Drug Resistance Markers In *E. vermicualris*

Identifying the source of resistance to Benzimidazoles in *Enterobius vermicularis*

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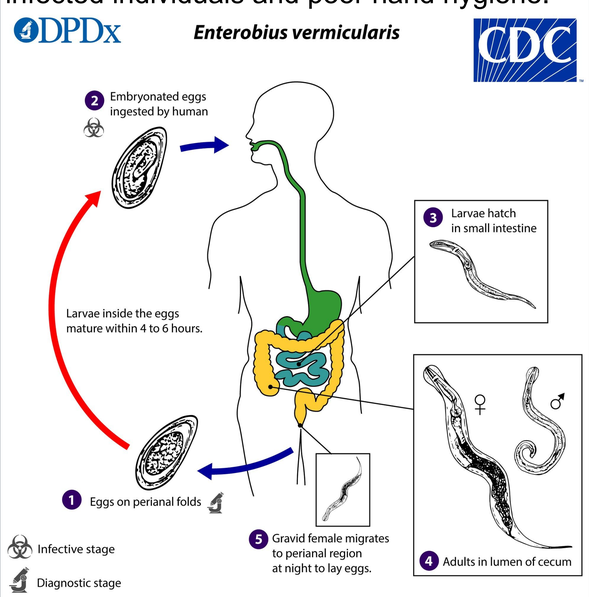
Introduction

Enterobius vermicularis commonly known as Pinworm or Threadworm is an intestinal helminth parasitic worm infectious to humans. At least 1 billion people are currently infected, 40% are asymptomatic and others generally only experience discomfort though this can cause serious psychological distress due to irritation from eggs being laid by the female worm on the perianal region. It is spread via the fecal-oral route, increased by contact with infected individuals and poor hand hygiene.

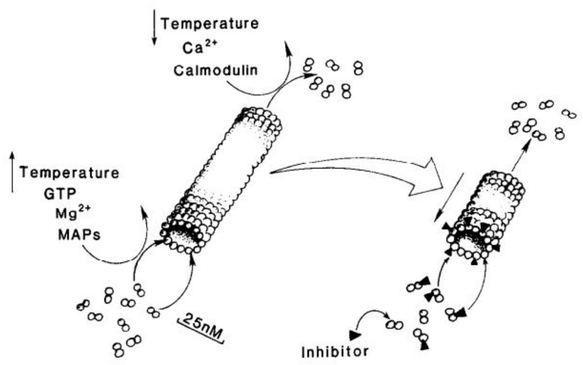


Life cycle of *E. vermicularis* within human host [1]

4 chemotherapy drugs are the sole approved treatment against *E. vermicularis*, and Benzimidazoles are the only drugs effective against larval and adult worm life stages. Mass drug administration hasn't been used to control *E. vermicularis* though resistance is expected to develop. The ideal long-term control against infectious disease are vaccines though despite late stage trails, infectious helminths including *E. vermicularis* lack any commercially available vaccines.

Benzimidazoles and Beta-tubulin

Benzimidazoles: Albendazole and Mebendazole as a single or repeated dose are the main treatment for enterobiasis. Uptaken by the parasite these bind to the colchicine binding site on Beta-tubulin, a subunit of the tubulin dimer protein that polymerise to form microtubules in dynamic equilibrium. Meaning the microtubules are continuously being formed and degenerating. Benzimidazoles cap the polymerising tail halting polymerisation causing degeneration of microtubules and inhibiting any further synthesis. This causes a breakdown of cell structure and transport systems leading to death of the parasite.



The dynamic equilibrium of microtubules and the effect of inhibitors Lacey 1990 [2]

Reinfection

After treatment patients are often reinfected with worms by autoinfection where worms and eggs re-enter the body or spread from incomplete cure rate or the environment. To avoid this, treatments must be repeated 1-2 weeks after the initial dose.

Long term control requires good sanitary conditions and regular testing as individuals are always at risk of reinfection.

Resistance

Due to use of these drugs over the last 30 years resistance has been reported in a range of helminth veterinary parasites and also fungal species. This resistance has been associated with 3 single nucleotide polymorphisms within the beta-tubulin gene.

167 (Phenylalanine → Tyrosine)
198 (Glutamic acid → Alanine)
200 (Phenylalanine → Tyrosine)

These SNP mutations are naturally common within helminth populations as they don't significantly alter the proteins function allowing for selection. Samples collected from London HTD from reported cases of *E. vermicularis* that have shown resistance to Benzimidazole treatment will be analysed against control samples to identify the source of resistance. It is suspected to be caused by the same SNP changes seen in other related helminth parasite species

Methods

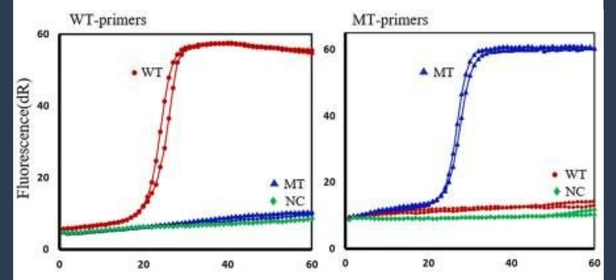
DNA extraction methods for helminths are not well explored with most commercial kits lacking advise to optimise yield from helminth species. The free-living model worm *P. redivivus* in a distinct clade to *C. elegans* will be used to optimise methods used by previous studies into helminth genetics for use on the limited samples.

A. P. redivivus on a microscope slide, B. *E. vermicularis* female left, male right

Sequencing and developing clinical diagnostic

Of the 4 genes identified in the reference genome, Isotype-1 identified by phylogenetics expressed in all life stages will be amplified by PCR in its entirety. Isotypes 2-4 will only have their SNP occurring region sequenced. As well as full genome sequencing via illumina to analyse and compare all suspected regions where genetic resistance may occur.

Diagnostic tests identifying the SNP mutations associated with Benzimidazole resistance have been developed for a range of helminth parasites. By targeting the beta-tubulin genes using SmartAmp2 PCR targeted to the helminth beta-tubulin genes to quickly identify resistance-associated-SNPs.



SmartAmp2 assay to detect resistance associated SNP mutation in *T. trichiura* [3]

Resistance to Benzimidazoles has spread to a large range of human and veterinary parasites. Tracking the resistance to these drugs will inform on future treatments extending the effectiveness of drugs to allow more time for effective vaccines or other long term preventatives to be developed.

References

1. Centers for Disease Control and Prevention. Enterobiasis. www.cdc.gov/parasites/pinworm
2. Lacey, E. (1990). Mode of action of benzimidazoles. Parasitology Today, 6(4), 112–115.
3. Rashwan, Nour et al. Rapid Genotyping of β-tubulin Polymorphisms in Trichuris trichiura and Ascaris lumbricoides. PLoS neglected tropical diseases vol. 11,1 e0005205. 12 Jan. 2017,