culex*, *Aedes* and *Anopheles* species (reviewed in Fontaine et al., 2011). Concerning *Glossina* genus, recent studies have shown that high IgG responses directed against whole saliva extracts (WSE) of *G. m. morsitans*, *G. fuscipes fuscipes* and *G. palpalis (p.) gambiense* were observed in populations from HAT endemic areas in Uganda (Caljon et al., 2006), Democratic Republic of Congo (Poinsignon et al., 2007, 2008) and Guinea (Dama et al., 2013) respectively. These data suggest that the antibody response to saliva antigens can be used to evaluate exposure to tsetse flies as for other blood sucking arthropods. Among possible applications of this new sero-epidemiological tool is the monitoring of area-wide tsetse eradication campaigns that are being implemented across Africa (Schofield and Kabayo, 2008). The IgG response to tsetse saliva had never been yet evaluated in cattle toward which most efforts in terms of vector control are made. Furthermore the use of bovines provides the opportunity to develop "natural" experimental models of exposure that are most valuable tools to perform kinetics and specificity studies.

In order to evaluate the value of the cattle antibody responses to tsetse saliva as a biomarker of exposure, we compared IgG responses directed against WSE from *G. p. gambiense* in serum samples collected from cattle living in tsetse free and infested areas from Burkina Faso. In addition the kinetics of the anti-saliva response was monitored in

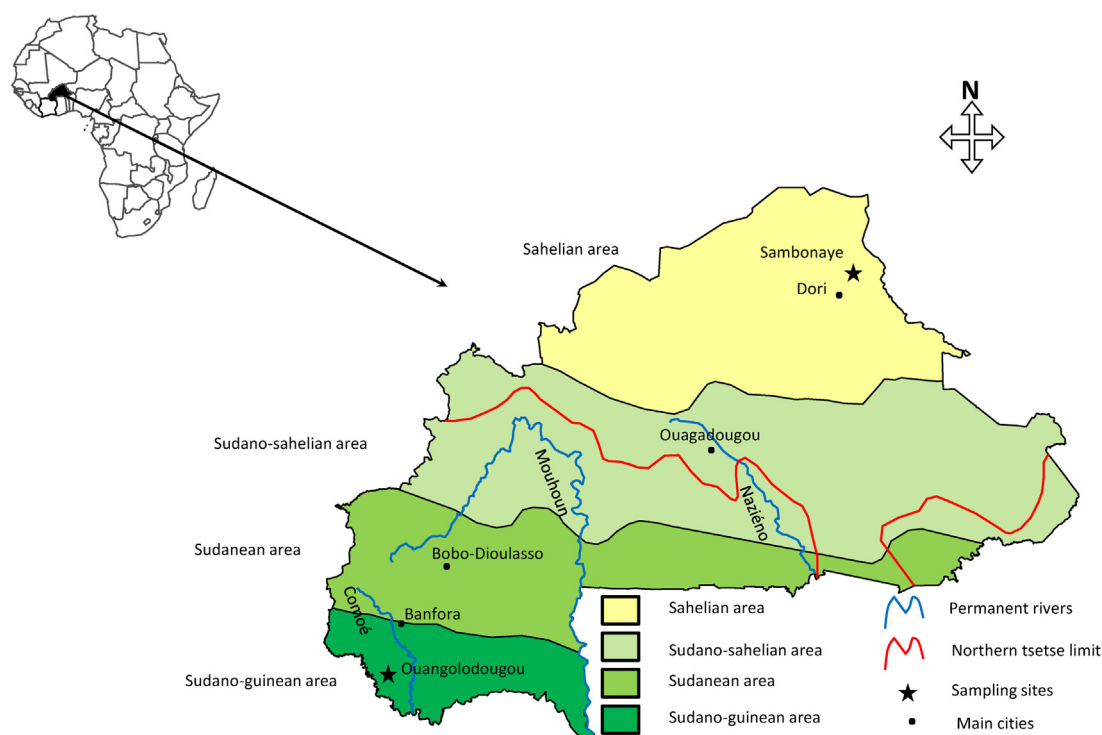


Fig. 1. Study areas. Agro-climatic map of Burkina Faso indicating the study sampling sites and the northern limit of tsetse flies.

cows experimentally exposed to *G. p. gambiensis*, *G. m. submorsitans* and to other blood sucking arthropods feeding on cattle (*Tabanidae* spp, *Stomoxys* spp, *Amblyomma* (A.) *variegatum* and *Anopheles* (An.) *gambiae*) to assess the specificity of the response. Results are discussed in the perspective of developing a biomarker of cattle exposure to tsetse flies.

2. Materials and methods

2.1. Cattle from tsetse free and infested areas

For the purpose of the study, 162 bovine samples that were collected in the framework of previous studies and preserved at -80°C in the bovine serum cryobank at Centre International de Recherche-Développement sur l'Élevage en zone Subhumide (CIRDES, Bobo-Dioulasso, Burkina Faso) were selected. Forty eight samples of them came from non exposed sedentary zebu (*Bos indicus*) living in Sambonaye located well above the northern limit of tsetse flies (Courtin et al., 2010) in Burkina Faso (Fig. 1). The remaining 114 samples came from a cohort of 53 cross-bred bovine (baoule-zebu peul) from Ouangolodougou in a tsetse infested agro-pastoral area from South-West Burkina Faso. These animals, belonging to 10 different owners, were followed up in time in the framework of a previous study aimed at assessing AAT incidence in this area (Dayo et al., 2010). Four animals were sampled four times, 14 animals three times, 21 twice and 14 once. In this area the climate is of Sudano-Guinean type with two main seasons: a dry season (from November to May) and a rainy season (from June to October). Serum samples collected

from cattle were taken from 2003 to 2005 at various time of the year (83 during the rainy season and 31 during the dry season). We had access to the parasitological status at the time of sampling, determined by the Buffy-coat technique (Murray et al., 1977) for all animals. The study area is covered by woodland savannah mixed with riverine forests along the river courses. Main tsetse species are *G. p. gambiensis* and *G. tachinoides* which favorite biotope is riverine vegetation and *G. m. submorsitans* exclusively found in woody savannah and especially in protected areas. *G. medicorum* is also present but at very low densities (Pagabeleguem et al., 2012).

