Leiden University
Medical Center

Diagnosis of schistosomiasis in migrants:
New diagnostic approaches at the horizon

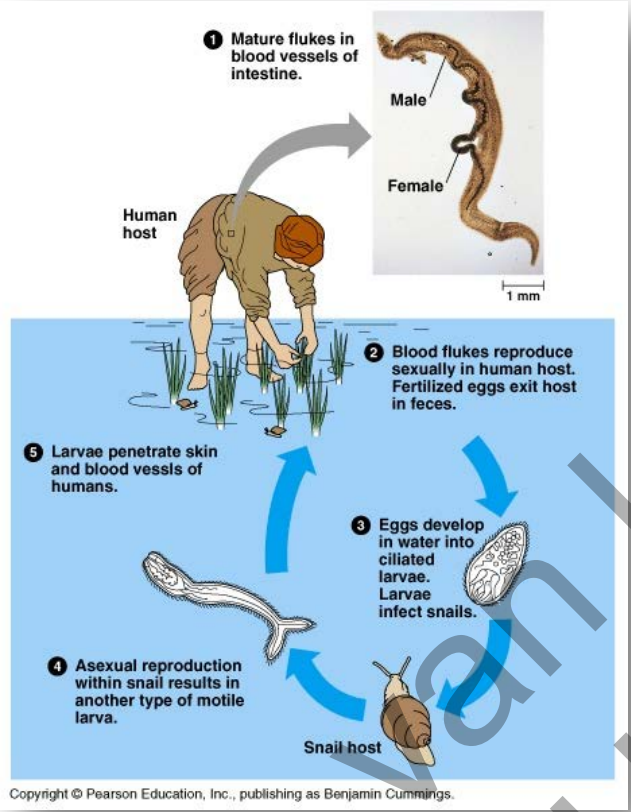
24 November 2017

Lisette van Lieshout (LUMC)

With special thanks to Eric Brienen, Pytsje Hoekstra, Claudia de Dood, Jutte de Vries, Rebecca van Grootveld, Meta Roestenberg, Paul Corstjens and Govert van Dam

Colleagues from Maastricht UMC & Deventer hospital

Schistosoma - Blood parasites



Schistosomiasis:
Globally ≈258 M infected cases (WHO), >SS Africa

Imported cases in NL:
several hundreds a year

- traveller
- ex-pats
- migrants from endemic countries

Newly emerging risk groups within Europe: Refugees & Visiting Relatives and Friends

Diagnostic challenges in schistosomiasis

Case 1 17Y (M) recent migrant, Somalia (Deventer Zkh)

- Intermittent haematuria
- LUMC: serology negative => D: Egg seen in urine

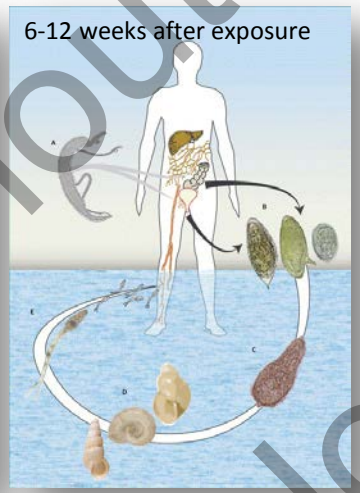
Case 2 21Y (M) recent migrant Sierra Leone (Maastricht UMC)

- Intermittent haematuria, urine microscopy neg but Sh according to urologist
- LUMC: serology negative

Case 3 25Y (M) recent migrant Eritrea (LUMC)

- Several clinical issues, stress related, obstipation, weight loss, mild eosinophilia
- Serology positive (IFA only), => microscopy: urine and faeces neg

Schistosoma diagnostics: microscopy

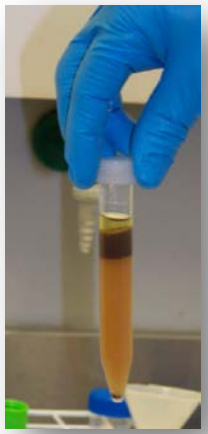


Surveys: eggs/10m eggs per gram (epg)

Urine filtration

Kato - slides

Glyc. sedimen.



