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Human IgG Antibody Response to *Glossina* Saliva: An Epidemiologic Marker of Exposure to *Glossina* Bites

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Abstract. The evaluation of human antibody response specific to arthropod saliva may be a useful marker of exposure to vector-borne disease. Such an immunologic tool, applied to the evaluation of the exposure to *Glossina* bites, could be integrated in the control of human African trypanosomiasis (HAT). The antibody (IgG) response specific to uninfected *Glossina fuscipes fuscipes* saliva was evaluated according to the vector exposure and trypanic status in individuals residing in an HAT-endemic area. A high level of anti-saliva IgG antibodies was only detected in exposed individuals, whether infected or not by *Trypanosoma brucei gambiense*. In addition, the evaluation of specific IgG response represented spatial heterogeneity according to studied sites. These results suggest that the evaluation of anti-saliva IgG could be an indicator of *Glossina* exposure and thus could be integrated in other available tools to identify populations presenting risks of HAT transmission.

INTRODUCTION

Trypanosoma brucei gambiense, the causative agent of the chronic form of human African trypanosomiasis (HAT), is transmitted by infected *Glossina* bites during the blood meal. The disease occurs in West and Central Africa, where 60 million individuals are exposed to the *Glossina* vector. Considered as a neglected disease, many efforts are conducted under WHO recommendations by systematic screening of populations to diagnose infected individuals and to control HAT transmission. These campaigns have shown their potential efficacy in several countries by reducing HAT incidence, such as in the Democratic Republic of Congo (DRC).[1] The weak sensibility and specificity of the useful diagnostic tools requires urgent attention to develop new diagnostic tools distinguishing *Glossina* exposure, early detection of infection, and HAT morbidity stages. The entomological methods are currently the referent methods to evaluate the densities of *Glossina* population but their major limit is the application to the field conditions as a large scale. The use of a geographical information system seems to be an adequate tool to detect favorable ecologic location for colonization of *Glossina* vector.[2]

HAT morbidity results from complex interactions between the parasite, tsetse fly vector, and human host. Salivary proteins of *Glossina* are injected during the bite to favor the correct blood feeding by using their pharmacologic properties (vasodilatators, anti-platelet aggregation, and blood coagulation inhibitors).[3] In addition, some salivary proteins are immunogenic in inducing a specific immune response with the production of antibodies (Ab).[4,5] Studies on different vectors (*Triatoma, Aedes, Phlebotomus, Anopheles*) have suggested that the evaluation of human specific Ab response to saliva and/or to recombinant salivary protein could evaluate the exposure of individuals to vector bites and thus could be an indicator of risk to pathogens transmission.[6–9] Few studies have explored the immune properties of *Glossina* saliva.[10] Previous study indicated that IgG response to *Glossina morsitans morsitans* saliva and to specific salivary Tsal proteins were detected in exposed and infected Ugandan populations.[5] Recently, our team highlighted the detection of immunogenic salivary proteins of four *Glossina* species. In individuals living in the Bandundu area (DRC) endemic for sleeping sickness, we showed that the profile of these immunogenic proteins was dependent to the *Glossina* species (vector or not) and to the trypanic status of individuals.[4] As a next step and to strengthen our hypothesis to elaborate a marker of *Glossina* exposure based on the saliva immunogenicity, the objective of this study was to evaluate, using enzyme-linked immunosorbent assay (ELISA), the IgG Ab level specific to *Glossina fuscipes fuscipes* saliva according to the vector exposure and to the trypanic status of individuals.

MATERIALS AND METHODS

Studied population. The study was conducted using sera from individuals living in the HAT-endemic area of Bandundu in the DRC.[11] The status of 71 individuals included in the study was defined using serologic, parasitologic, and molecular studies as previously described.[4] These results led to defining two groups of exposed individuals (Exp group): 1) the ENI group, which included exposed but uninfected individuals (N = 52; 8–62 years old) and 2) the EI group, which included exposed and infected patients in the first and second stage of the disease (N = 19; 6–59 years old). A negative control (Nexp group) included individuals (N = 37; 21–76 years old) who live in *Glossina* spp.–free area (personnel from Lapeyronie Hospital, Montpellier, France, and autochthons from Reunion island). The study adhered to the ethical principles defined by the Helsinki Declaration and was reviewed and approved by the local Ethical Committee of the DRC (Public Health Ministry 2001). All individuals enrolled in this study signed an informed consent form.

Saliva collection. *Glossina fuscipes fuscipes* is the main *T. brucei gambiense* vector in the Bandundu area. The salivation technique used enabled analysis of biologic material similar to saliva injected in the vertebrate host during natural blood feeding.[4] Whole saliva extract (WSE) samples from uninfected male and female *G. fuscipes fuscipes* bred in an insectarium (Unit Research 177, IRD) were collected, as previ-

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ously described.[12] Briefly, the *Glossina* were enclosed in a tube and placed above a drop of salivation buffer (10 mmol/L HEPES, 150 mmol/L NaCl, and 5 mmol/L EDTA, pH 7.2) on warm slides (37°C). After 10 minutes of salivation, the saliva solution was collected and stored at −80°C before use. The protein concentration of the saliva solution for female sex (150 μg/mL) and for male sex (250 μg/mL) was evaluated by a bicinchoninic acid test (BCA Protein Assay Kit; Pierce, Rockford, IL). Mixed WSE was done by pooling equal quantities of proteins of both sexes.

