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Sparse serological evidence of *Plasmodium vivax* transmission in the Ouest and Sud-Est departments of Haiti

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ABSTRACT

Background: Plasmodium vivax infections, while quite prevalent throughout South and Central America, are virtually non-existent in Haiti, where *P. falciparum* infections are detected in over 99% of malaria cases. Historically, few cases of *P. vivax* have been reported in Haiti; all of which were identified by microscopy and none were confirmed by molecular diagnostics. To further examine the transmission of *P. vivax* in Haiti, a cross-sectional seroepidemiological study was conducted.

Methods: Whole blood was collected from 814 community members and school children ranging in age between 2 and 80 years-of-age from four locations in the Ouest and Sud-Est Departments of Haiti. After separation of serum, samples were screened for antibodies toward *P. vivax* apical membrane antigen (AMA-1) and merozoite surface protein-1_{19} (MSP-1) using an indirect enzyme-linked immunosorbent assay (ELISA).

Results: Of all participants screened, 4.42% (36/814) were seropositive for AMA-1, 4.55% (37/814) were seropositive for MSP-1, 7.99% (65/814) were seropositive to either antigen, and only 0.98% (7/814) were seropositive for both antigens. Seroconversion rates (SCR) for AMA-1, MSP-1, either AMA-1 or MSP-1, and for both AMA-1 and MSP-1 estimated from the cross-sectional seroprevalence indicated rates of *P. vivax* transmission of less than 1% per year.

Conclusion: Given the lack of historical evidence of *P. vivax* infections on the island of Hispaniola, the sparse serological evidence of antibodies toward *P. vivax* identified in the current study further support the notion that the transmission of *P. vivax* malaria might be extremely low or even completely absent in Haiti.

1. Introduction

Malaria remains the world's most important parasitic disease, with an estimated 200 million cases and over 400,000 deaths each year (WHO, 2015). Malaria is primarily caused by infection from four species of *Plasmodium* parasites (*P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*), of which *P. falciparum* and *P. vivax* are responsible for the majority of cases (CDC, 2013). *P. vivax* infections, while quite prevalent throughout South and Central America, are rare in Haiti, where *P. falciparum* is responsible for over 99% of malaria cases (Lindo et al., 2007; Agarwal et al., 2012). However, the introduction of *P. vivax* from the surrounding endemic countries could sustain undetectable and recurrent transmission, which could undermine current efforts to eliminate malaria from the island of Hispaniola (Boncy et al., 2015). After a thorough review of the literature available on *P. vivax* transmission in Haiti, only two suspected cases were identified in the past 50 years (Lindo et al., 2007; PAHO, 2006). Both reports originated from refugees and travelers returning from Haiti, where *P. vivax* was only identified by microscopy, which has been demonstrated to be of poor predictive value in Haiti (Patrick

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Kachur et al., 1998); neither confirmed the presence of *P. vivax* by polymerase chain reaction (PCR). Supporting this notion even further, a recent study conducted in the Ouest Department, found no evidence of *P. vivax* in patients with mixed malaria infections (*P. falciparum* and other *Plasmodium* species) as identified by rapid diagnostic tests (Weppelmann et al., 2013). Given the lack of evidence of *P. vivax* cases reported in Haiti from passive surveillance (Jelinek, et al., 2000; WHO, 2015), we conducted this cross-sectional seroepidemiological survey in the Ouest and Sud-Est Departments to investigate the transmission of *P. vivax* in Haiti. The objective of this study was to measure the proportion of community members between the ages of 2–80 years-of-age with antibodies toward two *P. vivax* antigens in order to more accurately characterize the likelihood of both recent and historical *P. vivax* infections in Haiti.

2. Materials and methods

2.1. Ethical approval

This research was approved by the Haiti Ministry of Health Ethical Review Committee, the University of Florida Institutional Review Board, and the Office of Research Protections, United States Army Medical Research and Materials Command.

2.2. Sample collection

Between February and May 2013, a convenience sample of non-febrile participants between the ages of 2 and 80 years-of-age (n = 814) were enrolled from four different sample collection sites in the Ouest and Sud-Est departments of Haiti (Fig. 1). The sampling included two school based enrollments of healthy children from the Christianville School in Gressier and the Hosana Baptist School in Jacmel, individuals seeking care from a mobile clinic in the rural community of Chabin, and from healthy family members of patients seeking care in the Portail Leogane Clinic in Jacmel. Detailed demographic information on the participants is presented in Table 1 by the site of enrollment. After collection of 3 ml of blood by venipuncture, serum was separated by centrifugation at 6000 RPM for two minutes, and stored at −80 °C at the University of Florida Field Laboratory located in Gressier, Haiti prior to transportation to the Emerging Pathogens Institute at the University of Florida in Gainesville, Florida, USA for serological analyses.

