Leiden University Medical Center

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# Wat kunnen we diagnostisch leren van de *Controlled Human Infection Models*?

*22nd November 2018*
*Lisette van Lieshout (LUMC)*

*Slides:*
*Meta Roestenberg, Marijke Langenberg, Marie-Astrid Hoogerwerf*
*Mark Dekker, Chelsea Gootjes*
*Paul Corstjens and Govert van Dam*

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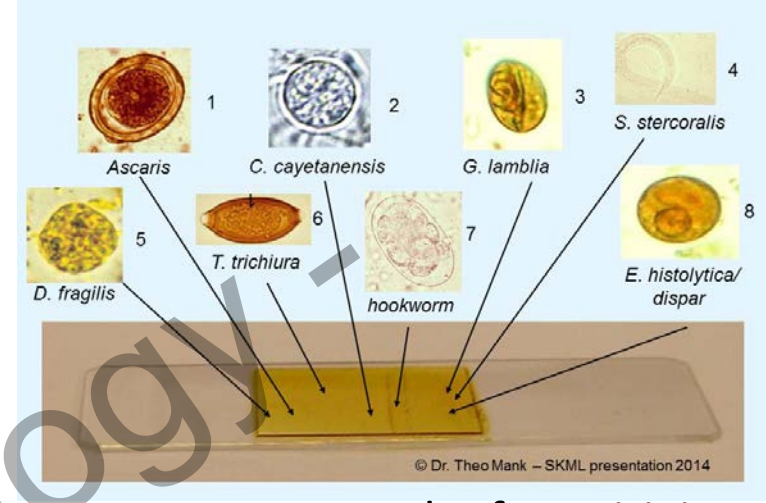
# Diagnosis of intestinal helminths infection
# Globally: key role microscopy

Glyc. sedimen.




Lack of sensitivity

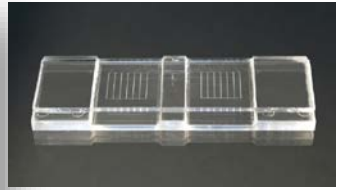
Surveys: eggs/10mL

eggs per gram (epg)

Urine filtration

Kato - slides

Mini-Flotac



Mc-Master

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# Schistosomiasis

World-wide: 200-250 million cases

Diagnosis: population surveys vs individual test-and-treat

Imported cases in the Netherlands:
several hundreds a year

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# Key diagnostic questions in schistosomiasis

How soon after exposure positive?

Infection yes/no?
When?

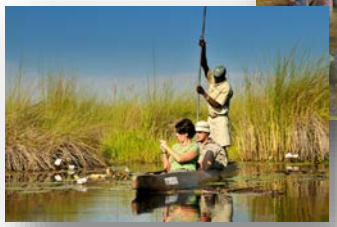
Worm burden?

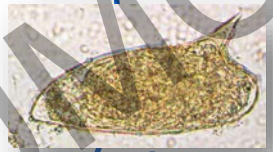
*S. mansoni*

*S. haematobium*

*S. japonicum*

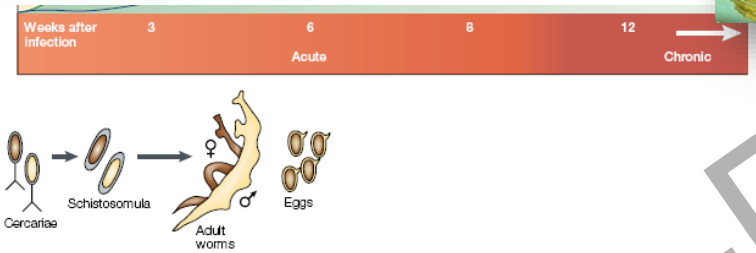
Worm species?





Morbidity indication?

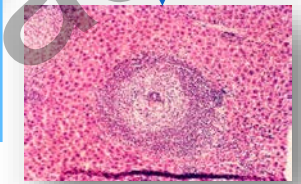
Effect of treatment?




*Biomphilaria*

*Bulinus*

*Oncomelania*



Symptoms of chronic infection; still viable worms?

