Proposal: Parasitologic correlates of genomic differences in *Plasmodium ovale wallikeri* and *Plasmodium ovale curtis*i

Applicant: Melissa Phuong, McMaster University

Supervisor: Dr. Andrea K. Boggild, University of Toronto

Background

Plasmodium ovale is one species of malaria parasite that is pathogenic to humans[1]. Prevalent in West Africa[1], *P. ovale* infections can be challenging to diagnose in part due to the typical presentation of clinically mild disease and low burden of parasites. Consequently, conventional diagnostic tools, such as microscopy of blood films and rapid antigen-based diagnostic tests (RDT), have limited performance in detecting *P. ovale* infection[2,3], often necessitating the use of molecular techniques to confirm the diagnosis[4].

Two genetically distinct sub-species of *P. ovale* exist: *P. ovale curtisi* (classic type) and *P. ovale wallikeri* (variant type). At present, it is unknown if the performance of malaria diagnostic tests may be affected by the sub-species causing the infection. Consequently, investigating if parasite burden, RDT performance, molecular diagnosis and expression of virulence factors differ between the two sub-species may have important implications for the laboratory diagnosis and management of those infected with *P. ovale*.

Objectives

The aim of this project is to understand parasitologic correlates of genomic differences in the two sub-species of *P. ovale*. RDT performance and thin film microscopy results. Gene sequences of virulence factors between *P. o. curtisi* and *P. o. wallikeri* will be compared to better understand how genetic differences may contribute to clinically relevant parasitologic parameters.

Methods

P. ovale-positive, whole-blood specimens that were previously examined at Public Health Ontario Laboratories (PHOL) over the last five years and banked at −80°C following diagnostic testing will be identified. The parasitemia and RDT performance that were recorded upon initial processing will also be retrieved from the biobank database for analysis.

Using 200 µl of frozen whole blood per specimen, the DNA of these specimens will be extracted using the DNA Minikit blood or body fluid spin protocol (Qiagen, Germantown MD). The extracted DNA will then be eluted with 60 µl AE buffer and stored at −20°C prior to use.

Human beta-2-microglobulin (B2MG) extraction control and confirmatory *Plasmodiu*m genus real-time PCR (qPCR) assays will be conducted. To confirm microscopy diagnosis and eliminate the possibility of a mixed-infection, *P. falciparum*/*P. vivax* species-specific duplex and *P. malariae*/*P. ovale* species-specific duplex qPCR as previously described by Khairnar et al.[4], Shokoples et al.[5] and Phuong et al.[6] will be carried out.

18S gene copy numbers can be quantified by using serial dilutions of a *P. ovale* clone and including them in each run of the *P. malariae*/*P. ovale* species-specific duplex qPCR assay. The logarithm of the gene copy number will then be plotted against Ct values for each concentration

of the clone. A linear regression can be constructed from this graph, and the resulting equation will calculate the gene copy number for each banked specimen.

Endpoint PCR of the virulence target regions will be conducted, and amplicons will be visualized on agarose gels to confirm amplification prior to sequencing. PCR products will then be purified and Sanger sequenced with a BigDye Terminator v3.1 cycle sequencing kit (Life Technologies). Sequenced products will be purified and analyzed by an ABI 3130xl genetic analyzer, and the results from each sample will be aligned using Vector NTI software (Life Technologies). Alignments of *P. ovale* sequences from all cases will be performed using Mega5.2 software[7].

Reported parasitemia, RDT result, PCR result, 18S gene copy number and the sequences of chloroquine resistance transporter (*crt*), dihydrofolate reductase (*dhfr*), dihydropteroate synthase (*dhps*), tryptophan-rich antigen (*tra*) and reticulocyte binding protein 2 (*rbp2*) between both sub-species will be compared to elucidate those that are clinically relevant. Regarding statistical analysis, continuous and categorical variables will be compared using Student's t-test and Yates' corrected Chi-squared analysis respectively. The level of significance will be set at $p<0.05$, and all computations will be performed using GraphPad Prism or SigmaStat software.

Anticipated Significance

The goal is to improve the diagnostic accuracy of testing at PHOL for *Plasmodium ovale* and to elucidate the role of genetic differences in the two parasite sub-species to diagnostic performance characteristics. There is also the intent to contribute to the larger scientific community in the form of a peer-reviewed publication, and presentation at a scientific conference.

References

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