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Mini Review

Molecular Monitoring of Schistosomiasis Transmission

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Abstract

Schistosomal repeated genomic sequences were identified and their amplification by PCR and LAMP was developed for molecular detection of snails infected by *Schistosoma mansoni* and *S. haematobium*. Patent and prepatent snail infections were identified. Inter-repeat amplification was introduced for differentiating snails infected by *S. haematobium* from those infected by animal schistosomes. A year-long screening of *S. haematobium* infection in snails was carried out and the dynamics of prepatent and patent infections in multiple water bodies was determined. Matters concerning application of molecular mass screening were suggested.

INTRODUCTION

Schistosomiasis transmission occurs through the environment by skin-invasive larvae developing in fresh water snails. Reinfection is very common and related to environmentally dependent conditions including long term environmental contamination with schistosome ova by humans due to the disease chronicity, wide distribution of the intermediate snail host, vigorous multiplication cercariae within the snails at suitable environmental conditions, and water contact habits of humans.

Repeated drug administrations, although resulting in much reduced infection and morbidity, does usually not achieve transmission-aborting reduction in the rate of infected snails. The under-input in schistosomiasis transmission-control seems to contradict the success of schistosomiasis eradication in some areas in China by snail control alone [1]. Yet, environmental aspects, important for transmission of schistosomiasis (and of other environmentally dependent diseases-EDIDs) are usually not handled by health authorities. As it becomes increasingly difficult to monitor residual infection in previously treated and retreated human populations, mass monitoring of snail infection and snail control becomes increasingly relevant. The cost of snail control vs. the burden of disease, re-monitoring, and retreatment is often discussed, but developmental input for finding more suitable tools for snail control seem to lag.

Herewith presented is a brief review of the work of Joseph Hamburger and his colleagues on molecular monitoring of snail infection by schistosomes.

METHODS, RESULTS AND DISCUSSION

For mass screening of snail infection rates the traditional method of cercarial shedding is not suitable as prepatent infections cannot be detected and the cercariae shed cannot be differentiated by species from animal schistosomes. Previous molecular detection attempts were based on identification of ribosomal or mitochontrial genes, but for large scale monitoring we have centered on the identification of highly repeated genomic DNA sequences in schistosomes, and adapted PCR and LAMP (Loop Mediated Isothermal Amplification) for this purpose.

Schistosoma mansoni

In order to identify highly repeated genomic sequences we have used radioactively labelled fragmented genomic DNA for hybridization with *S. mansoni* genomic libraries. Library colonies exhibiting strongest hybridization signals were selected for sequence analysis. Using the SmaI restriction enzyme for DNA fragmentation in these studies, we have identified schistosomal repeated genomic DNA sequence of *S. mansoni*, and named it the Sm1-7 repeated sequence [2]. The Sm1-7 is a tandemly arranged repeated sequence composed of 121 base-pair long repeat units, and the corresponding arrays represent about 12% of the schistosomal genome. By targeting it for amplification by PCR, a highly sensitive detection of *S. mansoni* DNA became possible. A simple procedure for extracting snails was involved as a first step, and the extracts could be saved at ambient temperature for later testing. Detection sensitivity of 1fg was achieved by PCR and snails harbouring prepatent as well as patent infection were identified [3,4]. Detection of prepatent snail infection is a significant addition to an accurate measurement of infection rates in snails, and the proportion of prepatent infection can likely indicate the intensity of fresh water contamination before and after a circle of mass treatment of humans.

Schistosoma haematobium

A cloning strategy, similar to the one described above for *S. mansoni*, was used for cloning of *S. haematobium* highly repeated sequences. In this case the DraI restriction enzyme was employed for DNA fragmentation, and a repeated sequence of *S.*

haematobium, we have named the DraI repeat, was identified. These are 120bp long repeat units arranged in tandem, and representing about 15% of the S. *haematobium* genome [5].

As in the case of *S. mansoni*, PCR employing DraI primers enabled identification of prepatent as well as patent *S. haematobium* infection in host snails. This led us to carry out large-scale and year-long monitoring of infected snails in multiple transmission sites in Coastal Kenya [6]. In perennial sites snails and prepatent infections were present all year-round but patent infections were much fewer and were found only after the rainy season. In temporary (seasonal) sites snails were present with prepatent or patent infections only after the rainy season. It therefore appears that the best time for snail control in this area is after the rainy season. Ripening of prepatency to patency appears to be dependent on environmental conditions (yet undefined), but in many cases patency did not appear at all. Whether this is due to snail mortality or to other environmental effects has not been determined. Prepatency rates seemed to correspond to human infection rates and average intensity. This correspondence remains to be studied as it may present a marker of infection in the human population contaminating the water.