Evaluation of human IgG Ab levels. An ELISA technique was carried out using WSE from uninfected mixed male and female *G. fuscipes fuscipes*, and sera were tested for IgG Ab as previously described for *Anopheles*.[9] Maxisorp plates (Nunc, Roskilde, Denmark) were coated with mixed WSE (2 μg/mL) in carbonate/bicarbonate buffer and saturated with blocking buffer (Pierce). Individual sera were incubated (1:120) in phosphate-buffered saline (PBS)-Tween 1%, and IgG detection was performed using a mouse anti-human IgG biotinylated mAb (BD Pharmingen, San Diego, CA). Peroxidase-conjugated streptavidin was added (Amersham, Les Ulis, France), and colorimetric development was carried out using [2,2'-azino-bis (3-ethylbenzthiazoline 6-sulfonic acid] diammonium (ABTS; Sigma, St Louis, MO) in 50 mmol/L citrate buffer (pH 4) containing 0.003% H_2O_2. Absorbance (OD) was measured at 405 nm. In addition, the absence of significant Ab detection in wells without antigen was verified (ODn). Individual results were expressed as the ΔOD value calculated according to the formula: ΔOD = ODx − ODn, where ODx represented the individuals OD value with antigens wells. A subject was considered as an "immune responder" if its ΔOD was higher than ΔOD mean + (3 × SD) in the unexposed group (Nexp; ΔOD = 0.389).

Statistical analysis. All data were analyzed with the GraphPad Prism software (GraphPad, San Diego, CA). After verifying that values did not assume a Gaussian distribution, the non-parametric Mann-Whitney *U* test was used for comparison of Ab level between two independent groups and the non-parametric Kruskal-Wallis test for comparison between more than two groups. All differences were considered significant at $P < 0.05$.

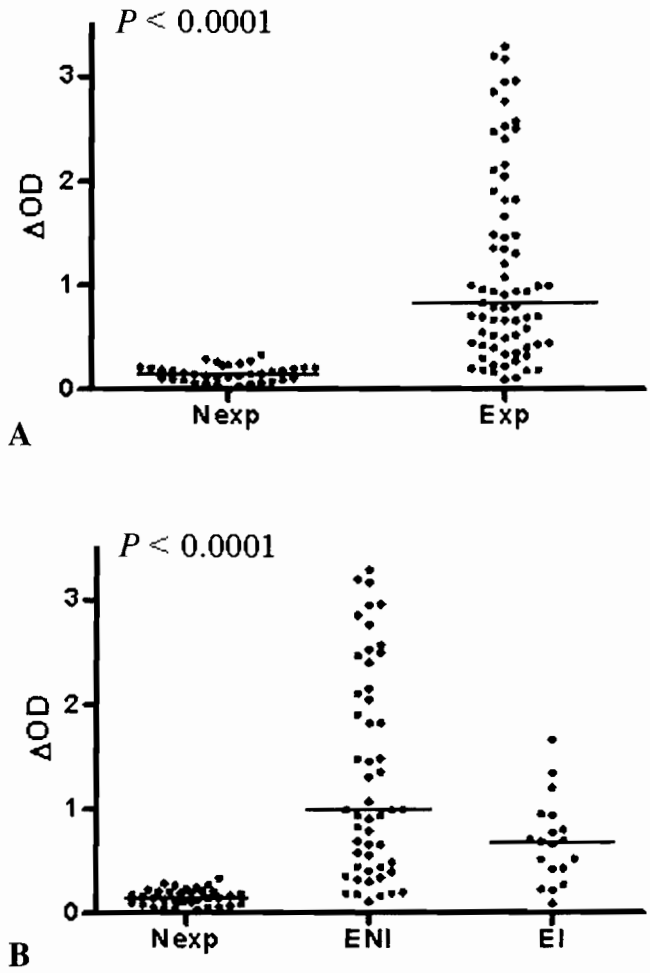

FIGURE 1. Individual IgG Ab levels specific to saliva of the *G. fuscipes fuscipes*. Individual ΔOD results are presented, and bars indicate the median value for each group. The results are presented according to the exposure status **A**, either exposed (Exp) or not (Nexp) to *Glossina* bites, and according to HAT infected status **B**, either exposed but uninfected (ENI) or exposed and infected (EI). Statistical significance between three groups is indicated (non-parametric Kruskal-Wallis test).

