



Final Report

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4.1 Final publishable summary report

4.1.1. Executive Summary

Plasmodium Vivax (*P.vivax*) is the most common and widely distributed human malaria parasite and causes up to 80 million cases annually, with the majority occurring in Asia and the Western Pacific, Central and South America and the Middle East. It has been estimated that globally 93 million pregnancies occurred in areas endemic for *P.vivax* in 2007.

While the effects of *Plasmodium Falciparum* (*P. falciparum*) malaria in pregnancy have been well characterised and are responsible for considerable maternal and infant morbidity and mortality, surprisingly little is known about the burden and impact of *P.vivax* infection on maternal and foetal health. Some studies indicate adverse effects of *P.vivax* malaria in pregnancy; yet, they all provide only partial information on the epidemiological and clinical aspects of the infection in pregnancy and none on the mechanisms involved. This gives us a disconcerting and incomplete picture of the true burden and impact of *P.vivax* in pregnancy.

The **PregVax Consortium** started back in 2008 to address those knowledge gaps in *P.vivax* infection during pregnancy. It has brought together eleven research institutions and some of the best multidisciplinary scientists and experts on *P.vivax* malaria from a variety of countries and regions across the world. Under the umbrella of this consortium a cohort observational study of pregnant women from five *P.vivax* endemic countries (**Brazil, Colombia, Guatemala, India and Papua New Guinea**) broadly representing most of the world's *P.vivax* infections has been conducted. This study describes the epidemiological and clinical features of *P.vivax* malaria in pregnancy following standardized methods for data collection. In addition, the PregVax Consortium has been working to determine whether there are pregnancy specific *P.vivax* immune responses and characterize genotypically and phenotypically the parasites of the placenta.

In an unprecedented effort, almost 10,000 pregnant women have been enrolled at the different project sites during their routine antenatal care visits and followed-up at the health facility until delivery. Recruited women have been: 1696 in Brazil, 2039 in Colombia, 2009 in Guatemala, 2050 in India and 1679 at PNG. Deliveries attended and included in the study have been: 905 in Brazil, 783 in Colombia, 1025 in Guatemala, 1332 in India and 935 at PNG. This means that about 53% of the women participants in the study were followed up until delivery showing a low rate of loss to follow-up during the study. In the context of this large cohort of pregnant women, almost 98.000 study visits have occurred. A database has been built up that includes information from near 98.000 questionnaires and almost 77.000 biological samples have been collected. Find a summary in Annex I.

Although *P.falciparum* is the most deadly species and the subject of most malaria-related research and literature, more attention should be given to *P.vivax*. So far, with PregVax study, some progress has been made and further evidence is becoming available. This recent evidence suggests that ***P.vivax* malaria can have deleterious effects on the health of the mother and the neonate. More accurate data of vivax malaria during gestation are essential to improve its clinical management and to guide control policies.** Furthermore, elucidating the mechanisms involved in the pathology of *P.vivax* in pregnancy **will help to develop specific control tools such as more effective drugs and vaccines.**

4.1.2 Summary Description of the Project Context and Objectives

Despite the reality that *P.vivax* is the most widely distributed human malaria parasite, it has been the focus of surprisingly little research. *Vivax* malaria accounts for approximately 70 to 80 million cases worldwide per year, and for more than half of the malaria cases reported in Latin America, the Middle East and Asia. Of the total *P.vivax* cases, 65% occur in Southern Asia and the Western Pacific, 12.5% in South America and 4.5% in Central America². While *P. falciparum* in Africa, the region with the highest concentration of *falciparum* malaria cases, affects primarily children, *P.vivax* affects all age groups. Very little is known about the burden of *P.vivax* in pregnancy and its impact on maternal and foetal health. Furthermore, the health and economic impact associated with *P.vivax* malaria has not been established, but it is likely to be considerable.

PregVax was devised in order to respond to all these knowledge gaps on *P.vivax* from an epidemiological perspective, including evaluation of burden and impact, pregnancy-specific immunological responses and histopathological changes in the placenta. Furthermore, ancillary studies have been conducted to assess the economic impact of *P.vivax* infection during pregnancy.

Objectives and milestones have been classified in workpackages, being fundamentally:

- WP1 - Epidemiology: To determine the burden (prevalence and incidence) of *P.vivax* malaria in pregnancy in different epidemiological settings and its impact on pregnancy outcomes (mother and newborn).
- WP2 - Immunology: To study naturally acquired antigen-specific antibody and cellular immune responses in pregnant women infected with *P.vivax* parasites
- WP3 - Genotyping and Molecular Diagnosis: To evaluate the presence of submicroscopic infections by molecular diagnosis and to characterize the genetic structure and transmission dynamics of *P.vivax* parasites in different epidemiological regions
- WP4 – Adhesion: To study the adhesive features of *P.vivax* infected red blood cells eluted from the placenta and from the peripheral blood, including rosetting
- WP5 - Histopathology: To evaluate the placental histological changes associated with *P.vivax* infection during pregnancy and their possible impact on maternal and foetal health, to investigate whether *P.vivax*-infected erythrocytes accumulate in the placenta and to evaluate whether there is an immune cell reaction in the placenta and to evaluate its cell composition.
- WP6 - Management: To coordinate the technical activities of the project and the overall financial and administrative management, in order to accomplish the milestones of WP1-5, and to disseminate knowledge within the scientific community, raise public interest on the project and public health in general and generate public awareness and participation.

