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#### TITLE

**ENVIRONMENTAL DNA (EDNA) APPROACH FOR DETECTING SCHISTOSOMIASIS TRANSMISSION FOCI IN A MODERATE ENDEMIC AREA IN BRAZIL**

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#### ABSTRACT

Schistosoma sp. detection is challenging, mainly in moderate and low-endemic areas where the monitoring is hampered by difficulties in snail collection, particularly in the dry season. Also, snail sampling, shipment, and parasitological detection require trained personnel. Thus, there is a need to improve tools for surveillance of transmission foci, as targeted in the WHO/NTD road map. In response to these challenges, we used the environmental DNA (eDNA) approach to monitor schistosomiasis transmission areas in Brazil, where only *Schistosoma mansoni* is present. This approach involves analyzing water samples from potential endemic locations to detect traces of parasite DNA, facilitating the assessment of active transmission foci. The eDNA assay was standardized for local conditions by evaluating the specificity of qPCR primers and probe targeting the mitochondrial gene cytochrome c oxidase (coi) of *S. mansoni* for detecting the parasite DNA. Following, water from snail breeding tanks containing *Biomphalaria glabrata* infected or not with *S. mansoni* was used for eDNA extraction. Specific eDNA detection was done by three previously described molecular techniques for *S. mansoni* detection: Low-Stringency PCR (LS-PCR), Loop-mediated isothermal amplification (LAMP), and quantitative PCR (qPCR). After standardization, the approaches were used to investigate samples from five water body collections in a schistosomiasis moderate endemic area from a rural District in Northeastern Minas Gerais, Brazil, where an epidemiological study is in progress (personal communication). A malacological survey was also carried out. The qPCR primers and probe specifically amplified *Schistosoma* sp. DNA but no other trematodes occurring in Brazil. Additionally, all three molecular assays amplified *S. mansoni* DNA only from eDNA samples collected from tanks with infected snails. Using the traditional light exposure, we identified snails shedding cercariae at collection point 3 during the malacological survey. However, the eDNA approach allowed us to identify potential transmission foci in collection points 3 and 4 using eDNA field sampling associated with LAMP and qPCR assays. The LS-PCR assay could not detect parasite DNA in any collection point. In summary, our study illustrates the potential of eDNA detection approach in a moderate endemic area in Brazil, as an effective strategy for monitoring schistosomiasis endemic areas and identifying active *S. mansoni* transmission foci. This approach may be used in the detection of *S. mansoni* in non-human animal hosts, an under studied area, particularly in Brazil. Yet, LAMP technique demonstrated promising results as a more feasible molecular technique, to be used as the detection method in the field.

#### KEYWORDS

Bilharziasis; *Schistosoma mansoni*; Environmental Surveillance; quantitative PCR (qPCR); Loop-Mediated Isothermal Amplification (LAMP); Low-Stringency PCR (LS-PCR)

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