2.2. Experimental exposure of cows to *Glossina* spp. and other hematophagous arthropods

2.2.1. Animals

All animal experiments were carried out in compliance with the CIRDES ethical review comity (03-2011/CE-CIRDES). Two batches of cows were bought and kept in the CIRDES mosquito net-protected cowshed. The first batch of animals consisted of six taurine-zebu crossbred cows (2–3 years-old) bought in the surroundings of Bobo-Dioulasso. All animals were parasitologically and serologically negative for AAT and had a low level of antibody response against *G. p. gambiensis* (see Section 2.4). The second batch of cows consisted of 10 zebras (2–3 years-old) from a tsetse free area in Northern Burkina Faso (Dori). Upon arrival all animals were given multivitamins (stress-vitam), were treated for ecto-parasites (vadfène), bacterial infections (Tenaline 20%), helminthes (Ivomec) and trypanosomes

(diminazene aceturate at 7 mg/kg) and were vaccinated against hemorrhagic septicemia, contagious bovine pleuropneumonia, and blackleg disease, in order to avoid inter-recurrent infections. All animals were kept for two months in the cowshed before starting experiments.

2.2.2. Blood sucking arthropods used for experimental exposure

Tsetse flies: Newly emerged unfed male and female (ratio 1:1) flies from the *G. p. gambiensis* and *G. m. submorsitans* CIRDES colonies were used for experimental exposure.

Ticks: *A. variegatum* unfed nymphs were obtained from the CIRDES colony.

Mosquitoes: Three to five days old females *An. gambiae sensu stricto* were kindly provided by the “Institut de Recherche en Sciences de la Santé” (IRSS, Bobo-Dioulasso, Burkina Faso). Mosquitoes were starved for 24 h before experimental exposure.

Tabanids and stomoxes: Female specimens of Tabanidae species (*Tabanus taeniola*, *Tabanus biguttatus*, *Chrysops distinctipennis*, *Atylotus agrestis*, *Tabanus par* and *Tabanus gratus letrouducus*) and male and female specimens of Stomoxys species (*Stomoxys calcitrans* and *Stomoxys nigra*) were captured in the countryside around Bobo-Dioulasso using N’ZI and monoconical traps. We were concerned with keeping the proportion of the different species constant for experimental exposure although this was not always possible depending on the captures of the day.

For experimental exposure, blood sucking insects were placed in mosquito net cages that were attached on a shaved area of the cow’s flanks for two hours in the dark. For ticks, nymphs were fixed on the cow’s ear. The ear was then wrapped in a cloth to prevent them from escaping and to be collected after feeding. Ticks were left for 6 days before removal. The next exposure on the second ear occurred after a one week break. All arthropods were collected and visually examined to insure that feeding was successful after exposure. Feeding rates were almost always >70% for all the species used for experimental exposures.

2.2.3. Exposure experiments

In the first experiment each of the six crossbred cows were exposed to *G. p. gambiensis* (20 flies, twice a week), *G. m. submorsitans* (20 flies, twice a week), *Tabanidae spp* (20 flies, twice a week), *Stomoxys spp* (20 flies, twice a week); *An. gambiae* (50 mosquitoes, twice a week) and *A. variegatum* (25 nymphes, every two weeks) respectively. Experimental exposure to *Anopheles* bites lasted for 12 weeks while exposure to other blood sucking arthropods lasted for 23 weeks. For all animals peripheral blood samples (10 ml) were collected in dry vacutainers taken from the jugular vein once a week during all the exposure period. Blood samples were centrifuged at $2000 \times g$ for 15 min at room temperature after clotting and sera were collected and stored at -80°C until use. The two cows exposed to tsetse bites (*G. p. gambiensis*, *G. m. submorsitans*) were kept in the cowshed and monitored during 10 more weeks after the end of experimental exposure.

In the second experiment, the 10 zebus were kept in three groups: (i) a high exposure group ($n=4$) in which animals were exposed to 50 tsetse flies (*G. m. submorsitans*)

twice a week during 11 weeks; (ii) a low exposure group ($n=4$) in which animals were exposed to 10 tsetse flies (*G. m. submorsitans*) once a week during 11 weeks; and (iii) a control group ($n=2$) made of unexposed animals. Serum samples were collected from each animal once a week (as described above) all through the experimental exposure period.

2.3. Collection of tsetse saliva

To produce whole saliva extracts, 10–12 days-old male and female flies from the *G. p. gambiensis*, *G. m. submorsitans* and *G. tachinoides* colonies at CIRDES were used. The tsetse saliva was collected as described previously (Poinsignon et al., 2007) by a salivation technique that does not requires the dissection of salivary glands to avoid the presence of non salivary antigens in WSE. Briefly, tsetse flies (4–6 flies) were enclosed in 50 ml Falcon tubes closed by a mosquito net and placed above a drop of salivation buffer (10 mM HEPES, 150 mM NaCl and 5 mM EDTA, pH 7.2) on warm slides (37°C). The buffer was collected after 10 min of salivation and pooled according to tsetse species. A solution of protease inhibitor (Mannheim, Germany, Roche diagnostics) was added (1:25) to WSEs and protein concentration was measured with the BCA (bicinchoninic acid) Protein Assay Kit (Pierce, Rockford, IL). WSE aliquots were then frozen at -80°C before use.