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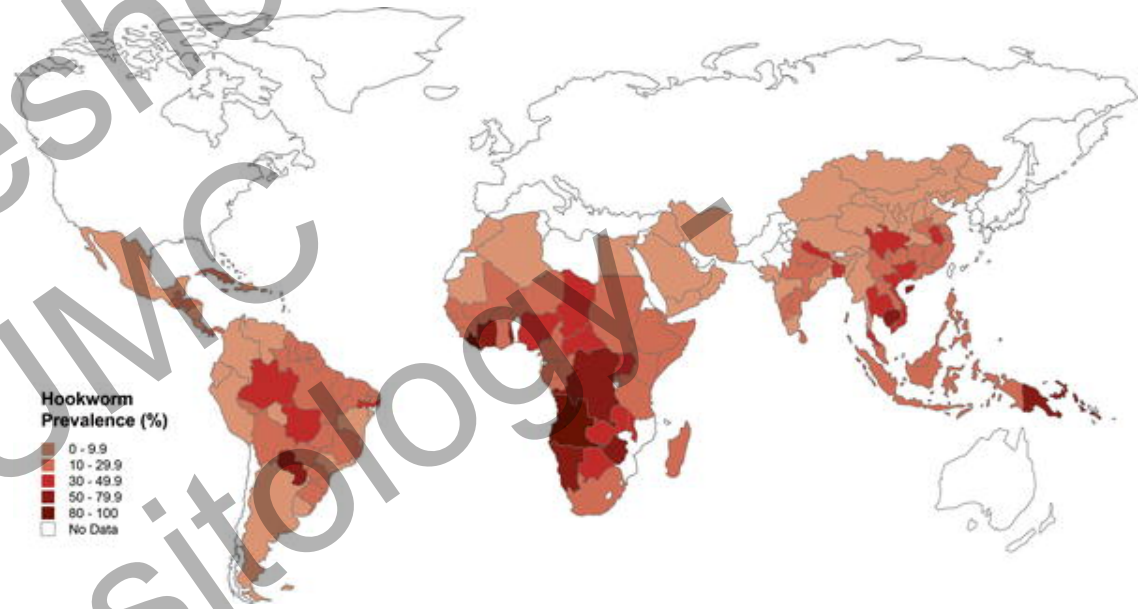
# Hookworm (*Necator americanus/Ancylostoma* spp.)

World-wide: ≈450 million cases

Mainly in developing countries;

Anaemia, blood and protein loss




Imported cases in the Netherlands???

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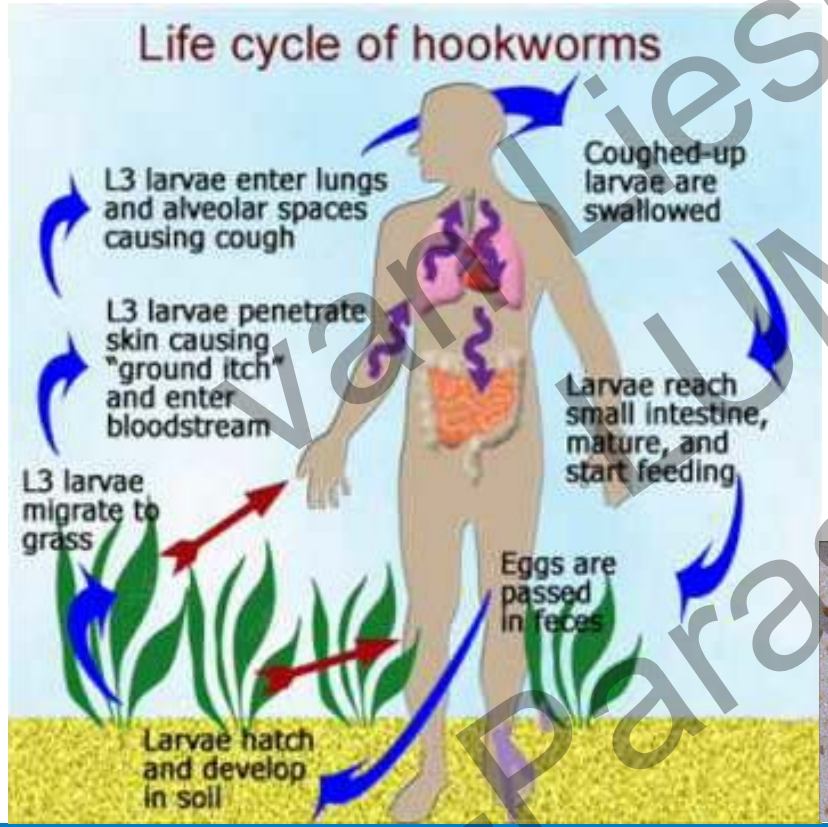
# Key diagnostic questions in hookworm infections

