See How They Squiggle and Squirm?!

- Intestinal worm infections among refugee children in Thailand

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Introduction

- Around 3.5 billion people are infected with intestinal worms worldwide
- Their impact is measure in disability adjusted life years ~ million worldwide
- The major intestinal worms are so called Soil-Transmitted Helminths

***Ascaris lumbricoides*, *Trichiura trichiuris* & Hookworm (*Necator americanus*, *Ancylostoma duodenale*)**

- Pregnant women and children in sub-tropical to tropical countries are at high risk of infection

Higher infection prevalence than malaria!

- Symptoms vary from diarrhea, malnutrition, stunting, wasting and iron deficiency to reduced cognitive development in children
- Causing factors are overcrowding, poor sanitation and little infrastructure
- Drugs (Benzimidazoles) are available, but only kill worms present inside the gut → re-infection rate is high due to infectious eggs in the environment

Karen ethnic population

- ~146,477 refugees in 7 camps
- Remote, mountainous and forested living conditions with high summer rainfalls
- Karen, Mon and Karenni minorities since 1984 in the Tak province

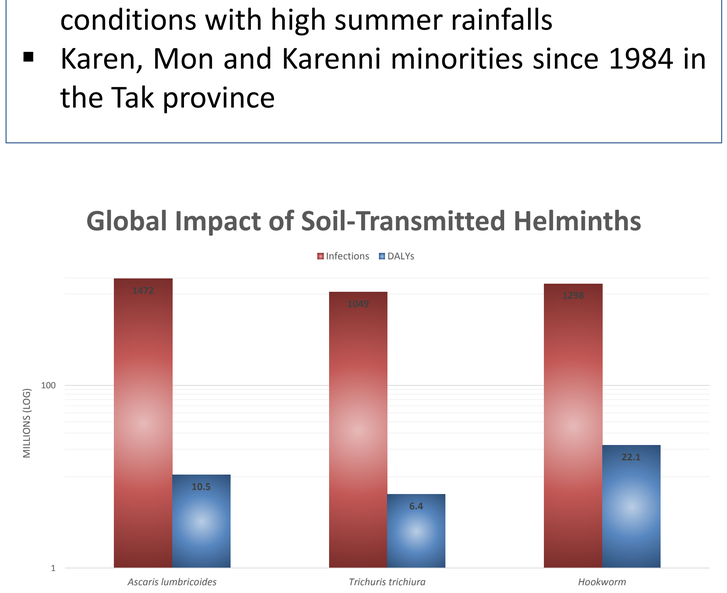

Figure 1. Global impact of Soil-Transmitted Helminths in number of infections and disability-adjusted life years[1,2].

Aims

1. How many pre-school children from the Karen community are infected with intestinal worms?
2. Which worm species cause infection?
3. Is multi-parasitism common (infection with more than one species)?
4. What are the acute and chronic health implications the children suffer from caused by intestinal worm infection?

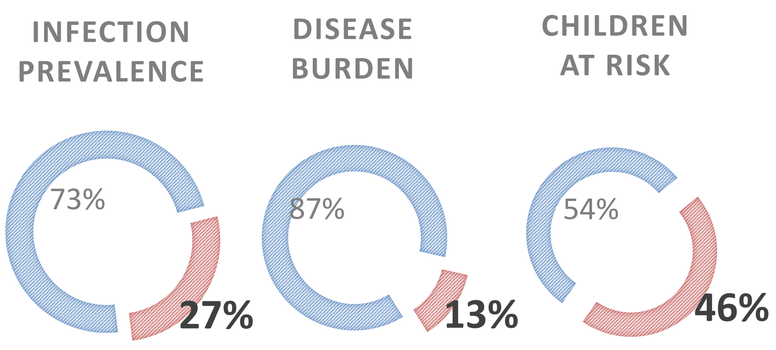

Figure 2. Global infection prevalence, disease burden and number of children at risk of infection of Soil-Transmitted Helminths[1,2].