2.4. Serological analysis of bovine specific IgG levels

Total IgG levels directed against tsetse saliva antigens were measured by indirect-ELISA. Briefly, Polysorp 96-wells plates (Nunc, VWR, France) were coated with 100 μl of WSE at 2 $\mu\text{g/ml}$ in carbonate/bicarbonate buffer for 2 h and 30 min at 37°C . After washing, and saturation with casein phosphate-buffered saline (PBS) buffer, serum samples (100 μl) were incubated at 37°C for two hours at 1/200 dilution in PBS-Tween20 (1%). IgG detection was performed by adding a sheep anti-bovine IgG (AbD Serotec, France) at 1/4000 dilution for two hours at 37°C followed by the addition of 2,2’-azino-bis (3-ethylbenzthiazoline 6-sulfonic acid) diammonium (ABTS, Sigma, St. Louis, MO) in 50 mM citrate buffer (pH 4) containing 0.003% H_2O_2 . Optical density (OD) was measured at 405 nm after two hours. Each sample was assessed in duplicate wells (antigen well) and in a blank well containing no antigen. Results are expressed as the ΔOD value according to the formula: $\Delta\text{OD} = \text{OD}_x - \text{OD}_n$, where OD_x represents the mean of antigen wells OD values and OD_n the OD value of the blank well. A high responder serum was tested on each plate to allow the normalization of results from the different plates.

2.5. Statistical analysis

All statistical analyses were performed with the JMP 7.0 software (SAS Institute Inc.). The non parametric Wilcoxon/Kruskal–Wallis test was used to compare the level of anti-saliva IgG responses between two independent groups. The factors affecting cattle tsetse saliva antibody responses were also determined by multivariate mixed linear regression models using specific IgG levels as

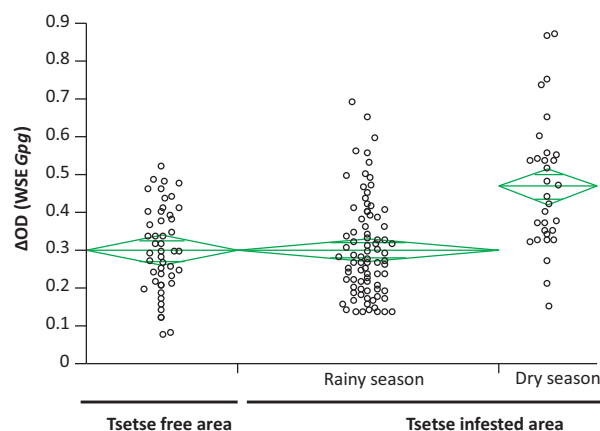


Fig. 2. Tsetse anti-saliva responses in cattle from tsetse free and tsetse infested areas. IgG levels measured by ELISA (ΔOD) to *Glossina palpalis gambiensis* (Gpg) whole saliva extracts (WSE) are shown for cattle sera collected in a tsetse free area (Northern Burkina Faso) and for sera collected in a tsetse infested area (South-West Burkina Faso) during the rainy and dry seasons. The line across each diamond represents the group mean, the vertical span of each diamond represents the 95% confidence interval (CI) for each group, overlap marks are drawn ($\sqrt{2}CI$)/2 above and below the group mean. The horizontal span of each diamond is proportional to the sample size in the group.

the response; the study covariates (season of sampling and parasitological status for cattle sampled in the field; and tsetse exposure levels and time of experimental exposure for cattle experimentally exposed to tsetse flies) as fixed effects and the animal number as a random effect. P -values ($p < 0.05$) were considered significant.

3. Results

3.1. Anti-saliva responses in cattle from different agro-pastoral zones

In a first attempt to evaluate the value of the cattle anti-tsetse saliva response as a biomarker of exposure to tsetse bites, IgG responses directed against tsetse WSE were measured by indirect-ELISA in two groups of cows living in well differentiated agro-pastoral zones (Fig. 2). A large inter-individual heterogeneity of antibody responses was observed in both groups of animals ranging from $\Delta OD = 0.07$ – 0.52 (mean $\Delta OD = 0.30 \pm 0.02$) in bovine from Northern Burkina Faso (tsetse free area) and from $\Delta OD = 0.13$ – 0.87 (mean $\Delta OD = 0.35 \pm 0.02$) in bovine from South-West Burkina Faso (tsetse infested area). The Anti-saliva IgG levels were not significantly different between sera collected during the rainy season in the tsetse infested area (mean $\Delta OD = 0.30 \pm 0.01$) and those collected in the tsetse free area ($p = 0.67$). However highly significantly higher specific IgG responses were observed in sera collected during the dry season (mean $\Delta OD = 0.47 \pm 0.03$; $p < 0.0001$).

In order to better characterize parameters that are associated with variation of *G. p. gambiensis* salivary antigen reactivity, a linear model of specific IgG levels was built including the season of sampling, the parasitological status and the owner as fixed effects, and the animal number

as a random effect (Table 1). Results of this analysis confirmed the strong season effect ($p < 0.0001$) and showed a significant association between high anti-saliva responses and the risk of infection by trypanosomes ($p = 0.01$).

3.2. IgG responses against *Glossina* spp. salivary antigens in cows experimentally exposed to the bite from different hematophagous arthropods

Immune reactivity to *G. p. gambiensis*, *G. m. submorsitans* and *G. tachinoides* WSE of animals experimentally exposed to tsetse flies (*G. p. gambiensis* and *G. m. submorsitans*) and to other blood sucking arthropods (tabanids, stable flies, mosquitoes and ticks) is shown in Fig. 3A, B, and C, respectively. An important induction of reactivity to WSE from all tsetse species was observed in the animal exposed to *G. p. gambiensis*. In contrast, IgG responses in the cow exposed to *G. m. submorsitans* showed less reactivity toward salivary antigens from the *palpalis* tsetse group (*G. p. gambiensis* and *G. tachinoides*) as compared to those from *G. m. submorsitans*. Anti-saliva IgGs started to increase in between the 6th and the 10th week of exposure (between 240 and 400 tsetse bites taking into account that in average 80% of blood meals were successful) in both cows. Anti-saliva responses then reached a plateau from week 16 to 21 and started to decrease steadily 5–6 weeks after the stop of exposure, returning to pre-exposure levels after 10 weeks.