Infection yes/no?
When?

How soon after exposure positive?

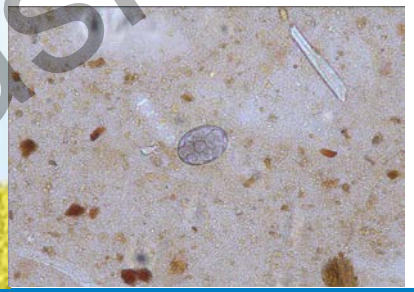



Worm burden?

Morbidity indication?

Worm species?

Effect of treatment?



Alternative for Kato??
Flotation procedures?
PCR?

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# Het diagnostisch verlanglijstje

*Lancet Infect Dis 2018* **Review**

## Experimental infection of human volunteers

*Meta Roestenberg, Marie-Astrid Hoogerwerf, Daniela M Ferreira, Benjamin Mordmüller, Maria Yazdanbakhsh*

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# Controlled Human *Schistosoma* infection (CoHSI)

Meta Roestenberg and team

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Not published

Not published

**Dosage of MALE *Schistosoma mansoni* cercariae**

| | Group A<br>3 volunteers | Group B<br>7 volunteers |
|---|---|---|
| Group 1 | 10 cercariae | |
| Group 2 | 30 cercariae | |
| Group 3 | 20 cercariae | 20 cercariae |

Jacqueline Janse and colleagues

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Janse *et al.*, JID (2018); Langenberg *et al.* (in press); Roestenberg *et al.*, (manuscript in preparation)

# CoHSI – model to study clinical symptoms & host responses

Not published

Marijke Langenberg and colleagues

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# Serology hookworm – not in clinical diagnosis
# Serology: anti-*Schistosoma* antibody detection

Commercial tests/in-house tests; a.o.

IHA – α-worms (AWA); ELISA – IgG α-eggs (SEA)

Some only for *S. mansoni*

Specificity?? (may differ per test)

~~Late(r) sero-conversion (may differ per test)~~

Occasionally false negative (chronic infections) (may differ per test)

No correlation with intensity of infection/clinical symptoms

Not for evaluation of treatment

**LUMC: in-house tests** (since 1982)

IFA – IgM α-worms (AWA)

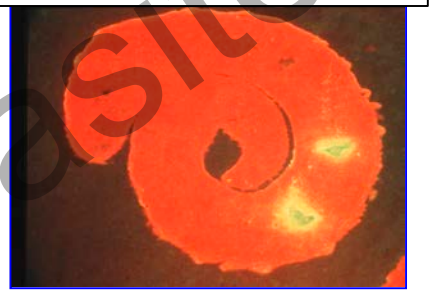
Based on T. Nash *et al.,* 1978 AJTMH

ELISA – IgG α-eggs (SEA)

≈ 1200 sera/year, mostly external

≈ 18% positive

Anti-adult worm antibodies



*IFA: sero-conversion ≈ 4-8 weeks*

Anti-eggs antibodies

*ELISA: less sens/spec.*

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# Sero-conversion 4-6 weeks after exposure

Not published

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Langenberg *et al.* (in press); Roestenberg *et al.*, (manuscript in preparation)