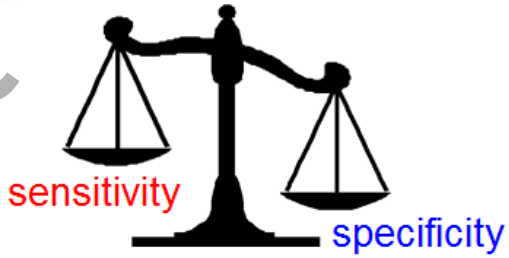
FEC

Serology: anti-*Schistosoma* antibody detection

Commercial tests/in-house tests

IHA – α-worms (AWA)

ELISA – IgG α-eggs (SEA)



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

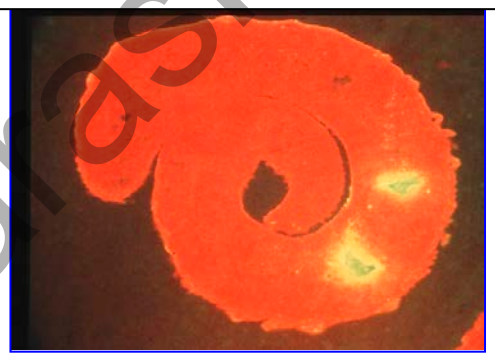
LUMC: in-house tests

IFA – IgM α-worms (AWA)

ELISA – IgG α-eggs (SEA)

≈ 1200 sera/y (≈ 18% pos)

Anti-adult worm antibodies



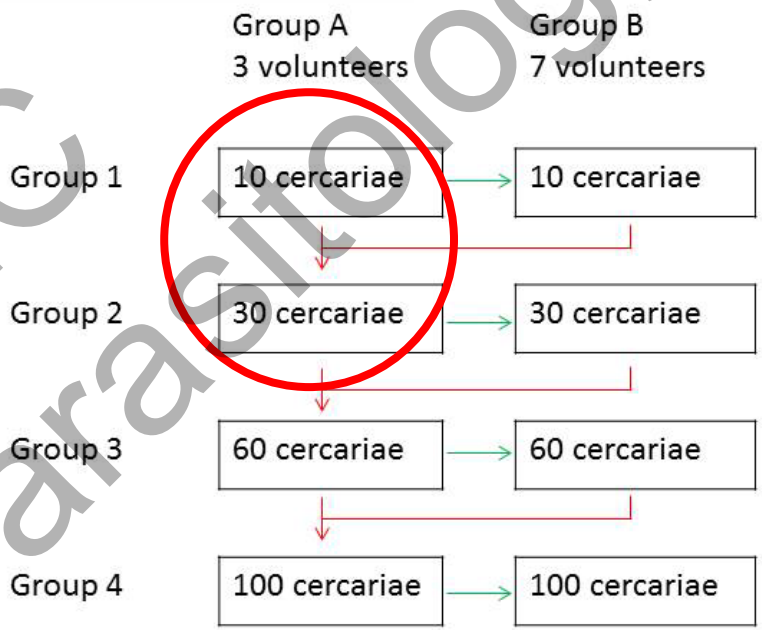
IFA: sero-conversion ≈ 4-8 weeks

Anti-eggs antibodies

ELISA: less sens/spec.

Review: Serological approaches for the diagnosis of schistosomiasis. Hinz et al., 2017 (MolCelProb 31:2)

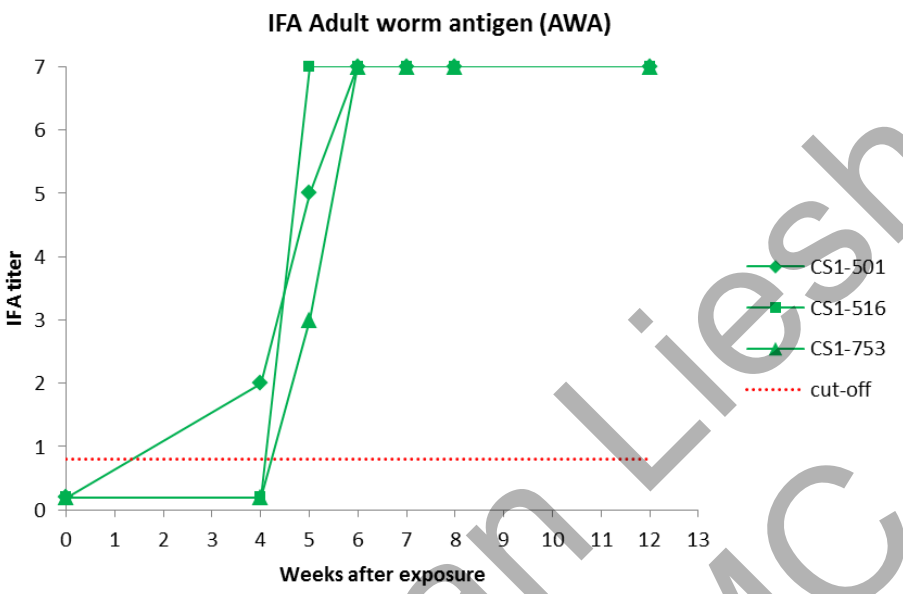
Controlled Human *Schistosoma* infection (CoHSI)