Experimental exposure of the three other cows to stable flies, mosquitoes and ticks did not induce any cross reactivity with none of the three tsetse species salivary antigens (Fig. 3). Nevertheless, cross reactivity was observed in the cow exposed to tabanids with WSE from *G. p. gambiensis* and *G. tachinoides* but not *G. m. morsitans*. In the view of these preliminary results obtained from a limited number of animals, we postulated that *G. m. submorsitans* salivary antigens were better suited to monitor tsetse contacts as it provided a good sensitivity to detect exposure to both *G. m. submorsitans* and *G. p. gambiensis* and appeared to be associated with a lower degree of cross reactions at least to tabanid species. Noteworthy, tabanids are major pests of cattle in the northern pastoral areas of Burkina Faso where they are found in very high densities (Pagabeleguem et al., 2012). This might have explained part of the intermediate responses observed initially in some animals from the tsetse free area (Fig. 2). To test this hypothesis, we selected serum samples from the tsetse free area ($n = 20$) and the tsetse infested area ($n = 20$) but having similar intermediate IgG levels ($0.26 \leq \Delta OD \leq 0.53$) to *G. p. gambiensis* WSE and repeated the ELISA test with *G. m. submorsitans* salivary antigens (Fig. 4). Confirming our hypothesis, highly significant differences were observed when *G. m. submorsitans* WSE were used ($p = 0.0007$), probably because these antigens provided a better specificity to detect exposure to tsetse bites.

3.3. Anti-saliva responses and level of exposure to tsetse bites

In the tsetse infested area, cattle displayed an important heterogeneity of anti-saliva responses. The fact that IgG levels are in part explained by the season and are associated

Table 1Season, parasitological status and cattle owner effects on *G. p. gambiensis* saliva specific IgG responses in South-West Burkina Faso.

Covariates		Means of ΔOD least squares ($\pm$ standard error)	p^a
Season	Rainy	0.29 $\pm$ 0.03	<0.0001
	Dry	0.50 $\pm$ 0.03	
Parasitological status	Negative	0.35 $\pm$ 0.03	0.01
	Positive	0.43 $\pm$ 0.03	
Cattle owner		–	0.20

Mixed linear regression model of *G. p. gambiensis* saliva specific IgG levels (ΔOD) with the season of sampling, parasitological status and cattle owner included as fixed effects and the animal number as a random effect. Model regression residues were normally distributed (Shapiro–Wilk W test, $p = 0.15$); model R -square = 0.63 ($p < 0.0001$).

^a p -Values <0.05 are bolded.

with positive parasitology suggested that the intensity of the anti-saliva response is related to the level of exposure to tsetse flies. Nevertheless, individual differences of cattle immune reactivity to salivary antigens are also undoubtedly implicated. Fig. 5 shows the results obtained from our three zebu groups submitted to different tsetse exposure levels. Although an important intra group variability of *G. m. submorsitans* WSE specific IgG levels was observed in

the two exposed groups (as indicated by standard errors), IgG response curves enabled to differentiate between the three groups of animals. Specific antibodies were detected earlier (between 2 and 4 weeks) in all cows from the two exposed groups as compared to the first experiments in taurine-zebu crossbred cows. Whereas the kinetics of the response appeared to be similar in both groups, the level at the plateau differed according to the exposure level. In a

