PvAMA1-MSP1: A novel chimeric recombinant protein for the evaluation of serological responses in the Peruvian Amazonia.

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BACKGROUND: Serological markers have been proposed as alternative tools to monitor and assess malaria intensity in unstable and low transmission settings. It is recommended to use multiple antigens for optimizing the sensitivity of the antibody detection against Plasmodium species and determining past malaria exposure. This work aimed to evaluate the antibody reactivity against a novel chimeric protein containing the Apical Membrane Antigen 1 (AMA-1_{66}) ectodomain and the 19 kDa carboxyl fragment of the Merozoite Surface Protein 1 ($MSP1_{19}$) of. *Plasmodium vivax* ($PvAMA1_{66}$-$MSP1_{19}$) on dried blood samples (DBS) from individuals living in the Peruvian Amazon.

MATERIALS AND METHODS: A total 823 DBS on filter paper were collected from individuals living in the Mazan district, and analyzed using indirect enzyme-linked immunosorbent assay (ELISA) to measure *P. vivax* immunoglobulin G (IgG) responses. Three antigens were used in the analysis: PvAMA1, $PvMSP1_{19}$, and their chimaera. To ensure a standardization of the sample results across ELISA plates, the percent positivity (PP) of each sample was calculated using the OD of the positive control serum as 100%. Then, the cut-off of PPs for seropositivity (indicating malaria exposure) was generated using a mixture model. Kappa (k) was used to evaluate agreement between serological results.

RESULTS AND CONCLUSIONS: The proportion of seropositivity according to the antigen used was: 27.2% (n=224) for $PvMSP1_{19}$, 33.7% (n=277) for PvAMA1, and 33.9% (n= 279) for $PvAMA1_{66}$-$MSP1_{19}$. Seropositivity to any of both independent antigens ($PvMSP1_{19}$ and/or PvAMA1) was 39.6% (n=326). An almost perfect agreement (k=0.867, p<0.001) was found between seropositivity levels to the chimeric protein $PvAMA1_{66}$-$MSP1_{19}$ and those to any of both independent antigens ($PvMSP1_{19}$ and/or PvAMA1). The results suggests that antibody responses to the novel recombinant protein $PvAMA1_{66}$-$PvMSP1_{19}$ provide similar information about malaria exposure than antibody responses to both single antigens, with the advantage of processing only one ELISA analysis per sample instead of performing two separated analyses per sample with single antigens.