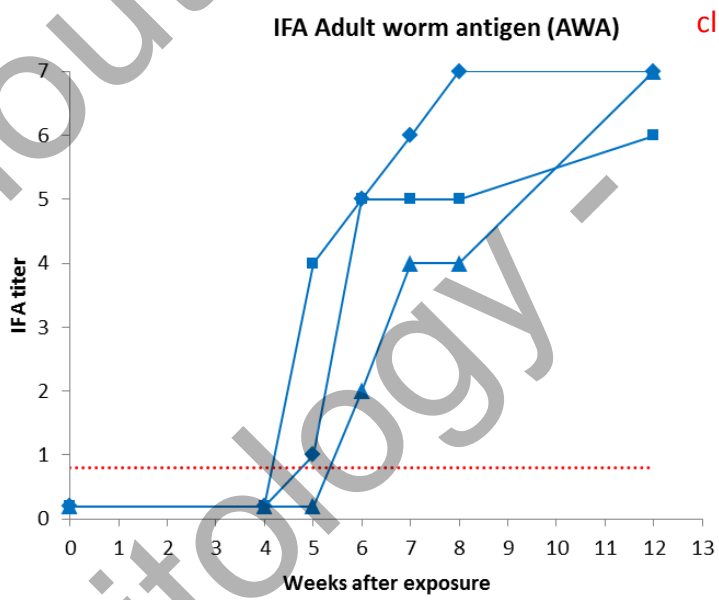
Roestenberg *et al.*, (manuscript in preparation)