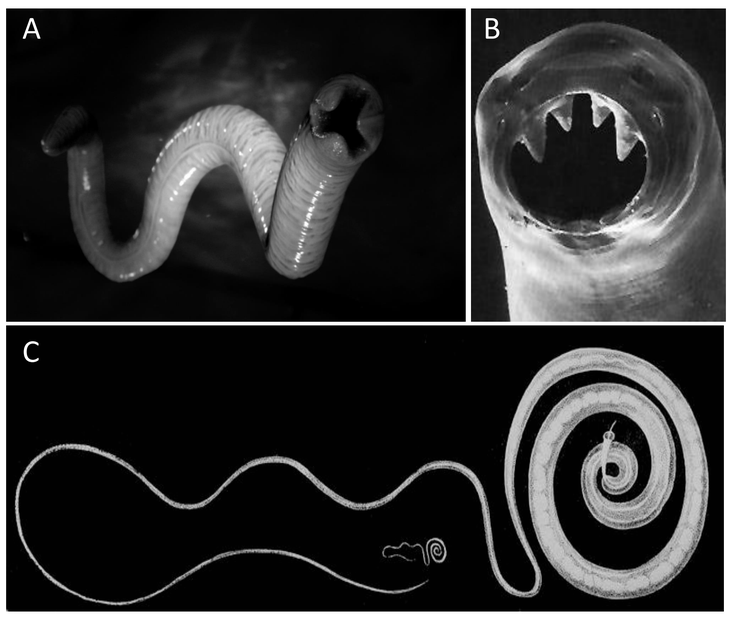

Figure 1. Three major Soil-Transmitted Helminths. A: *Ascaris lumbricoides*[2], B: *Ancylostoma duodenale*[3], C: *Trichuris trichiura*[4].

Hypotheses

- ❖ High worm infections are present in children <5 years.
- ❖ Many children are stunted, wasted and less far developed than compared to healthy children.
- ❖ Multi-parasitism is common.
- ❖ Drug resistances are emerging over time due to mass drug administration of Benzimidazoles.

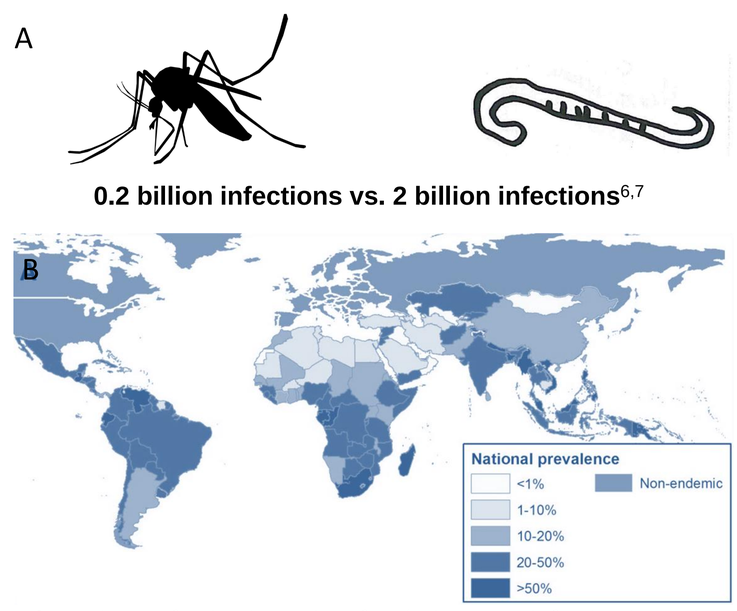

Figure 3. A: Infection intensity of malaria versus infection intensity of Soil-Transmitted Helminths worldwide. B: Global infection prevalence of Soil-Transmitted Helminths[1].

Methodology

- 💩 Data sampling
  - a) Anthropogenic = Weight, height, upper-arm circumference
  - b) Morbidity = Health records of the last 12 months from a local hospital
  - c) Socioeconomic = Sex, age, date of birth, household conditions, toilet availability, vehicle, parent's education/occupation
- 💩 Sampling of stool assisted by parents.
- 💩 DNA extraction from stool using the PowerSoil DNA extraction kit by MoBio®.
- 💩 Multiplexed-Tandem Polymerase Chain Reaction assay to amplify a specific gene of interest which is similar enough among worm species to amplify all DNA samples in one reaction but different enough to differentiate between worm species[9].