For studies in rural areas where transmission occurs, and local laboratories have only basic operational capabilities, we have introduced LAMP for simpler molecular monitoring of snail infection [7]. As temperature cycling used for PCR is not required for LAMP, the expensive and expertise-dependent temperature cycler is not required. A common water bath is sufficient for the isothermal amplification by LAMP. Although laboratory technicians untrained in molecular biology could run the LAMP technique independently [8], it comes to mind in a retrospect that for mass screening over a long period of time they will likely require supervision by an expert, either directly or by cell phone. This possibility remains to be examined. Anyway, a field laboratory team can easily master the technique used for snail extraction in dilute NaOH, and deliver the extracts in ambient temperature to a central facility for DNA amplification by PCR, Real-time PCR or LAMP. Also, dot blotting of amplification products and hybridization with labelled marker sequences has been successfully attempted. Separation between the collection of snails and their extraction in field laboratories, and molecular testing in suitable facilities can be practically important for mass monitoring of transmission. Mass snail collection in the field by community members should be attempted for mass collection for monitoring and for mass clearance of snails.

Monitoring rates of snail infection with S. haematobium in areas where animal schistosomes are present in the same environment

Snail hosts of *S. haematobium*, (bulinid snail species) are present in water bodies in many endemic areas. They can also be infected by schistosomes related to *S. haematobium* that infect ruminants. Therefore differential identification of *S. haematobium* is required for accurate monitoring of snails infection rates by the human schistosome. Of these animal schistosomes, *S. bovis*, present in the Middle-East and in many areas in Africa, and *S. mattheei* present in Southern Africa are the most abundant animal schistosomes. These as well as, *S. magrebowei, S. currasoni,* and *S. intercalatum* cannot be differentiated by DraI PCR as they all contain the DraI sequence.

For species-specific identification of *S. haematobium* we have identified and characterized the *S. haematobium* repeated sequences Sh73 and Sh77 (73bp and 77bp long, respectively). Both were present in all schistosomes of the *S. haematobium* group members tested [9]. An inter-repeat sequence was then amplified by PCR using one primer designed based on a newly identified repeated sequence Sh73 and another one based on the DraI repeat. The inter-repeat PCR is a novel concept previously rarely used, and here adapted for schistosomes-species identification. Simple PCR using these primers enabled differentiation of *S. haematobium* from *S. bovis*, and the other animal schistosome, by clearly different banding patterns and with detection sensitivity of 100 fg, sufficient for identifying a single parasite. The possibility of differentiating *S. haematobium* from related schistosomes by one step PCR presents an advantage over other approaches described in the literature which involved nested PCR or the use restriction enzymes for obtaining identifiable fragments.

As all repeated sequences so far described in the *S. haematobium* genome are also present in the genomes of animal schistosomes, further studies were undertaken in order to identify a species-specific sequence of *S. haematobium* that may further facilitate its specific identification. Most recently [10], the arrangement and association between DraI, sh73 and Sh77 repetitive sequences in both *S. haematobium* and *S. bovis* genomes was clarified. Most importantly, a DNA fragment of 64bp length (termed the Sh64bp repeat linker) that links DraI repeat with the sh73bp repeat was identified in the *S. haematobium* genome, and another unique fragment of 30bp was identified in *S. bovis* and was named Sb30bp repeat linker. The exact location of the DraI sequence in relation to the Sh73 and Sh77 sequences was found, and the location of the Sh64 was likewise determined as connecting between these sequences. Thus, the DraI repetitive sequences array is connected in its 3' end to the repeat linker, to which the sh73bp and sh77bp repeats are connected at the 5' end in an alternating manner. So, the linker appears to be an integral part of the arrays of the various repeated sequences. The presence of repeat linkers in the other animal schistosomes remains to be demonstrated.

We have designed, from the identified repeat linker sequences, species specific oligonculeotides that can be used for specifically identifying *S. haematobium* and *S. bovis*, using DNA hybridization tools. Initially these species-specific olygonucleotides were used in reverse line blot (RLB) hybridization for *S. haematobium* and *S. bovis* differentiation. Using it for hybridization with multiple dot-blots of snail extracts was preliminarily and successfully tested for mass screening.

CONCLUSIONS

Large-scale schistosomiasis elimination activities are expected to intensify soon, and repeated drug administrations are likely to be used for it. Yet, this is still expected to leave residual transmission. Therefore, the matter of including mass molecular monitoring of snail infection as an integral component of eradication is likely to become more central. Based on available research results, some lines of required

attention and development are hereby presented, and following suitable refinements the available methods and organizational frameworks can be used for mass molecular monitoring.

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