# Sero-conversion 4-6 weeks after exposure – minimal effect PZQ treatment

Not published

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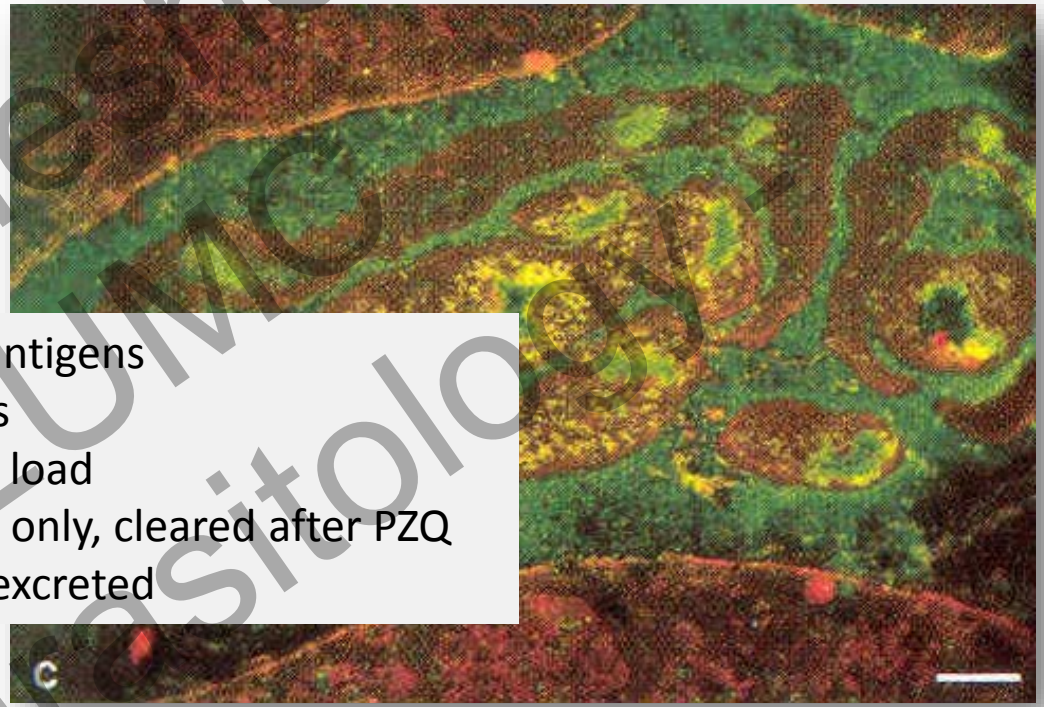
Langenberg *et al.* (in press); Roestenberg *et al.*, (manuscript in preparation)

# *Schistosoma* Circulating Cathodic Antigen (CCA)
# *Schistosoma* Circulating Anodic Antigen (CAA)

CCA in worm gut

McAb fluorescence microscopy

CAA excreted by worm in liver vein




Van Dam *et al.*

Gut-associated antigens
Glyco-conjugates
Reflects parasite load
Active infections only, cleared after PZQ
Serum => urine excreted

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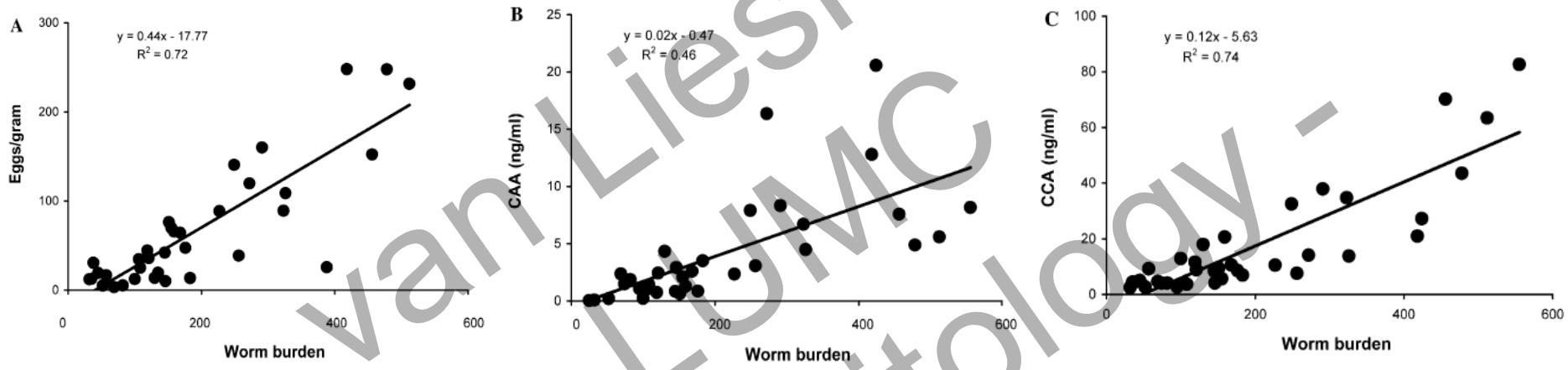
# Schistosomiasis – intensity of infection