MT-PCR advantages

Higher sensitivity and specificity than usual PCR assay
Internal control with known standard
User friendly robotic platform

Note

Ethic approval was obtained through collaborator's local HREC (Thailand) and will be reviewed by the WEHI HREC (Australia). Faecal samples will be collected and processed after the standard precaution guidelines by the World Health Organization.

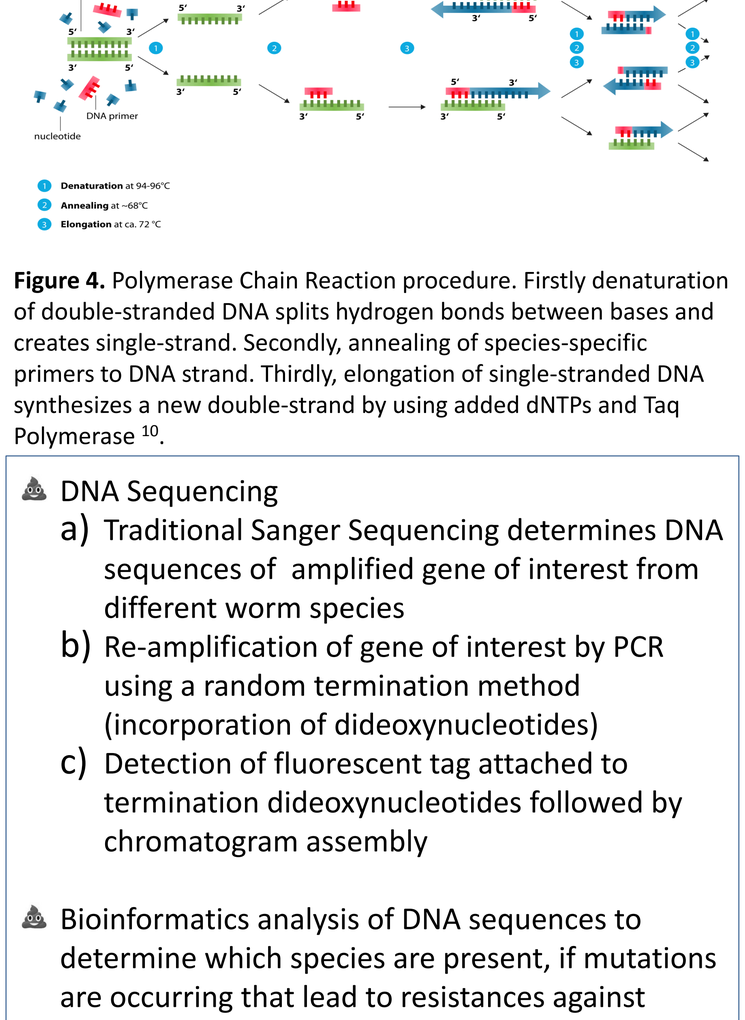

Figure 4. Polymerase Chain Reaction procedure. Firstly denaturation of double-stranded DNA splits hydrogen bonds between bases and creates single-strand. Secondly, annealing of species-specific primers to DNA strand. Thirdly, elongation of single-stranded DNA synthesizes a new double-strand by using added dNTPs and Taq Polymerase [10].

- 💩 DNA Sequencing
  - a) Traditional Sanger Sequencing determines DNA sequences of amplified gene of interest from different worm species
  - b) Re-amplification of gene of interest by PCR using a random termination method (incorporation of dideoxynucleotides)
  - c) Detection of fluorescent tag attached to termination dideoxynucleotides followed by chromatogram assembly
- 💩 Bioinformatics analysis of DNA sequences to determine which species are present, if mutations are occurring that lead to resistances against drugs and similarity comparison between found sequences and known references.

TO BE CONTINUED...

References

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