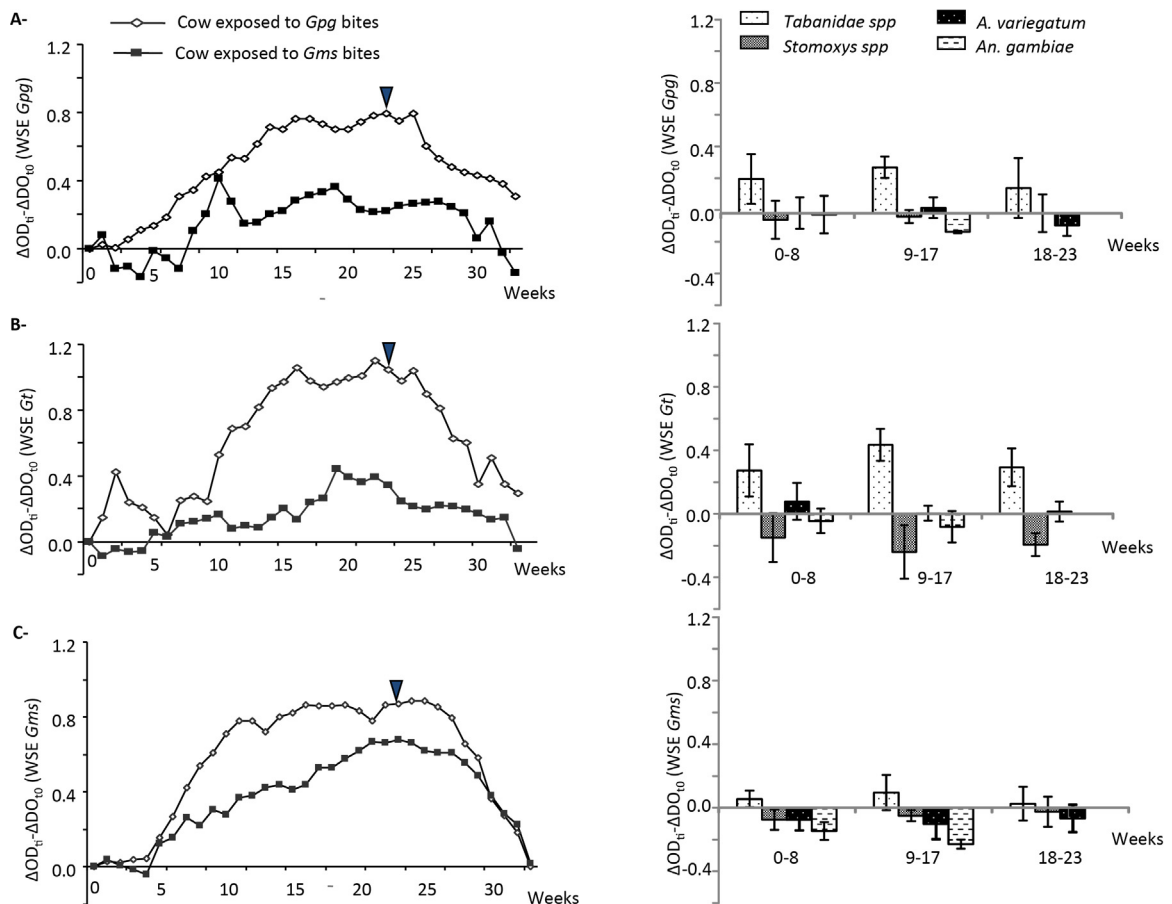


Fig. 3. Monitoring anti-saliva responses in cattle exposed to tsetse and other blood sucking arthropods. Six taurine-zebu crossbred cows were either exposed to *Glossina palpalis gambiensis* (Gpg), *Glossina morsitans submorsitans* (Gms), tabanids, stable flies, mosquitoes or ticks and monitored weekly. Variations in IgG levels ($\Delta OD_{ti} - \Delta OD_{t0}$) to whole saliva extracts (WSE) from Gpg, *G. tachinoides* (Gt) and Gms are shown in A, B and C, respectively. The weekly monitoring of IgG responses in the two cows exposed to tsetse flies are shown on the graphs on the left of the figure. Histograms on the right represent the mean IgG levels (calculated from week 0–8; 9–17 and 18–23) in the four other animals exposed to *Tabanidae* species (spp.), *Stomoxys* spp., *Anopheles* (An.) *gambiae* and *Amblyomma* (A.) *variegatum*. On histograms, vertical bars (above and below) represent the standard deviation of the mean. The ▼ sign indicates the stop of experimental exposure.

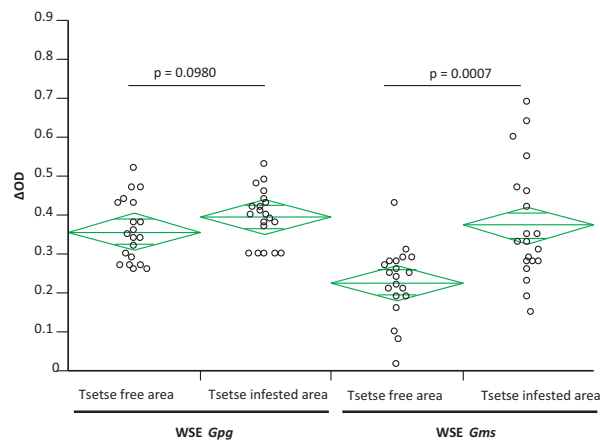


Fig. 4. Antibody responses to *G. m. submorsitans* and *G. p. gambiensis* WSE in cattle from tsetse free and infested areas. IgG responses (ΔOD) to *G. morsitans submorsitans* (*Gms*) WSE of 20 animals from the tsetse free area and 20 animals from the tsetse infested area displaying intermediate response to *G. palpalis gambiensis* (*Gpg*) WSE are shown. The line across each black diamond represents the group mean, the vertical span of each diamond represents the 95% confidence interval (CI) for each group and overlap marks are drawn ($\sqrt{2}CI$)/2 above and below the group mean. P-values calculated by the non parametric Wilcoxon/Kruskal–Wallis test are shown.

mixed linear regression model (Table 2) including the animal number as a random effect, both the time of exposure (in weeks) and the exposure group were significantly associated with increased anti-saliva responses ($p < 0.0001$ and $p = 0.0031$ respectively).

4. Discussion

The host antibody response induced by the saliva of blood sucking arthropods has provided new perspectives to evaluate host–vector contacts and specific anti-salivary immunoassays are now more and more widely used in epidemiological studies to assess the disease risk or the impact of vector control measures (Fontaine et al., 2011).

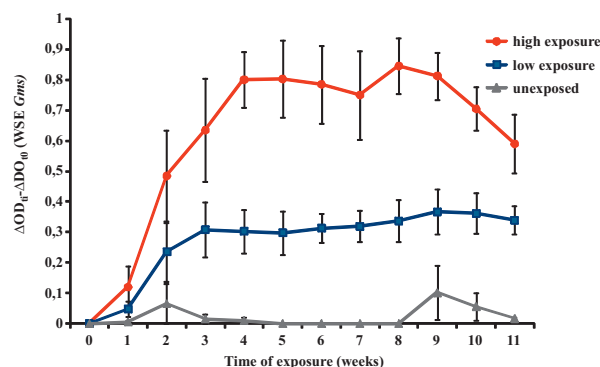


Fig. 5. Monitoring of the tsetse anti-saliva response in cattle exposed to different tsetse exposure levels. The graph represents the evolution of IgG responses ($\Delta OD_{ti} - \Delta OD_0$) to *Glossina morsitans submorsitans* (*Gms*) according to the time of exposure in three groups of cows: high exposure group ($n = 4$; 50 *Gms* flies twice a week); low exposure group ($n = 4$; 10 *Gms* flies once a week); unexposed group ($n = 2$). Vertical bars above and below the lines are the standard errors of the group mean.

Table 2

Association between IgG levels directed against *G. m. submorsitans* WSE and the level of exposure to tsetse bites.

Variables		Means of ΔOD least squares ($\pm$ standard error)	p^a
Exposure group	Control	0.02 $\pm$ 0.09	
	Low	0.27 $\pm$ 0.07	
	High	0.61 $\pm$ 0.07	0.0031
Time of exposure (weeks)		–	<0.0001

Mixed linear regression model of *G. m. submorsitans* saliva specific IgG levels (ΔOD) with the exposure group and time of exposure in weeks as fixed effects and animal as a random effect. Model regression residues were normally distributed (Shapiro–Wilk W test, $p = 0.09$); model R -square = 0.72 ($p < 0.0001$).

