

Population genetic diversity of samples from the 2012 and 2015 Zambia malaria indicator surveys

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Background

Malaria indicator surveys (MIS) have been regularly implemented in Zambia since 2006 to assess the prevalence of malaria across the country.

Data have been used to track intervention impact and highlight spatial and temporal heterogeneity.

As Zambia targets elimination, and prevalence is reduced to very low levels, the ability to continue to track progress through the MIS is reduced due to the large sample size required. Other tools are therefore needed to continue to track progress.

Population genetics could provide a means to assess transmission intensity and therefore progress when prevalence becomes very low. This is based on the assumption that the amount of genetic crossing between parasite haplotypes and therefore the parasite population diversity declines along with reduced transmission intensity.

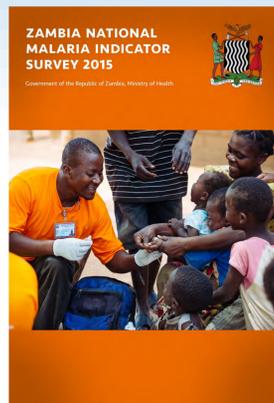
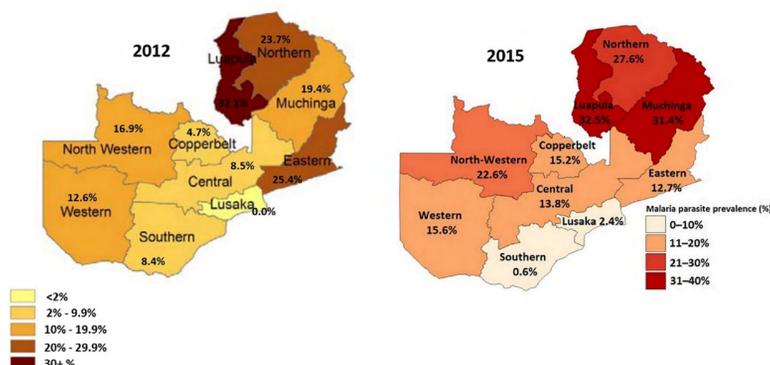


Figure 1. Malaria parasite prevalence among children under five years of age, 2012 and 2015, Zambia



Methods

Samples were selected from provinces with a range of different *Plasmodium falciparum* prevalence.

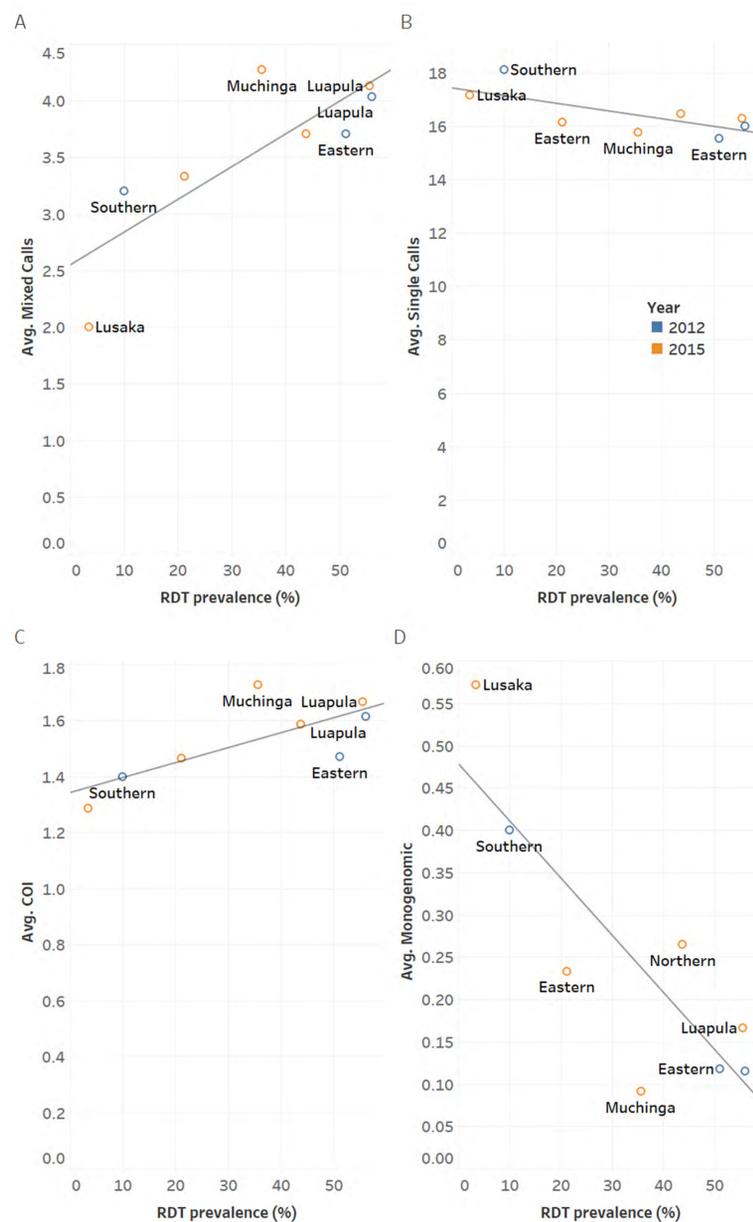
Parasite DNA was extracted from dried blood spots (DBS) from all rapid diagnostic test (RDT)-positive samples using Promega DNA IQ Casework Pro Kit for Maxwell 16 or Qiagen MiniPrep kits.