RESULTS AND DISCUSSION

The anti-saliva IgG level was compared between individuals exposed (Exp = ENI + EI groups) or not (Nexp group) to *Glossina* bites (Figure 1A). Eighty percent of the exposed individuals were "immune responders" for anti-saliva IgG. The diagnostic value of this specific ELISA test presents the following characteristics: sensibility = 80%, specificity = 100%, positive predictive value = 100%, and negative predictive value = 73%.

A high level of specific IgG Abs was observed in exposed individuals (median = 0.827), whereas baseline IgG level was very low in the Nexp group (median = 0.148). The difference in specific IgG response was highly significant between both groups ($P < 0.0001$). The individuals from the Nexp group, despite living in an area free from *Glossina*, could be exposed to others hematophagous arthropods (*Aedes* spp., *Culex* spp., or *Anopheles* spp.), especially those from Reunion Island.[13] The very low baseline of specific IgG level in the Nexp group could indicate the absence of immune cross-reactivity of salivary proteins between *Glossina* and others arthropods. Nevertheless, possible cross-reactivity of tsetse saliva with other *Brachycera* flies, such as tabanids or stable flies, which are more closely related to tsetse than mosquitoes can not be excluded. Altogether, the results suggest that the anti-saliva IgG response could be a selective marker of exposure to *Glossina* bites. In addition, the level of anti-saliva IgG Ab was compared between the Nexp group and infected (EI group) or uninfected (ENI group) individuals (Figure 1B). The specific IgG response remained significantly higher in the ENI group compared with the Nexp group ($P < 0.0001$). However, the anti-saliva IgG response was significantly lower in the EI group compared with the ENI group ($P = 0.0371$). In a subanalysis according to age (< 35 and > 35 years of age), the difference between the EI and ENI groups was only significant for individuals < 35 years of age (data not shown). Despite several parameters (history of exposure, environmental factors, etc) that could explain this difference, these results

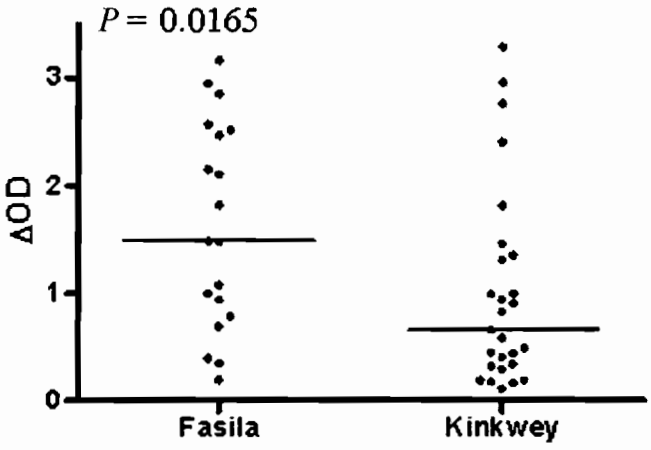

FIGURE 2. Individual IgG Ab levels specific to saliva of the *G. fuscipes fuscipes* in ENI group according to studied villages. Individual ΔOD results are presented, and bars indicate the median value for each group. The results of the exposed but uninfected individuals (ENI group) are shown according to their dwelling place in the region of Bandundu (DRC). Statistical significance between both groups is indicated (non-parametric Mann-Whitney *U* test).

suggest a possible influence of an immuno-suppression phenomenon in HAT-infected individuals as previously described.[14] Nevertheless, anti-saliva IgG level remained considerably higher in the infected (EI group) than in the uninfected (Nexp group) individuals ($P < 0.0001$). This result indicates that the anti-saliva Ab response could be a useful marker of *Glossina* exposure even if individuals are immunosuppressed because infected.

The anti-saliva IgG response was compared only in ENI individuals according to their dwelling villages with the objective of evaluating the possible spatial heterogeneity of exposure to *Glossina* bites (Figure 2). The specific IgG Ab levels differed significantly ($P = 0.0165$) between the two studied sites. This first approach suggests that the evaluation of anti-saliva IgG response could discriminate the difference of *Glossina* exposure according to studied site, as previously described for *Aedes* exposure.[7] Nevertheless, further studies including entomologic assessments of precise *Glossina* exposure are necessary to confirm these results according to different levels of exposure.

This study indicated that IgG response to whole saliva could be a potential marker of exposure to *Glossina* bites and, probably, an indicator of the spatial heterogeneity of exposure. However, an optimal marker, in terms of specificity to *Glossina* species (i.e., to avoid cross-reactivity with others arthropods) would be obtained by identifying immunogenic salivary proteins specific 1) to *Glossina* species vector of *Trypanosoma brucei gambiense* and 2) to infected bites.

This study, in complement to previous results identifying species-specific immunogenic salivary proteins and evaluating Ab response to specific recombinant proteins, highlights the potential use of anti-salivary protein Ab response as an immuno-epidemiologic indicator of *Glossina* bite exposure.[4,5] This tool could allow the mapping and delimitation of foci where the HAT disease prevails and could be integrated into WHO strategies on HAT control in Africa.

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