A duplex tetra-primer ARMS-PCR assay to discriminate three species of the Schistosoma haematobium group: Schistosoma curassoni, S. bovis, S. haematobium and their hybrids

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BACKGROUND: The use of applications involving single nucleotide polymorphisms (SNPs) has greatly increased since the beginning of the 2000s, with the number of associated techniques expanding rapidly in the field of molecular research. Tetra-primer amplification refractory mutation system-PCR (T-ARMS-PCR) is one such technique involving SNP genotyping. It has the advantage of amplifying multiple alleles in a single reaction with the inclusion of an internal molecular control. We report here the development of a rapid, reliable and cost-effective duplex T-ARMS-PCR assay to distinguish between three Schistosoma species, namely Schistosoma haematobium (human parasite), Schistosoma bovis and Schistosoma curassoni (animal parasites), and their hybrids. This technique will facilitate studies of population genetics and the evolution of introgression events. METHODS: During the development of the technique we focused on one of the five inter-species internal transcribed spacer (ITS) SNPs and one of the inter-species 18S SNPs which, when combined, discriminate between all three Schistosoma species and their hybrid forms. We designed T-ARMS-PCR primers to amplify amplicons of specific lengths for each species, which in turn can then be visualized on an electrophoresis gel. This was further tested using laboratory and field-collected adult worms and field-collected larval stages (miracidia) from Spain, Egypt, Mali, Senegal and Ivory Coast. The combined duplex T-ARMS-PCR and ITS + 18S primer set was then used to differentiate the three species in a single reaction. RESULTS: The T-ARMS-PCR assay was able to detect DNA from both species being analysed at the maximum and minimum levels in the DNA ratios (95/5) tested. The duplex T-ARMS-PCR assay was also able to detect all hybrids tested and was validated by sequencing the ITS and the 18S amplicons of 148 of the field samples included in the study. CONCLUSIONS: The duplex tetra-primer ARMS-PCR assay described here can be applied to differentiate between Schistosoma species and their hybrid forms that infect humans and animals, thereby providing a method to investigate the epidemiology of these species in endemic areas. The addition of several markers in a single reaction saves considerable time and is of long-standing interest for investigating genetic populations.
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