## Egg counts and worm antigen CAA and CCA correlate to worm burden




- *good correlations of egg counts, CAA and CCA to worm burden*
- *lower worm numbers are detected by CAA and CCA*

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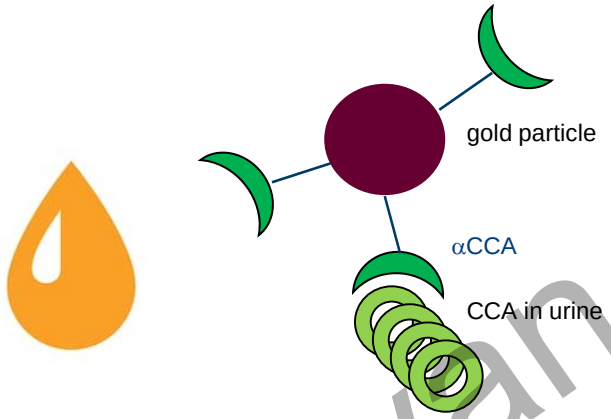
# POC-CCA test principle
# Aiming for field applicable & user friendly

Govert van Dam and team




Urine test *S. mansoni*
Less for other species
Sensitivity > 1x stool microscopy
Spec ≈98% (traces? batch variation)
Endemic settings, o.a.:
Colley *et al.*, (2013, 2017)
Lamberton *et al.*, (2014)
Post-treatment monitoring

POC-CCA
No diagnostic value in CoHSI

http://rapid-diagnostics.com/

rapidmedical diagnostics

*Silveira et al PLOS NTD 2016*

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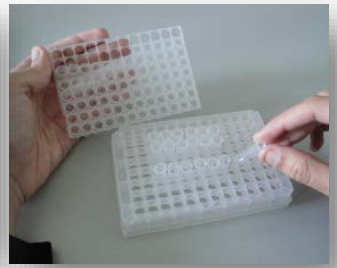
# Ultra-sensitive UCP Lateral Flow CAA assay (Up-Converting Phosphor technology)

**Optional: dry reagents**



**Incubate**

**Add LF strips, run for 1 h**

Govert van Dam and team

Paul Corstjens and team

## Sample pre-treatment

Mix serum or urine with equal volume 4% (w/v) TCA

Spin

(Concentrate supernatant)

Spin

Add 20 µL supernatant to 100 µL assay-buffer (UCP-McAb)

Mix

Incubate 1 h at 37 °C, shaking

**Read strips, quantitate (AWA-TCA dilutions as standard)**

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Corstjens *et al.*, JCM (2008); see review Corstjens *et al.*, Parasitology (2014)

# Summary of available CAA assays

| Required volume | Lower detection limit |
|---|---|
| | wet-assay (=> dry, more batch variation) |
| Serum 20 µl | 10 pg/ml |
| Serum 500 µl | 1 pg/ml (0.1- 3 pg/ml) |
| Urine 10 µl | 10 pg/ml |
| Urine 250 µl | 1 pg/ml |
| Urine 2 ml | 0.1 pg/ml (0.03- 0.3 pg/ml) |
| Urine 7,5 ml | 0.02 pg/ml |

Not published
> 1 worm detected in baboon serum by CAA

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# CoHSI – model : CAA in serum (0.5 mL assay)

Not published

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Langenberg *et al.* (in press); Roestenberg *et al.*, (manuscript in preparation)

# CoHSI – model : CAA in serum (0.5 mL assay)

Not published

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# CAA – levels comparable to low endemic area