Antibody responses in CoHSI

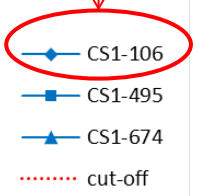
10 cercariae



30 cercariae

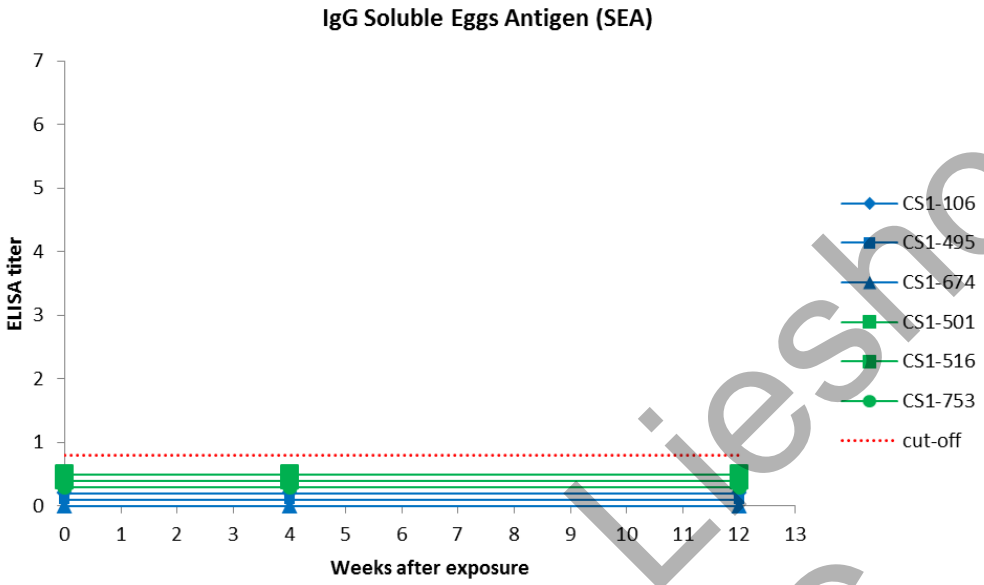


Katayama-related clinical symptoms

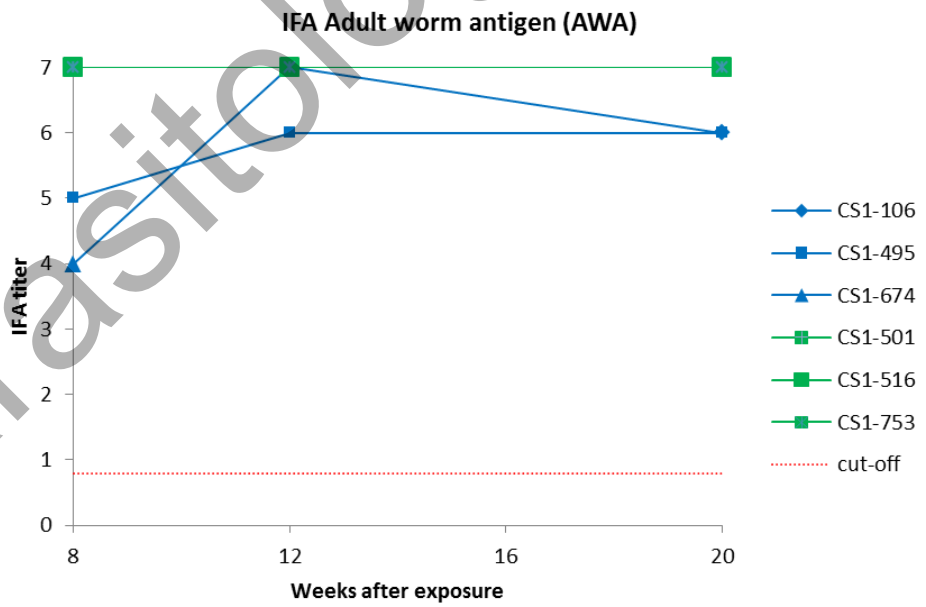


Roestenberg, Van Dam, Corstjens, Van Lieshout *et al.,* (manuscript in preparation)

Antibody responses in CoHSI



PZQ treatment at week 12



CoHSI confirms seen in LUMC clinical data:

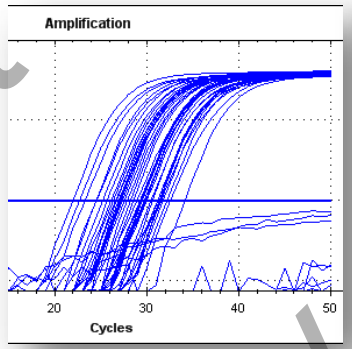
- highly sensitive and specific in first time exposed cases
- sero-conversion 4-8 weeks (IFA)

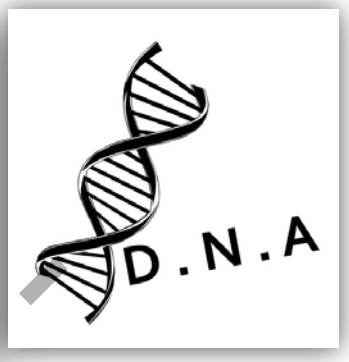
- no correlation with intensity of infection/ clinical symptoms
- not for evaluation of treatment

What to do with occasionally false negatives in chronic infections??

Roestenberg, Van Dam, Corstjens, Van Lieshout *et al.,* (manuscript in preparation)

Schistosoma real-time PCR





Range of in-house tests published

- Spec. 100%
- Ct ≈ intensity
- Stool, urine, clinical samples (serum/plasma)

LUMC:

ITS-2, genus (S.m./S.h.) Obeng *et al.*, 2008

- Stool, urine , sperm, biopsies (not serum)
- Endemic populations:

  Aryeety *et al.* (2013); Pillay *et al.*, (2014); Vinkeles *et al.*, (2014)

  Meurs et al., (2015, 2017) & unpublished data

  Sens. >>> microscopy, not 100%

Schistosoma real-time PCR

Routine diagnostics, since 2007 (≈ 200 stool/y & ≈20 urines/y)

Own LUMC patients: besides microscopy

Case 1 17Y (M) Somalia, haematuria (Deventer)

- Only serum

Case 2 21Y (M) Sierra Leone, haematuria (Maastricht)

- Urine => LUMC: PCR pos (Ct 30) - & Sh+ microscopy
- Feces => LUMC: PCR pos (Ct 25) - & microscopy n.d.