4.1.3. Main Scientific & Technical results/foregrounds

The project has been successful in defining a clearer framework of the true burden of *P.vivax* infection among pregnant women and its impact on maternal and foetal health. In order to accomplish this, the PregVax project has set up an unprecedented and unique multi-country research platform, a very large cohort of women enrolled during gestation and followed up until delivery. After 5 years of intensive work, the PregVax project has got to its last stage. Definitive results from

² Mendis, K., Sina, B.J., Marchesini, P., and Carter, R. The neglected burden of *Plasmodium vivax* malaria. *Am J Trop Med Hyg* 2001; 64(1-2 Suppl): 97-106.

the project will be soon available. Nevertheless, among the preliminary findings there are already key data:

- The **prevalence of *P. vivax* malaria detected by microscopy** during pregnancy **was low across sites**. However, molecular diagnosis (by RT-PCR) detected substantially more *P.vivax* and *P.falciparum* infections than microscopy, suggesting a **high proportion of submicroscopic infections**. To what extent these submicroscopic infections may have an impact on maternal and foetal health is not yet known since no study had previously attained this research question. Furthermore, they emphasise the importance of understanding the role of asymptomatic carriers on transmission. Submicroscopic infections are becoming a key issue in low endemicity settings and in a context of malaria control and elimination. The PregVax project is by far in the best position to respond to these knowledge gaps.
- Under the umbrella of the PregVax project, **the first *P.vivax* congenital malaria case in Guatemala, and the first in Latin America**, with genotypical, histological and clinical characterization has been described. The findings show that maternal *P.vivax* infection still occurs in areas that are in the pathway towards malaria elimination, and can be associated with detrimental health effects for the neonate. It also highlights the need in very low transmission areas of not only maintaining, but increasing awareness of the problem and developing surveillance strategies to detect the infection, particularly in most vulnerable groups of the population.
- So far, evidence of the presence of *P.vivax* in the placenta had been scarce and inconclusive. This information is relevant to understanding whether *P.vivax* affects placental function and how it may contribute to poor pregnancy outcomes. A histopathological and molecular study of placental infection with *P.vivax* conducted as part of PregVax objectives has shown that ***P.vivax* can be associated with placental infection and might be able to sequester in the placenta**. Placental inflammation was not observed in *P.vivax* placental malaria cases differently to what has been well described for *P.falciparum* infection; what suggests that other causes of poor delivery outcomes must be associated with *P.vivax* infection.
- Cytoadhesion phenotypes with a clinical impact in *P.vivax*. Cytoadhesion of infected erythrocytes (IE) may contribute to mild *P.vivax* sequestration although its physiological impact remains still unknown. In this study we aimed to describe clinically relevant cytoadhesive phenotypes of *P.vivax* IEs isolated from pregnant and non-pregnant malaria patients in the Brazilian Amazons. It was seen that **Rosetting and cytoadhesion to CSA in *P.vivax* infections may negatively impact the health of infected hosts**.

4.1.4. Potential Impact

Despite global efforts, malaria continues to represent a huge health burden, particularly to most vulnerable populations, pregnant women and infants. While the effects of *falciparum* infection are well-known, at least in sub-Saharan Africa, and specific policy recommendations have been developed, the reality is that little focus has been devoted to *P.vivax*, both in research, advocacy and more importantly in specific policy guidance.

This project provides accurate data on the burden and impact of *vivax* malaria during gestation. This is essential to improve its clinical management and to guide malaria in pregnancy control policies in low transmission settings. Besides, elucidating the mechanisms involved in the pathology of *P.vivax* in pregnancy, as for instance, the histopathological and molecular features of placental *P.vivax*

infection, and the cytoadhesion phenotypes with a clinical impact in *P.vivax*, investigated as part of this project, may help to develop specific control tools such as more effective drugs and vaccines.

Main results of PregVax project are expected to inform and guide key global recommendations for malaria control during pregnancy in low transmission settings. This is aligned with the current goal of the World Health Organization aimed to develop an evidence-driven *Global Strategic Plan for P.vivax Control and Elimination* that will be an integral part of the *Global Technical Strategy for Malaria Control and Elimination, 2016-2025*. PregVax project will genuinely contribute to identify the most adequate approach in clinical management, epidemiological surveillance, and preventive strategies for malaria control during pregnancy in areas where *P.vivax* malaria transmission occurs.

4.1.5. Website and Contacts

PregVax Project Logo:



PregVax website: www.pregvax.net

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Annex I: General figures for PregVax Cohort.

		Brazil	Colombia	Guatemala	India	PNG	Total
ANC Scheduled Visits	1 st ANC Visit (Enrolment)	1696	2039	2009	2050	1679	9473
	2 nd & 3 rd ANC Visit (Follow up visits)	1537	1754	1324	604	2358	7577
Non-Scheduled Visits	Through Passive Case Detection	118	202	63	53	838	1274
Deliveries		905	783	1025	1332	935	4980
Questionnaires in the database		~17000	~18000	~20000	~22000	~21000	> 98000

Annex II: PregVax in Action.



Fig. 1: Dr. Ordi showing results from WP5 during the 3rd PregVax Investigators Meeting held at New Delhi (Jan 2012). Fig. 2: Antenatal clinic visit in Tierra Alta, Colombia (Jun 2011). Fig. 3: Group picture at the 3rd PregVax Investigators Meeting held at New Delhi (Jan 2012). Fig. 4: Reading of a blood thick smear slide by a trained microscopist at Madang, PNG (Nov 2012). Fig. 5: Retrieving data in Fray Bartolomé de las Casas, Guatemala (Jun 2011). Fig. 6: Assessment of a newborn to a study woman in Fray Bartolomé de las Casas, Guatemala (Sept 2011). Fig. 7: A post-partum household visit of a study women in Manaus, Brazil (Jul 2011). Fig. 8: Enrolment of a pregnant women at the antenatal clinic in Bikaner, Rajasthan, India (Nov. 2010). Fig. 9: Internal seminar of PregVax work package leaders to share knowledge on *P.vivax* malaria, Barcelona, Spain (Apr. 2012).