Not published

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Langenberg *et al.* (in press); Roestenberg *et al.*, (manuscript in preparation)

# Hookworms: Product dossier

All larvae released according to GMP Guidelines

Production

Collect faeces → Microscopy (Kato smear) negative for hookworm eggs
PCR positive for:
*Norovirus, Sapovirus, Astrovirus, Adenovirus Rotavirus*
*Salmonella, Shigella, Campylobacter, Yersinia*
*Entamoeba histolytica, Giardia, Cryptosporidium*
Bacterial culture positive for:
*Staphylococcus aureus*
*Gentamicin-resistant Enterobacteriaciae*

Culture 7-10 days

Collect larvae → Microscopy negative for *Necator americanus* L3 larvae
Viability <80%
PCR positive for:
*Ankylostoma duodenale, Strongyloides stercoralis*
Bacterial/fungal culture positive for pathogenic bacteria particularly: *Staphylococcus aureus*
*Pseudomonas aeruginosa*
*B-hemolytic streptococci*

Prepare 50 L3 CHHI dose on gauze → Microscopy negative for Necator americanus L3 larvae
Viability <80%

Release

Termination of process

Meta Roestenberg and team

Eric Brienen and colleagues

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# Controlled Human Hookworm Infection Leiden (CHHIL)

Marie Astrid Hoogerwerf and colleagues

Not published

CHHIL (2017):
N=4; Infection with 50 L3 larvae
Dose divided over both arms and legs
Weekly follow-up for 12 weeks
On demand faeces donation after week 12

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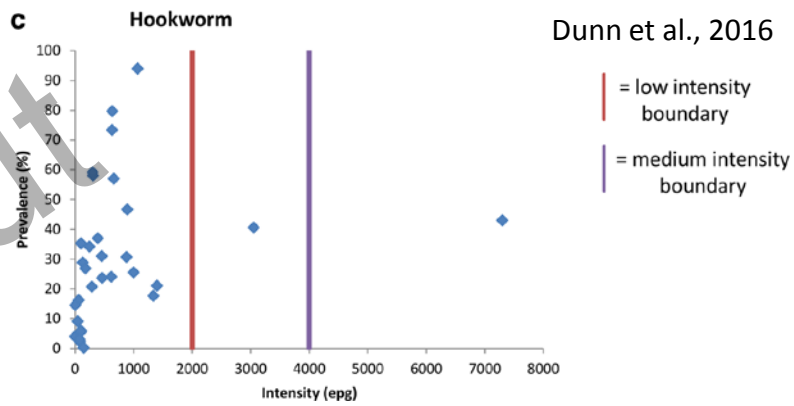
Not published




Dunn et al., 2016

First positive week 7 (also in PCR)
All positive week 9
Flotations no improvement

Plateau phase +/- week 13
Posterior mean ~1000 epg in plateau phase

Variability: Low variability in measurements of same volunteer on same day due to standardised Kato's => improves reliability of model

High variability between days and between individuals

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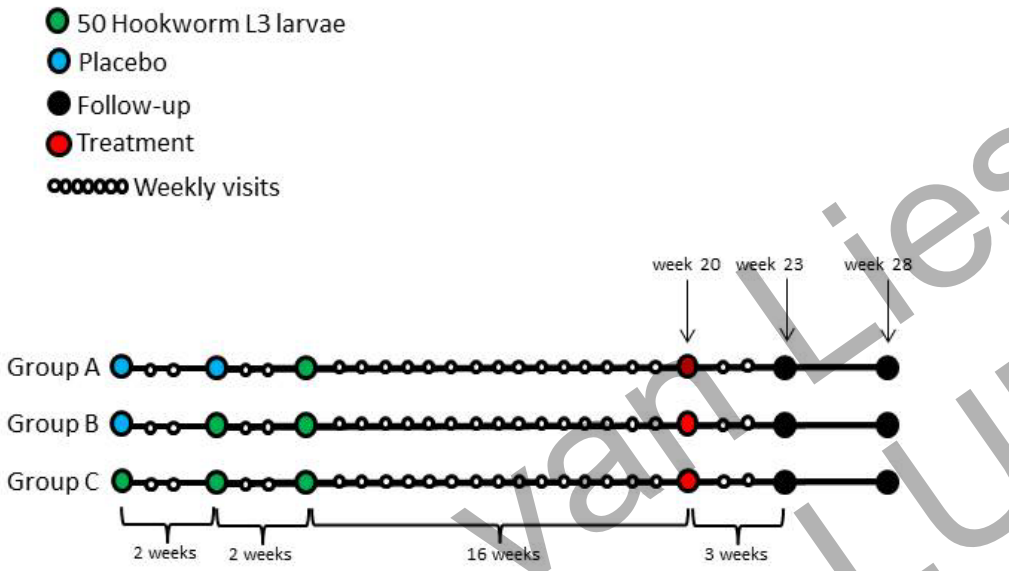
# Repeated Controlled Human Hookworm Infection (ReCHHI)



