Hybridization in UroGenital Schistosomiasis (HUGS): A novel real-time PCR assay, with high resolution melt profiling, useful for the detection of hybrid schistosomes in Malawi

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Introduction

The ability of schistosomes to form inter-species mating pairs with resultant hybrid or introgressed offspring has been previously described. Such studies have relied upon sequence analysis of nuclear genome (nDNA) and/or the mitochondrial genome (mDNA) loci. Although highly accurate, sequence analysis of large numbers of targeted specimens is prohibitive, being both time-consuming and overtly costly.

We have developed a rapid, low-cost two-tube real-time (rt)PCR multiplex assays, with high resolution melt profiling, screening both mDNA and nDNA loci. We have conducted a large-scale examination of recently collected material from two communities in Nsanje and Mangochi Districts.

Methods

Species-specific primers producing unique high-resolution melt-peaks were designed for mDNA gene targets (tRNA Lys, ND4 and ND6) for six schistosome species (*S. mattheei, S. currassoni, S. bovis, S. haematobium, S. mansoni* and *S. margrebowei*). To compliment the mDNA qPCR, a single-plex nDNA rtPCR targeting a 168bp variable region of the ITS2 gene was also developed.

Comparison of the specific melt-peaks produced by each assay can be used to distinguish individuals of different species and determine if they have any mixed species parental signatures, especially maternal mitochondrial carriage.

Preliminary results

A combined total of 1,012 urine filter samples obtained from ~2,400 people were screened using the mDNA and nDNA qPCR assays. This resulted in the identification of 77 putative mixed infections and/or hybrid cases, equating to an overall prevalence of 7.3%.

Furthermore, our assays identified gross under-reporting of mixed species infections with ectopic *S. mansoni* (n=95) versus microscopy (n=6), including 18 individuals with markers for triple-species infections.

Conclusion

With new assays, our ability to screen natural populations of schistosomes for introgressed forms expands. Our study indicates the presence of various hybrid schistosomes capable of infecting local communities of endemic areas of Malawi in sub-Saharan Africa.

Further analysis of individual eggs on FTA cards is ongoing to clarify the distinctions between mixed infections versus introgressed genotypes. We also aim to complement these qPCR assays with development of discriminatory sex-specific loci to better interrogate the directional basis of hybridisation between male (ZW) and female (ZZ) genders.