Monitoring drug efficacy and anthelmintic resistance in human STH: an update from Starworms

Bruno Levecke, Piet Cools, Johnny Vlaminck and Jozef Vercruysse
Background
## Neglected Tropical Diseases - NTDs

<table>
<thead>
<tr>
<th>Diseases</th>
<th>DALYs (x 1000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil-transmitted helminthiasis</td>
<td>3,378</td>
</tr>
<tr>
<td>Schistosomiasis</td>
<td>2,613</td>
</tr>
<tr>
<td>Lymphatic filariasis</td>
<td>2,075</td>
</tr>
<tr>
<td>Dengue</td>
<td>1,892</td>
</tr>
<tr>
<td>Onchocerciasis</td>
<td>1,356</td>
</tr>
</tbody>
</table>
Soil-transmitted helminths

**STH**

- *Ascaris lumbricoides*
- *Trichuris trichiura*
- *Ancylostoma duodenale*  
  *Necator americanus*
Soil-transmitted helminths
Soil-transmitted helminths

**Trichuris trichiura**

1. Unembryonated eggs passed in feces.
2. 2-cell stage
3. Advanced cleavage
4. Embryonated eggs are ingested

**Hookworms**

1. Eggs in feces
2. Rhabditiform larva hatches
3. Filariform larva penetrates skin
4. Filariform larva
5. Adults in small intestine

- = Infective Stage
= Diagnostic Stage
Soil-transmitted helminths

- Fecal-oral / per-cutaneous
  - Environmental contamination = important

<table>
<thead>
<tr>
<th>Species</th>
<th>Length (mm)</th>
<th>Daily egg output per female worm</th>
<th>Location in host</th>
<th>Lifespan (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large common roundworm</td>
<td>150-400</td>
<td>200,000</td>
<td>Small intestine</td>
<td>1</td>
</tr>
<tr>
<td>Ascaris lumbricoides</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whipworm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trichuris trichiura</td>
<td>30-50</td>
<td>3000-5000</td>
<td>Caecum and colon</td>
<td>1.5-2.0</td>
</tr>
<tr>
<td>Hookworms</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Necator americanus</td>
<td>7-13</td>
<td>9000-10,000</td>
<td>Upper small intestine</td>
<td>5-7</td>
</tr>
<tr>
<td>Ancylostoma duodenale</td>
<td>8-13</td>
<td>25,000-30,000</td>
<td>Upper small intestine</td>
<td>5-7</td>
</tr>
</tbody>
</table>

Table 2: Characteristics of the soil-transmitted helminths: adult worms of greatest public-health significance
Soil-transmitted helminths

- 20% of the world is infected
Control

• School-based deworming = cornerstone
Control

• School-based deworming = cornerstone

Commonly used drugs

- Albendazole (1x 400 mg)
- Mebendazole (1x 500 mg)

Frequency

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 50 %</td>
<td>2x year</td>
</tr>
<tr>
<td>≥ 20 % &amp; &lt; 50%</td>
<td>1x year</td>
</tr>
<tr>
<td>&lt; 20%</td>
<td>Not recommended</td>
</tr>
</tbody>
</table>
ALB and MEB are equally efficacious, except for hookworms

<table>
<thead>
<tr>
<th></th>
<th>ALB</th>
<th>MEB</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. lumbricoides</em></td>
<td>99.9</td>
<td>97.6</td>
</tr>
<tr>
<td><em>T. trichiura</em></td>
<td>64.5</td>
<td>63.1</td>
</tr>
<tr>
<td>Hookworm</td>
<td>96.2</td>
<td>79.6</td>
</tr>
</tbody>
</table>
Control

- Coverage increased with ≥300% between 2008 – 2016
- ≥65% of children in need of MDA worldwide have been treated
Challenges
The challenges

- Drugs alone will not do the job
  - Sanitation prevents infection

<table>
<thead>
<tr>
<th></th>
<th>OR</th>
<th>95% CI</th>
</tr>
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<tbody>
<tr>
<td><em>A. lumbricoides</em></td>
<td>0.54</td>
<td>0.43 – 0.69</td>
</tr>
<tr>
<td><em>T. trichiura</em></td>
<td>0.58</td>
<td>0.28 – 0.75</td>
</tr>
<tr>
<td>Hookworm</td>
<td>0.60</td>
<td>0.48 – 0.75</td>
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</table>
The challenges

• Drugs alone will not do the job
  – Sanitation prevents infection
The challenges