P. falciparum samples were genotyped using a 24-point single nucleotide polymorphism (SNP)-based TaqMan barcode assay^[1]

Complexity of infection (COI) was determined using COIL^[2] (<http://portals.broadinstitute.org/infect/malaria/coil/>).

Results

Figure 2. Barcode characteristics against *Plasmodium falciparum* prevalence by province for samples collected in the 2012 (blue) and 2015 (orange) MIS. (A) Average number of mixed calls. (B) Average number of single calls. (C) Average COI. (D) Average number of monogenomic (i.e., no mixed calls/single haplotype infections)



Barcoded samples (Table 1) from provinces with a higher prevalence of *Plasmodium falciparum* were found to have (Figure 2):

- A higher number of mixed cells on average.
- A lower number of monogenomic infections (i.e., single haplotype detected).
- A higher complexity of infection.

Acknowledgements

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Table 1. Characteristics of samples collected in the 2012 and 2015 MIS *

Province	Enrolled	RDT-positive	RDT prevalence (%)	Number barcoded*	Year
Eastern	492	124	51.1	17	2012
Luapula	356	114	56	26	2012
Southern	370	31	10	10	2012
Eastern	229	29	21.2	30	2015
Luapula	562	183	55.5	30	2015
Lusaka	198	7	3.5	7	2015
Muchinga	181	57	35.6	33	2015
Northern	293	81	43.8	34	2015

*Only samples where ≥ 16 of the barcode loci were defined were included.

Conclusions

Barcoded samples showed a clear relationship between parasite prevalence and measures of multiplicity of infection (Figure 2) consistent with samples from higher prevalence provinces containing, on average, a higher number of haplotypes. This was expected as the number of haplotypes is related to the level of malaria transmission.

While the association is clear, it was not as dramatic as expected (e.g., the COI at the lowest prevalence [3.5% in Lusaka, 2015]) was 1.29, compared to 1.67 in Luapula with a prevalence of 55.5%. Unfortunately, travel information was not collected as part of the MIS and so any relationship between travel and these indicators could not be assessed.

These barcoded samples will form a library/baseline of parasites from around the country that may be relevant to comparisons in the future. For example, it is hoped that these barcodes could ultimately be used to differentiate between imported and local infections as elimination is approached.

Next steps

Identify any PCR-positive/RDT-negative samples, barcode and compare with the RDT-positive samples already analysed.

References

1. R. Daniels et al. "A general SNP-based molecular barcode for *Plasmodium falciparum* identification and tracking," *Malar J*, vol. 7, p. 223, 2008.
2. K. Galinsky et al. "COIL: a methodology for evaluating malarial complexity of infection using likelihood from single nucleotide polymorphism data.," *Malar J*, vol. 14, no. 1, p. 4, 2015.