A novel immunoassay to measure human blackfly exposure in onchocerciasis endemic areas

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Background: Blackflies of the *S. damnosum sensu lato* (s.l.) complex are the main vectors of human onchocerciasis in Africa. Vector biting rates and heterogeneity in exposure to vector bites are important epidemiological determinants. To date, the only way to estimate blackfly biting rates and exposure patterns is by performing human landing catches (HLCs). Since this is a labour-intensive technique associated with ethical concerns, novel alternatives to measure blackfly exposure are required. We aimed to develop IgG- and IgM-based immunoassays to quantify human antibody responses to blackfly saliva in onchocerciasis endemic communities and to examine demographic patterns in human blackfly exposure using the measured anti-blackfly antibody responses.

Methods: Blackflies were collected by HLCs in an onchocerciasis-endemic area in Ghana from which 940 salivary glands were dissected. The salivary glands were used as antigen to develop two enzyme-linked immunosorbent assays (ELISA) to measure anti-*S. damnosum* s.l. salivary IgG or IgM antibodies. Also, blood samples from 958 people (aged 5 to 95 years) living in 4 endemic villages were collected. Samples from people living in Accra, a blackfly-free area, were used as negative controls together with samples from blackfly-free locations in Sudan. The relationship between anti-blackfly antibodies and village, sex, and age was investigated using generalized linear models.

Results: Both immunoassays were successfully developed and were able to differentiate negative controls from village residents. Overall, males showed a significantly lower IgG response than females. A significant decline in antibody response with increasing age was observed for both IgG and IgM responses. The decline in IgG appeared steeper for adult males than adult females.

Discussion/Conclusion: In the present study, human IgG and IgM immunoassays were successfully developed as a novel tool to quantify exposure to blackfly bites and better understand patterns of exposure. Anti-blackfly antibody responses were shown to differ between sexes and to decline with age. This trend has been previously observed for *Anopheles* mosquitoes, indicating an age-associated desensitization in areas with high biting pressures.