- Targeting only kids will not do the job.
The challenges

Combat STH

Anthelminthic Resistance
Starworms
Starworms

- Stop Anthelmintic Resistant Worms
- Bill & Melinda Gates Foundation project
- 3 WHO Collaborating Centers
• Work packages
  – **WP1**: Validation of diagnostic tools to monitor effectiveness of drugs and spread of AR
  – **WP2**: Establishment of a surveillance system to monitor the global patterns of effectiveness of drugs and AR in STH programs
  – **WP3**: Development of supporting tools for planning, analysis and following-up of the effectiveness of drugs and spread of AR
Work packages

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Starworms

• Study design
  – School aged children (5 – 14 years of age)
  – 4 study sites with different history of drug pressure
Starworms

• Study design
  – Clinical efficacy trial (albendazole)

Day 0

- Stool examination

Day 14 – 21

- Stool preservation
- qPCR
- pyrosequencing
- LAMP
- ddpPCR

- qPCR
- pyrosequencing
- LAMP
- ddPCR
Starworms

- Differences in sensitivity, but similar drug efficacy estimates

<table>
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<th>Method</th>
<th>Ascaris (n = 441)</th>
<th>Se (%)</th>
<th>RR (%)</th>
</tr>
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<tbody>
<tr>
<td>qPCR</td>
<td></td>
<td>90.0</td>
<td>97.4</td>
</tr>
<tr>
<td>2x Kato-Katz</td>
<td></td>
<td>73.0</td>
<td>98.0</td>
</tr>
<tr>
<td>1x Kato-Katz</td>
<td></td>
<td>71.9</td>
<td>98.1</td>
</tr>
<tr>
<td>Mini-FLOTAC</td>
<td></td>
<td>63.3</td>
<td>97.6</td>
</tr>
<tr>
<td>FECPAK(^G2)</td>
<td></td>
<td>58.9</td>
<td>98.8</td>
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Starworms

- Differences in sensitivity, but similar drug efficacy estimates

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Satisfactory | doubtful | reduced
Starworms

- Differences in sensitivity, but similar drug efficacy estimates

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<th>Method</th>
<th>Ascaris (n = 441)</th>
<th>Hookworms (n = 457)</th>
<th>Trichuris (n = 456)</th>
</tr>
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<tbody>
<tr>
<td></td>
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<td>RR (%)</td>
<td>Se (%)</td>
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<td>73.9</td>
</tr>
<tr>
<td>FECPAK\textsuperscript{G2}</td>
<td>58.9</td>
<td>98.8</td>
<td>52.4</td>
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Satisfactory: Green
Doubtful: Yellow
Reduced: Red
Starworms

- Other factors will determine choice method
Starworms

What is the diagnostic performance of the different methods?

- Pyrosequencing
- Loop mediated isothermal amplification
- Digital PCR
- Deep amplicon sequencing
• Differences across study sites due to anthelmintic resistance?

<table>
<thead>
<tr>
<th>Drug efficacy (%)</th>
<th>Ascaris</th>
<th>Trichuris</th>
<th>Hookworm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pemba Island</td>
<td>96.8</td>
<td>-11.0</td>
<td>84.2</td>
</tr>
<tr>
<td>Lao PDR</td>
<td>99.2</td>
<td>36.7</td>
<td>96.1</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>99.9</td>
<td>52.9</td>
<td>96.3</td>
</tr>
<tr>
<td>Brazil</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
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</table>

Drug pressure: Satisfactory, doubtful, reduced
Starworms

- Differences across study sites due to anthelmintic resistance?
Starworms

- Follow-study in Tanzania
Starworms

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Starworms

- Study design same as in WP1
  - Countries / implementation units were drug pressure has been consistently high:

  - Africa
    - Ghana
    - Rwanda
    - Senegal

  - Asia
    - Bangladesh
    - Cambodia
    - Lao PDR
    - Vietnam

  - Americas
    - Dominican Republic
    - Nicaragua
Starworms

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Visual tutorials
The tutorials demonstrate different operational procedures used to monitor drug efficacy and the emergence of anthelmintic resistance, including, but not limited to diagnostic techniques.

ParaDrug
This tool provides support in analyzing, summarizing, interpreting and reporting drug efficacy data without the need of any prior knowledge on statistical software.

Starworms documents
The forms represent the standard operating procedures, the data record forms and the data entry forms used in the project.

ParaDesign 2.0
This tool provides the most cost-effective study design to be used in deworming programs without the need of prior knowledge on the underlying mathematical framework or any statistical software.

Virtual STH sample inventory
This virtual inventory allows you to upload your own studies or identify other studies and research groups that have access to samples related to STH research (DNA, eggs & worms, host stool, urine, serum, etc.).
Thank you for your attention

www.starworms.org