^a p -Values < 0.05 are bolded.

An example is given by two recent studies performed to assess the efficacy of insecticide-treated nets (ITNs) in a malaria hypo-endemic transmission area (Drame et al., 2010) and of vector control interventions against Chagas disease (Schwarz et al., 2011). The aim of this study was to evaluate if the cattle IgG responses directed against *Glossina* whole salivary antigens could serve as a biomarker of exposure to tsetse flies and to study its main characteristics in terms of kinetics, sensitivity and specificity by both field based and experimental approaches.

In the tsetse infested area in South-West Burkina Faso we found that *G. p. gambiensis* WSE specific IgG levels were much higher during the dry season compared to samples collected during the rainy season or in cattle from the tsetse free area. Such seasonal variation of antibody responses to blood sucking arthropod's saliva have been previously reported for mosquitoes (Elanga Ndille et al., 2012; Remoue et al., 2006) and sand flies (Hostomska et al., 2008), of which densities are largely influenced by the climatic conditions. At first sight, our results could appear surprising because tsetse flies are abundant and more widely dispersed during the rainy season (Cuisance et al., 1985). But, our data fit well with the herd management system in the study area which is a typical pastoral extensive breeding system of partly sedentary type. In this system cattle spend the rainy season in the vicinity of villages. During this period cattle are poorly exposed to tsetse flies as watering of animals is made around rain-created ponds where tsetse flies are absent. During the dry season animals are herded in transhumance in search of pastures and water along the permanent riverine network and are in close contacts with tsetse flies (Dayo et al., 2010; De la Rocque et al., 1999; Koné et al., 2011). In agreement with the hypothesis that anti-saliva responses are related to the level of cattle exposure to tsetse bites is the finding that animals displaying the highest IgG levels had an increased risk of being infected by trypanosomes ($p = 0.01$). These results are well in line with those of a previous longitudinal epidemiological survey carried out in the area, showing that the dry hot season is associated with an important increase of AAT incidences (Dayo et al., 2010). Such associations between anti-saliva IgG levels and the risk of infection have previously been described in malaria (Andrade et al., 2009; Drame et al., 2010; Remoue et al., 2006), Lyme disease (Schwarz et al., 1991) and leishmaniasis (Rohousova

and Volf, 2006). Importantly however, whereas IgG levels against *G. p. gambiensis* WSE appeared to provide a good indicator of cattle–tsetse contacts, a number of animals in the tsetse free area displayed intermediate responses ($\Delta OD > 0.4$) suggesting that cross reactions with antibody directed against the saliva of other arthropods may have occurred in these animals.

Experimental exposure of animals to different tsetse species and other blood sucking arthropods has enabled to confirm the above findings and to investigate the antigenic properties of the saliva of the different tsetse colonies available at CIRDES (*G. m. submorsitans*, *G. p. gambiensis* and *G. tachinoides*). We were first able to show that the antibody response against tsetse salivary antigens was a dynamic process and that specific IgG responses decreased rapidly (within 10 weeks) to the initial pre-exposure levels once exposure was stopped. These results are well in line with the strong season effect observed in cattle from South-West Burkina Faso exposed to varying exposure levels to tsetse flies along the year. In addition, analysis of IgG responses to *G. m. submorsitans* enabled to differentiate between two groups of cows exposed to different intensities of tsetse exposure as shown in previous experimental studies led with other blood sucking arthropods such as sand flies (Vlkova et al., 2011) or triatomines (Schwarz et al., 2009). Our results thus indicate that in cattle, IgG responses to tsetse salivary antigens are at least partly related to the tsetse exposure level. Nevertheless important differences were also observed between animals of the same exposure group or breeds (zebu or taurine crossbreed cows) regarding both IgG levels and kinetics aspects. At the individual level, evaluation of tsetse contacts by anti-saliva antibodies may thus be impaired by other factors such as the genetic background of the cow or other factors affecting the responsiveness of B lymphocytes to salivary antigens. Nevertheless at the herd level IgG responses to tsetse salivary antigens appear to be indicative of the average level of tsetse exposure. Finally, we recommend the use of *G. m. submorsitans* WSE, at least in West Africa, as these antigens in contrast to those from tsetse species from the *palpalis* group, provided both a good sensitivity and specificity to detect exposure to tsetse bites regardless of the tsetse species used for exposure. Furthermore our results suggest that exposure to tabanids, which are important pests for cattle in many areas, has the potential to induce cross reacting antibodies with *G. p. gambiensis* salivary antigens. A fine analysis of the *G. m. submorsitans* immuno-sialome by a 2D-electrophoresis approach combined with mass spectrometry is now currently underway to identify specific immunogenic antigen candidates to develop a more standardized and specific tool to measure cattle exposure to tsetse bites.

With the PATTEC initiative, area-wide tsetse elimination campaigns are multiplying in sub-Saharan Africa with the objective of creating tsetse free area to promote breeding and the socio-economic development of these regions (Schofield and Kabayo, 2008; Vreysen et al., 2013). The results presented above indicate that the cattle antibody response to tsetse salivary antigens can be used as a direct biomarker of cattle exposure to tsetse flies. It thus constitutes an alternative tool complementary to the evaluation

of tsetse densities by conventional trapping methods. The fact that cattle breeding is largely of pastoral type, results in a high mobility of herds in many regions. Therefore tsetse apparent densities measured by trapping at fixed sites are in many cases poorly representative of cattle exposure to tsetse flies. Monitoring specific IgG responses to tsetse salivary antigens thus appear a more adapted method to evaluate cattle exposure to tsetse bites. Furthermore the rapid kinetics of apparition and disappearance of tsetse saliva antibodies are compatible with the monitoring of vector control campaigns (i) to assess their efficacy to reduce cattle–tsetse contacts and (ii) to detect early re-invasion phenomena after interventions.

Conflict of interest

The authors declare that they have no competing interests.