Not published

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# ReCHHI

Not published

Infection with 1x50L3 larvae lower egg counts

Infection with 3x 50 larvae does not result in higher egg counts than 2x 50L

Variability lower with repeated infection

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# Conclusions – diagnostic aspects of CHIM

CoHSI: First controlled human schistosome infection, model for testing novel products

- Seroconversion 5-6 weeks
- High antibody responses – also in very light infections
- Not for follow-up after treatment
- UCP-LF CAA serum; detectable 4-6 weeks post exposure; > 20 cercs
- Reflection of worm burden => still dynamic early in infection
- Follow-up after treatment (40 mg/kg PZQ is insufficient to achieve 100% cure)
- CoHSI worm burden, still low compared to endemic population

CHHIL/ReCHHI (data in analysis):

- Eggs 7-9 weeks; independent of worm burden
- Comparable to endemic population
- Variation!!!! Importance of Kato standardisation
- PCR slightly more sensitive than microscopy – variation!

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## The volunteers

Parasitology:
Maria Yazdanbakhsh
Lisette van Lieshout
Ron Hokke
Jacqueline Janse
Marijke Langenberg
Carola Feijt
Munisha Ganesh
Marie-Astrid Hoogerwerf
Jan-Pieter Koopman
Janneke Kos
Arifa Ozir
Angela van Diepen
Eric Brienen
Govert van Dam
Pytsje Hoekstra
Yvonne Kruize
Beatrice Winkel
Roos van Schuijlenburg
Mikhael Manurung
Abena Amoah
Miriam Casacuberta
Erliyani Sartono

Infectious diseases:
Leo Visser
Kitty Suijk
Gerdien Hardeman
Jos Fehrmann
Petra Verbeek-Menken
Martijn Bauer

Cell and chemical biology:
Paul Corstjens
Claudia de Dood

Animal facility:
Jan-Bas Prins
Ewout Speksnijder

IGFL:
Pauline Meij
Maarten Zandvliet
Anton Terwisscha
Inge Westra

Medical Microbiology:
Martha van Beek
Marianne Erkens
Pauline vd Plas
Els Wessels
Yvette van Aarle
Jutte de Vries
Christel van Zeijl

Gijsbert van Willigen

IMI lab:
Fijs van Leeuwen
Clarize Korne
Danny van Willigen
Mick Welling
Matthias van Oosterom

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www.controlledhumaninfection.org

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# Acknowledgements


**Volunteers**

PARA:
**Meta Roestenberg**
**Maria Yazdanbakhsh**
Jacqueline Janse
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Jan Pieter Koopman

Lisette van Lieshout
Eric Brienen
Yvonne Kruize
Erli Sartono
Michael Manurung
Mark Dekker
Chelsea Gootjes
Carola Feijth
Munisha Ganesh
Roos van Schuijlenburgh

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Martijn Bauer
Geert Groeneveld
Petra Verbeek
Jos Fehrmann
Kitty Suijck
Gerdien Hardeman

KFT:
Pauline Meij
Anton Terwisscha van Scheltinga
Inge Westra

EMC:
Luc Coffeng
Sake de Vlas

KML:
Marianne Erkens
Martha van Beek

MM:
Ed Kuijper
Bastian Hornung
Romy Zwittink
Quinten Ducarmon
Anoe Geelen

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