Case 3 25Y (M) Eritrea, obstipation, mild eosinophilia, active infection? (LUMC)

- Urine => PCR neg - & microscopy neg
- Feces => PCR pos (Ct 39) - & microscopy neg

Schistosomiasis
Need for diagnostic alternatives

Aiming for more field applicable/user friendly

1. Highly sensitive for monitoring intervention
2. Highly sensitive for travellers diagnostics
3. Reflect active infection (worm loads)

Circulating *Schistosoma* antigens CCA and CAA

Parasite load, active infection only, cleared after PZQ

Serum => urine excreted

Van Dam *et al.*, 2004

POC-CCA (commercially available)

- Urine test *S. mansoni* (less for other species)
- Sensitivity > 1x microscopy, spec 98% (traces?)
- Endemic settings (Colley *et al.*, (2013, 2017), Lamberton et al., (2014))
- Post-MDA monitoring

Urine POC-CCA for imported infections?

Clinical Infectious Diseases

MAJOR ARTICLE

Accuracy of Diagnostic Tests for *Schistosoma mansoni* Infection in Asymptomatic Eritrean Refugees: Serology and Point-of-Care Circulating Cathodic Antigen Against Stool Microscopy

Afona Chernet[1,2], Kerstin Kling[1,2], Véronique Sydow[1,2], Esther Kuenzli[1,2], Christoph Hatz[1,2], Jürg Utzinger[1,2], Lisette van Lieshout[3], Hanspeter Marti[1,2], Beatrice Nickel[1,2], Niklaus D. Labhardt[1,2], Andreas Neumayr[1,2]

[1]Swiss Tropical and Public Health Institute and [2]University of Basel, Switzerland; and [3]Department of Parasitology, Leiden University Medical Center, The Netherlands

107 newly arrived asymptomatic refugees

35% active *Schistosoma* infection

Sensitivity POC-CCA 91% (extensive microscopy) & additional cases

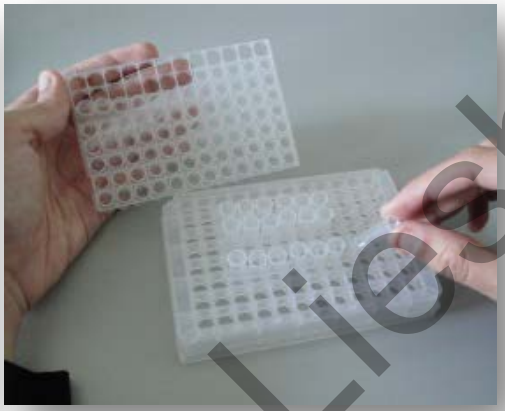
However:

- only *S. mansoni*
- observer dependent
- batch variation in specificity (see study Beltrame *et al*., PLosNTD 2017)
- validation in 60 consecutive urines (HTD London) – 1 positive only (migrant, Ab pos)
- LUMC: not right patient population

Chernet et al., Clin Inf Dis (2017)

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

Dry reagents



Sample pre-treatment

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

Spin

(Concentrate supernatant)

Spin

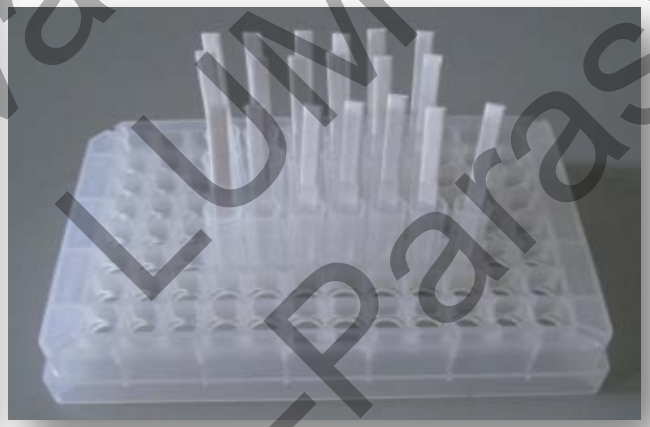
Add 20 µL supernatant to 100 µL assay-buffer (UCP-McAb)