Authors' contributions

MSB carried out the exposure experiments, immunological analyses and draft the manuscript. ZB conceived the study and participated in its design and coordination. AP participated to the study design and implementation of immunological assays and helped draft the manuscript. ED participated in animal experiments and immunological analyses. GKD participated in the design of the study and provided cattle serum samples from tsetse infested areas. IS and AS helped in the interpretation of results and drafting the manuscript. BB participated to the study design and coordination, analyzed the data and drafted the manuscript. All authors read and approved the final manuscript.

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References

- Andrade, B.B., Rocha, B.C., Reis-Filho, A., Camargo, L.M., Tadei, W.P., Moreira, L.A., Barral, A., Barral-Netto, M., 2009. Anti-*Anopheles darlingi* saliva antibodies as marker of *Plasmodium vivax* infection and clinical immunity in the Brazilian Amazon. *Malar. J.* 8, 121.
- Barclay, H.J., Hargrove, J.W., 2005. Probability models to facilitate a declaration of pest-free status, with special reference to tsetse (*Diptera Glossinidae*). *Bull. Entomol. Res.* 95, 1–11.
- Bouyer, J., Solano, P., Cuisance, D., Itard, J., Frézil, J.L., Authié, E., 2010. Control methods in Trypanosomiasis. In: Lefèvre, P.C., Blancou, J., Chermette, J., Uilenberg, G. (Eds.), *Infectious and Parasitic Diseases of Livestock*, vol. 2. Lavoisier, Paris, pp. 1936–1943.