2.3. Determination of previous exposure by ELISA

Serum samples were screened for antibodies toward *P. vivax* apical membrane antigen (AMA-1) and merozoite surface protein-1 (MSP-1) using an indirect enzyme-linked immunosorbent assay

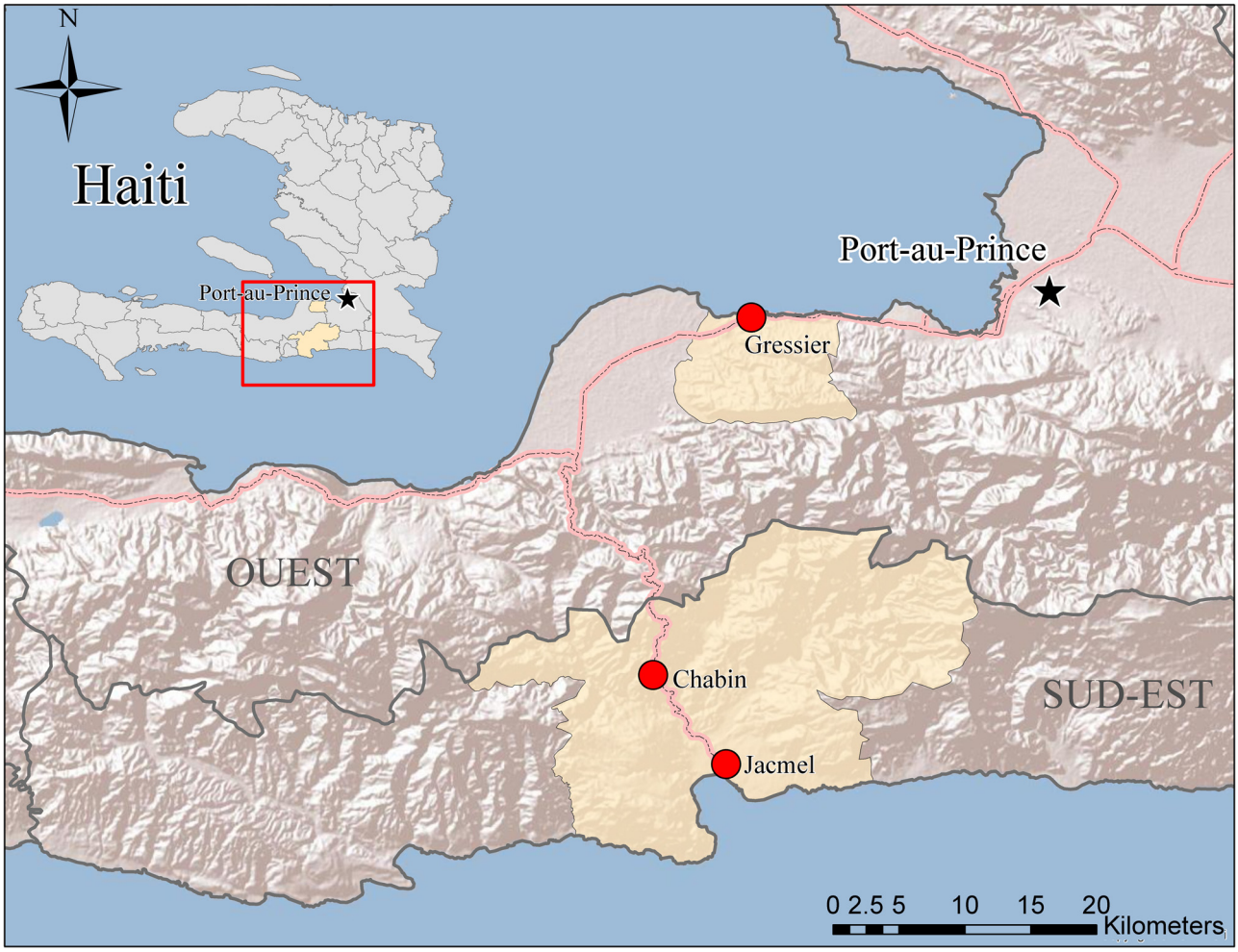

Fig. 1. Study enrollment and sample collection sites in Haiti. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
The study enrollment sites where blood samples were collected are located in the Ouest and Sud-Est Departments of Haiti and shown with respect to the national highway system (pink line). Enrollment sites (red dots) included Gressier (n = 506), Jacmel (n = 177), and Chabin (n = 131), which were located in the communes of Gressier and Jacmel (beige shaded regions enlarged map).