Tissue-based Species Level Molecular Identification of *Schistosoma haematobium* in Appendiceal Tissue

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INTRODUCTION

Schistosomiasis is a parasitic disease affecting over 230 million individuals worldwide and is a water-borne infection transmitted through freshwater snails as intermediate hosts (1).

Appendiceal involvement in schistosomiasis is rare, but it has been reported for the three major species commonly infecting humans (*S. haematobium, S. mansoni, and S. japonicum*) (*2,3,4).*

Often the diagnosis is made histologically following an appendectomy. However, many times the eggs found in the appendiceal tissue are calcified or degraded to a degree that a species-level diagnosis cannot be made morphologically.

CASE DESCRIPTION

A 37-year-old female presented to the emergency room complaining of a one-year history of intermittent abdominal pain. The patient lived in a refugee camp in Kenya for 26 years and immigrated to the United States in 2017. She reported occasional hematuria, but urinalysis was negative for red blood cells. White blood and absolute eosinophil count were within normal range. An appendectomy was performed due to the finding of appendicitis on an abdominal CT.

The appendix pathology showed calcified and degraded eggs consistent with Schistosoma (Figure 1), but a species could not be identified. Serum Schistosoma IgG was negative (BioAgilytix Diagnostics). The microscopic stool ova and parasites analysis were negative. A formalin-fixed paraffin tissue block from the appendectomy specimen was sent to the Laboratory of Parasitic Diseases at the National Institutes of Health (NIH) for quantitative polymerase chain reaction (PCR) molecular identification. DNA was extracted from two unstained biopsy slides using standard techniques for formalin-fixed, paraffin-embedded tissue and subjected to qPCR using *S. haematobium*- or *S. mansoni*-specific primers/probes.

The patient sample only amplified with the *S. haematobium*-specific primers/probes and not with the S. mansoni-specific primers/probes (no amplification). For both qPCR reactions, positive controls were positive and negative controls showed no amplification (Figure 2)

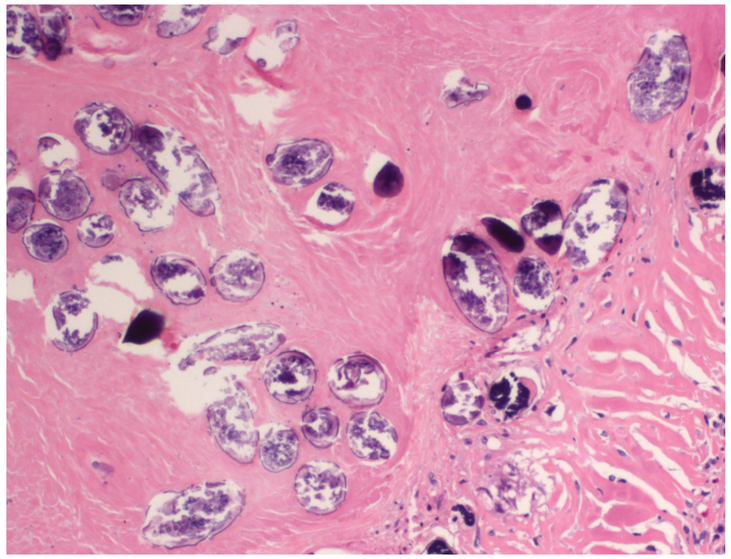

Figure 1: Degraded eggs of Schistosoma species seen within the wall of the appendix in muscularis propria and subserosa with associated granulomatous inflammation

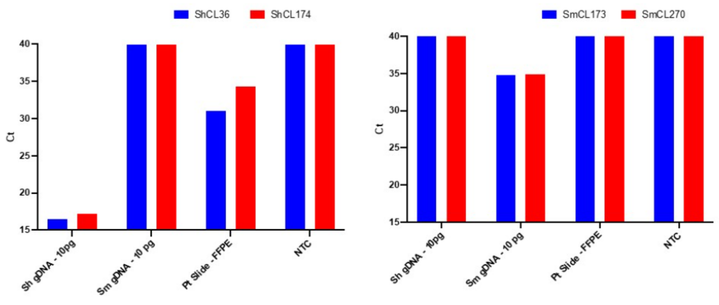

Figure 2: qPCR speciates eggs as *S. haematobium* - Left panel – Geometric Mean Ct values using 2 *S. haematobium* (Sh) specific targets ShCL36 (blue) and ShCL174 (red) against Sh gDNA, Sm gDNA, the patient sample (Pt Slide-FFPE), or the no template control (NTC). Ct values of 40 signify a lack of amplification. Right panel -– Geometric Mean Ct values using 2 *S. mansoni* (Sm) specific targetsSmCL173 (blue) and SmCL270 (red) against Sh gDNA, Sm gDNA, the patient sample (Pt Slide-FFPE), or the no template control (NTC). Ct values of 40 signify a lack of amplification.

DISCUSSION

Appendicitis caused by schistosomiasis is rare.

A definitive diagnosis is made by histopathologic examination showing schistosomal eggs. Differences in egg morphology can be used to distinguish between Schistosoma species. However, as in this case, the eggs can be found in the appendiceal tissue calcified and degraded to a degree that a species-level diagnosis cannot be made morphologically. Therefore, molecular identification can determine that the patient's schistosomiasis was caused by *Schistosoma haematobium*.

The mechanism of appendicitis in relation to schistosomal infection remains unclear and has been postulated to be due to granulomatous inflammation caused by eggs in submucosal, serosal layers and/or due to the mechanical obstruction and secondary infection resulting from fibrosis caused by calcified and degraded eggs.

Although the treatment for distinct species of Schistosoma may be similar, identification of species may help in epidemiological studies and in screening for long-term complications of the infection.

CONCLUSION

Schistosomiasis, an uncommon etiology of appendicitis, should be considered in differential diagnoses of patients with chronic abdominal pain and history of travel from African countries.

Tissue-based parasite identification of *S. haematobium* DNA provides a definitive species assignment for the causative agent presumably driving the appendiceal inflammation.

This technique further allows species-based therapeutics, should it be necessary. It could also be used as an epidemiological tool to determine species-specific incidence, which may aid in gaining funding for research and development.

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