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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 (Delespaux 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.*) *gambiensis* 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. gambiensis* 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