- Budd, L., 1999. DFID-Funded Tsetse and Trypanosome Research and Development Since 1980. Economic Analysis, Livestock Production Programme. NRInternational, Chatham Maritime, 123 pp.
- Caljon, G., Van Den Abbeele, J., Sternberg, J.M., Coosemans, M., De Baetselier, P., Magez, S., 2006. Tsetse fly saliva biases the immune response to Th2 and induces anti-vector antibodies that are a useful tool for exposure assessment. *Int. J. Parasitol.* 36, 1025–1035.
- Courtin, F., Rayaisse, J.B., Tamboura, I., Serdebeogo, O., Koudougou, Z., Solano, P., Sidibe, I., 2010. Updating the northern tsetse limit in Burkina Faso (1949–2009): impact of global change. *Int. J. Environ. Res. Public Health* 7, 1708–1719.
- Cuisance, D., Février, J., De Jardin, J., Filledier, J., 1985. Dispersion linéaire de *Glossina palpalis gambiensis* et *G. tachinoides* dans une galerie forestière en zone soudano-guinéenne (Burkina Faso). *Rev. Elev. Méd. Vét. Pays Trop* 38, 153–172.
- Dama, E., Cornelie, S., Somda, M.B., Camara, M., Kambiré, R.F., Jamonneau, C., Demetere, V., Seveno, E., Bengaly, M., Solano, Z., Poinsignon, P., Remoue, A., Belem, F., Bucheton, A.M.B., 2013. Identification of *Glossina palpalis gambiensis* specific salivary antigens: towards the development of a serologic biomarker of human exposure to tsetse flies in West Africa. *Microb. Infect.* 15, 416–427.
- Dayo, G.K., Bengaly, Z., Messad, S., Bucheton, B., Sidibe, I., Cene, B., Cuny, G., Thevenon, S., 2010. Prevalence and incidence of bovine trypanosomosis in an agro-pastoral area of southwestern Burkina Faso. *Res. Vet. Sci* 88, 470–477.
- De la Rocque, S., Bengaly, Z., Michel, J.F., Solano, P., Sidibé, I., Cuisance, D., 1999. Importances des interfaces spatiales et temporelles entre les bovins et les glossines dans la transmission de la trypanosomose animale en Afrique de l'Ouest. *Rev. Elev. Méd. Vét. Pays Trop* 52, 215–222.
- Delepiaux, V., Dinka, H., Masumu, J., Van den Bossche, P., Geerts, S., 2008. Five-fold increase in *Trypanosoma congolense* isolates resistant to diminazene aceturate over a seven-year period in Eastern Zambia. *Drug. Resist. Updat.* 11, 205–209.
- Desquesnes, M., Dia, M.L., 2003. *Trypanosoma vivax*: mechanical transmission in cattle by one of the most common African tabanids, *Atylotus agrestis*. *Exp. Parasitol.* 103, 35–43.
- Drame, P.M., Poinsignon, A., Besnard, P., Le Mire, J., Dos-Santos, M.A., Sow, C.S., Cornelie, S., Fomane, V., Toto, J.C., Sembene, M., Boulanger, D., Simondon, F., Fortes, F., Carnevale, P., Remoue, F., 2010. Human antibody response to *Anopheles gambiae* saliva: an immuno-epidemiological biomarker to evaluate the efficacy of insecticide-treated nets in malaria vector control. *Am. J. Trop. Med. Hyg.* 83, 115–121.
- Elanga Ndille, E., Doucoure, S., Damien, G., Mouchet, F., Drame, P.M., Cornelie, S., Noukpo, H., Yamadjako, S., Djenontin, A., Moiroux, N., Misse, D., Akogbeto, M., Corbel, V., Henry, M.C., Chandre, F., Baldet, T., Remoue, F., 2012. First attempt to validate human igg antibody response to Nterm-34kDa salivary peptide as biomarker for evaluating exposure to *Aedes aegypti* Bites. *PLoS Negl. Trop. Dis.* 6, e1905.
- Fontaine, A., Diouf, I., Bakkali, N., Misse, D., Pages, F., Fusai, T., Rogier, C., Almeras, L., 2011. Implication of haematophagous arthropod salivary proteins in host–vector interactions. *Parasites Vectors* 4, 187.
- Holmes, P.H., 1997. New approaches to the integrated control of trypanosomosis. *Vet. Parasitol.* 71, 121–135.
- Hostomska, J., Rohousova, I., Volf, V., Stanneck, D., Mencke, N., Volf, P., 2008. Kinetics of canine antibody response to saliva of the sand fly *Lutzomyia longipalpis*. *Vector Borne Zoonotic Dis.* 8, 443–450.
- Itard, J., Cuisance, D., Tacher, G., 2003. Trypanosomoses: historique-répartition géographique. In: Lefèvre, P.-C., Blancou, J., Chermette, R. (Eds.), Principales maladies infectieuses et parasitaires du bétail, Europe et régions chaudes, vol. 2. Lavoisier, Paris, pp. 1607–1625.
- Kabayo, J.P., 2002. Aiming to eliminate tsetse from Africa. *Trends Parasitol.* 18, 473–475.
- Koné, N., N'Goran, E.K., Sidibé, I., Kombassere, A.W., Bouyer, J., 2011. Spatio-temporal distribution of tsetse (*Diptera Glossinidae*) and other biting flies (*Diptera: Tabanidae* and *Stomoxinae*) in the Mouhoun River Basin, Burkina Faso. *Med. Vet. Entomol.* 25, 156–168.
- Moloo, S.K., Kabata, J.M., Gitire, N.M., 2000. Study on the mechanical transmission by tsetse fly *Glossina morsitans centralis* of *Trypanosoma vivax*, *T. congolense* or *T. brucei brucei* to goats. *Acta Trop.* 74, 105–108.
- Murray, M., Murray, P.K., McIntyre, W.I., 1977. An improved parasitological technique for the diagnosis of African trypanosomiasis. *Trans. R. Soc. Trop. Med. Hyg.* 71, 325–326.
- Pagabeleguem, S., Sangare, M., Bengaly, Z., Akoudjin, M., Belem, A.M., Bouyer, J., 2012. Climate, cattle rearing systems and african animal trypanosomosis risk in burkina faso. *PLoS One* 7, e49762.
- Poinsignon, A., Cornelie, S., Remoue, F., Grebaut, P., Courtin, D., Garcia, A., Simondon, F., 2007. Human/vector relationships during human African trypanosomiasis: initial screening of immunogenic salivary proteins of *Glossina* species. *Am. J. Trop. Med. Hyg.* 76, 327–333.
- Poinsignon, A., Remoue, F., Rossignol, M., Cornelie, S., Courtin, D., Grebaut, P., Garcia, A., Simondon, F., 2008. Human IgG antibody response to *Glossina* saliva: an epidemiologic marker of exposure to *Glossina* bites. *Am. J. Trop. Med. Hyg.* 78, 750–753.
- Remoue, F., Cisse, B., Ba, F., Sokhna, C., Herve, J.P., Boulanger, D., Simondon, F., 2006. Evaluation of the antibody response to *Anopheles* salivary antigens as a potential marker of risk of malaria. *Trans. R. Soc. Trop. Med. Hyg.* 100, 363–370.
- Ribeiro, J.M., Mans, B.J., Arca, B., 2010. An insight into the sialome of blood-feeding *Nematocera*. *Insect Biochem. Mol. Biol.* 40, 767–784.
- Rohousova, I., Volf, P., 2006. Sand fly saliva: effects on host immune response and *Leishmania* transmission. *Folia Parasitol. (Praha)* 53, 161–171.
- Schofield, C.J., Kabayo, J.P., 2008. Trypanosomiasis vector control in Africa and Latin America. *Parasites Vectors* 1, 24.
- Schwartz, B.S., Ford, D.P., Childs, J.E., Rothman, N., Thomas, R.J., 1991. Anti-tick saliva antibody: a biologic marker of tick exposure that is a risk factor for Lyme disease seropositivity. *Am. J. Epidemiol.* 134, 86–95.
- Schwarz, A., Juarez, J.A., Richards, J., Rath, B., Machaca, V.Q., Castro, Y.E., Malaga, E.S., Levy, K., Gilman, R.H., Bern, C., Verastegui, M., Levy, M.Z., 2011. Anti-triatomine saliva immunoassays for the evaluation of impregnated netting trials against Chagas disease transmission. *Int. J. Parasitol.* 41, 591–594.
- Schwarz, A., Sternberg, J.M., Johnston, V., Medrano-Mercado, N., Anderson, J.M., Hume, J.C., Valenzuela, J.G., Schaub, G.A., Billingsley, P.F., 2009. Antibody responses of domestic animals to salivary antigens of *Triatoma infestans* as biomarkers for low-level infestation of triatomines. *Int. J. Parasitol.* 39, 1021–1029.
- Shaw, A.P.M., 2004. Economics of African trypanosomiasis. In: Maudlin, I., Homes, P.H., Miles, M.A. (Eds.), *The Trypanosomiasis*. CABI Publishing, Wallingford, United Kingdom, pp. 369–402.
- Solano, P., Ravel, S., Bouyer, J., Camara, M., Kagbadouno, M.S., Dyer, N., Gardes, L., Herault, D., Donnelly, M.J., De Meus, T., 2009. The population structure of *Glossina palpalis gambiensis* from island and continental locations in Coastal Guinea. *PLoS Negl. Trop. Dis.* 3, e392.
- Van den Bossche, P., de La Rocque, S., Hendrickx, G., Bouyer, J., 2010. A changing environment and the epidemiology of tsetse-transmitted livestock trypanosomiasis. *Trends Parasitol.* 26, 236–243.
- Vikova, M., Rohousova, I., Drahota, J., Stanneck, D., Krudedewagen, E.M., Mencke, N., Otranto, D., Volf, P., 2011. Canine antibody response to *Phlebotomus perniciosus* bites negatively correlates with the risk of *Leishmania infantum* transmission. *PLoS Negl. Trop. Dis.* 5, e1344.
- Vreysen, M.J., Seck, M.T., Sall, B., Bouyer, J., 2013. Tsetse flies: Their biology and control using area-wide integrated pest management approaches. *J. Invertebr. Pathol.* 112, S15–S25.