Evidence summaries present new developments, innovations and research relevant to the work of National Malaria Control Programs in the Asia Pacific region. This brief was prepared by Dr Sarah Auburn from the APMEN Vivax Working Group Coordinating Team to provide a general overview. Country Partners are encouraged to use this information as a basis for ongoing discussions and planning within their own programs and the APMEN Vivax Working Group.

RATIONALE:
Imported malaria presents a major challenge to malaria elimination. Where local conditions support transmission, imported cases may hamper intervention efforts and enhance the risk of re-introduction in low or non-endemic settings. The introduction of new parasite strains against which individuals have no protective immunity enhances the risk of disease manifestation, and local outbreaks. In *P. vivax*, the ability of the hypnozoite stage to lie dormant in the liver for weeks or months before relapsing presents further challenges by enhancing the “mobility” of parasites, complicating the discrimination of imported from indigenous cases, and complicating geographic mapping of imported cases. Particularly in low endemic settings where the relative proportion of imported *P. vivax* cases is rising, effective tools are needed to 1) identify, and 2) map these cases.

INCLUSION CRITERIA
- Selected publications in the last decade
- APMEN Country Partner Technical Development Program results

LIST THE KEY STUDIES, PUBLICATIONS, TOOLS

ANTIGEN-ENCODING MARKERS
Several studies have attempted to identify and map imported *P. vivax* cases using genes encoding parasite surface proteins [1-3]. These genes have demonstrated some utility in identifying and mapping imported *P. vivax* infections in South Korea, where the majority of indigenous parasite strains were similar to each other [3]. However, in higher endemic settings where there are many different parasite strains and many infections carrying multiple strains, these markers may be less effective. Furthermore, being expressed on the parasite surface, some of the diversity in these genes may reflect selection from the host immune system rather than geographic ancestry.

MICROSATELLITE MARKERS
In contrast to surface protein markers, microsatellite markers are often neutral/ not under selection. As with the surface protein markers, microsatellite markers have demonstrated high utility in distinguishing imported from indigenous *P. vivax* infections in South Korea (APMEN Study 107-05 awarded Dr Jung-Yeon Kim). However, in central China, where there are many different parasite strains, microsatellite markers were less effective [4](APMEN study 107-02 awarded Prof Gao Qi). Further analysis in other populations is required to test the potential of microsatellites to map geographic origin. However, this function is likely to be limited as the high mutation rate of microsatellites constrains tracking of geographic ancestry.
MITOCHONDRIAL MARKERS

Two studies have used the mitochondrial genome to trace the geographic origins of *P. vivax* infections, selecting this genome because it is more historically conserved and less affected by selection pressures than the surface protein and microsatellite markers [5, 6]. Furthermore, hundreds of worldwide *P. vivax* mitochondrial sequences are accessible to the public (Genbank). In the most comprehensive study to date, Rodrigues et al. assessed the utility of mitochondrial markers to map imported cases collected in the US between 2004 and 2008 [6]. Using sequence data from 69 imported and 348 worldwide infections (from Genbank), they demonstrated effective mapping to the Americas, Southeast Asia, East Asia, and Melanesia. However, owing in part to limited data, parasite lineages from Africa, South Asia, Central Asia, and the Middle East could not be reliably mapped, highlighting the need for even more geographically comprehensive data sets to enable higher resolution mapping.

APICOPLAST MARKERS

A recent *P. falciparum*-based study highlighted the potential of markers in the apicoplast genome to identify and map imported infections [7]. As with the mitochondrion, the apicoplast genome is relatively conserved historically and is subject to few known selection pressures. Using mitochondrial and apicoplast sequence data on over 700 *P. falciparum* infections from 14 countries, the authors demonstrated that with just 23 variants, over 90% of test isolates could be mapped to the correct geographic region. A greater number of informative variants were identified in the apicoplast versus the mitochondrial genome. The authors discussed the potential to develop field-applicable assays to genotype the 23 variants locally.

IMPLICATIONS FOR POLICY OR PRACTICE

- A molecular tool to identify and determine the geographic origin of imported cases will assist policy makers to identify target populations for screening, and to assess the impact of local public health interventions on the parasite population.
- For a molecular tool, training would be required on appropriate collection and storage of samples at local health centres, and processing of samples and data analysis at the assigned reference lab(s).

RESEARCH GAPS

- A simple, cost-effective molecular tool is needed to effectively identify imported cases and determine their geographic origin.
- Analysis of the *P. vivax* sequence in isolates from a broad range of geographic locations and endemic settings will facilitate the detection of effective geographic markers.

ADDITIONAL INFORMATION THAT CAN AID THE TAKE UP OF NEW EVIDENCE

Country Partners are assisting in the country or regional analysis of *P. vivax*, using consensus genotyping methods. Novel approaches are being explored to progress genotyping methods and pool data across sites for comparative analysis (please contact Dr Sarah Auburn at Sarah.Auburn@menzies.edu.au for further information).
OVERVIEW OF THE EVIDENCE

<table>
<thead>
<tr>
<th>Strength of evidence</th>
<th>Antigen-encoding markers</th>
<th>Microsatellite markers</th>
<th>Mitochondrial markers</th>
<th>Apicoplast markers</th>
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<tbody>
<tr>
<td></td>
<td>Work well in South Korea. Not enough evidence for other endemic settings.</td>
<td>Work well in South Korea but not so well in central China. Not enough evidence for other endemic settings.</td>
<td>Difficult to map infections from Africa, South Asia, Central Asia, and the Middle East but data was limited in these regions.</td>
<td>Apicoplast is very informative in P. falciparum. 23 mitochondrial plus apicoplast SNP variants need to be genotyped in P. falciparum. Multiple options are available for genotyping these SNPs. Not enough data yet for Pvivax.</td>
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| Impact on malaria elimination |                      | Critical to confirm not indigenous transmission. Critical to identify at risk individuals or populations for target intervention/screening. | Critical to confirm not indigenous transmission. Critical to identify at risk individuals or populations for target intervention/screening. | Critical to confirm not indigenous transmission. Critical to identify at risk individuals or populations for target intervention/screening. |

REFERENCES


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