Mix

Incubate 1 h at 37 °C, shaking

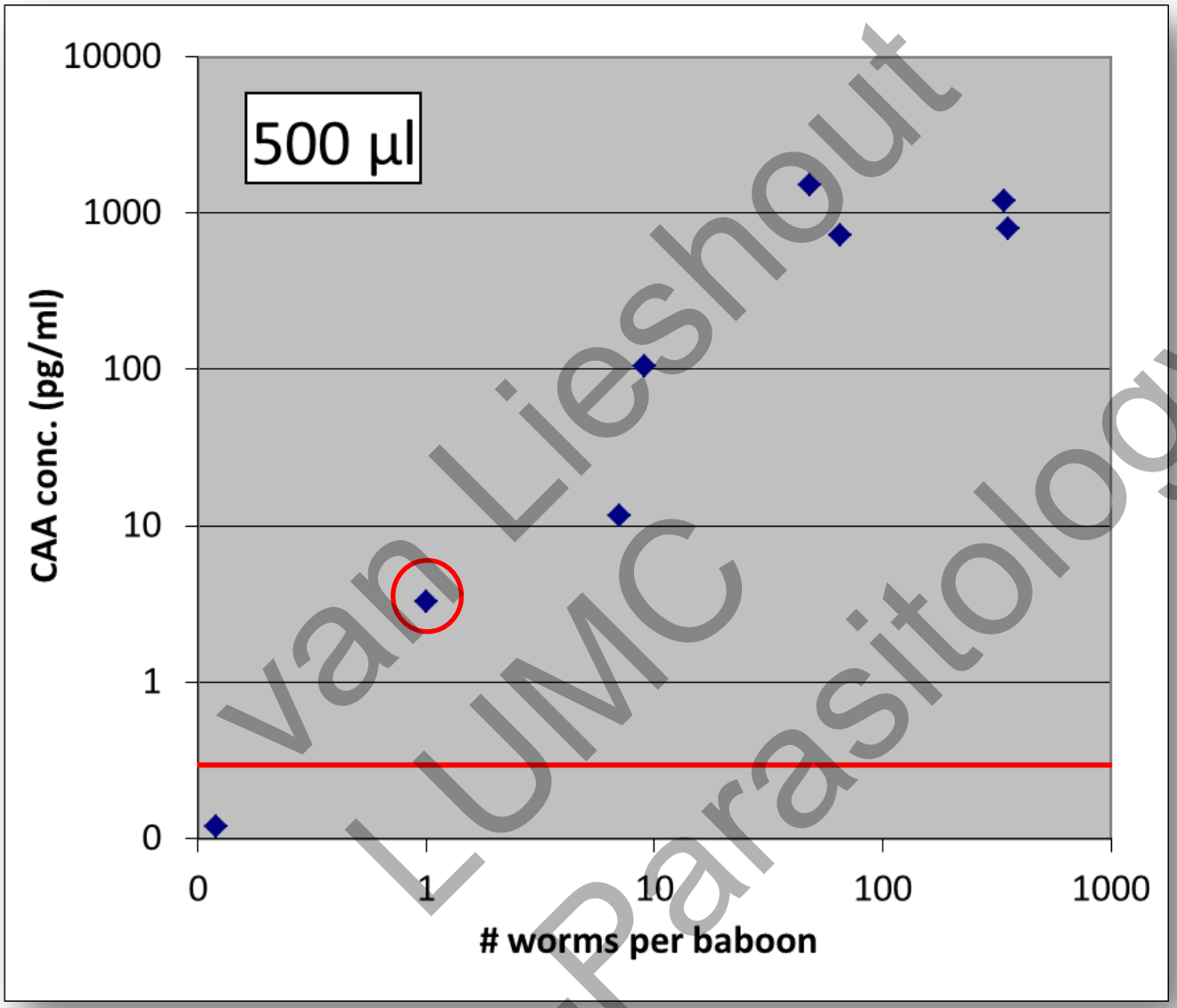
Incubate

Add LF strips, run for 1 h



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

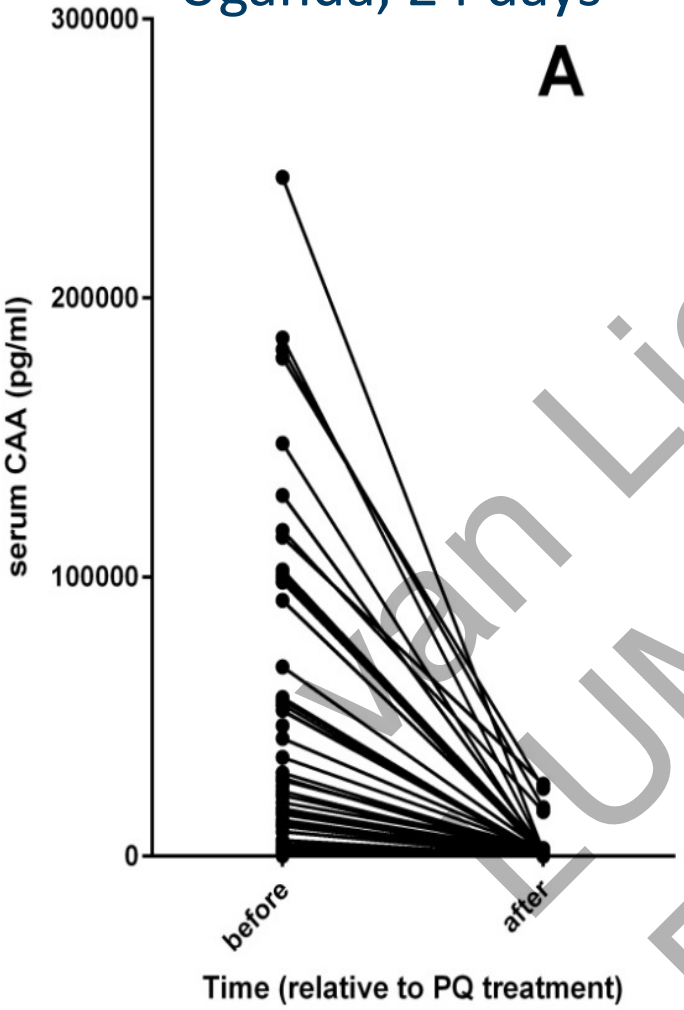
Serum CAA levels vs worm numbers concentrating 500 µL + TCA => 20 µL



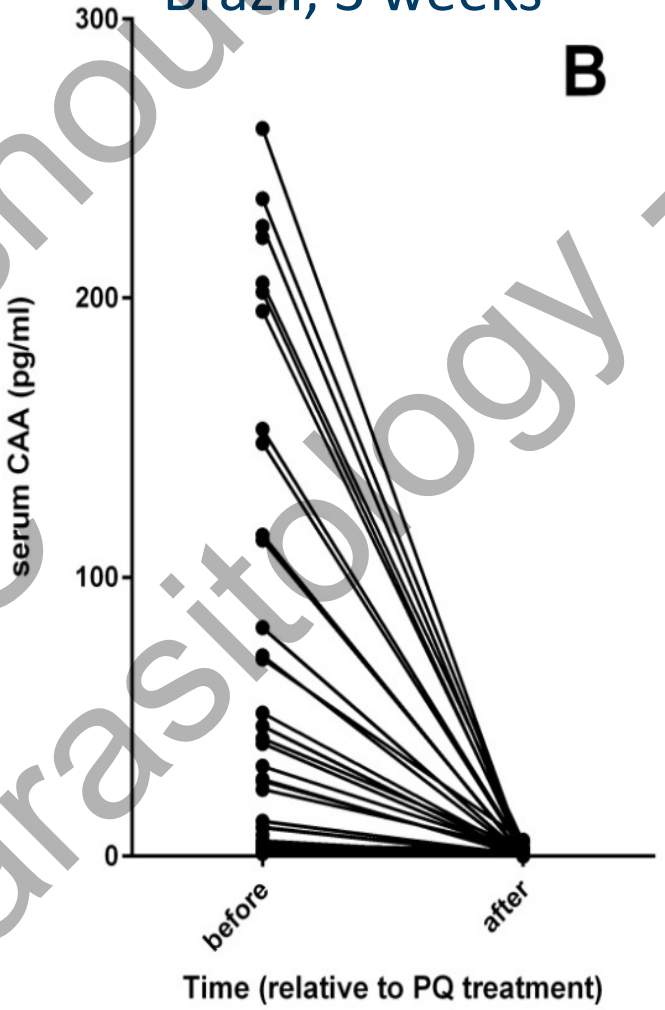
Corstjens *et al., (*2014); Van Dam *et al* (personal data)

Serum CAA in endemic areas
single dose of 40-60 mg/kg praziquantel

Uganda, 24 days



Brazil, 3 weeks



Van Dam & P. Corstjens *et al.,*

CAA levels in routine diagnostics
0.5 mL serum assay

Retrospective analysis LUMC, London (HTD) & Liverpool (LSTM),

- Confirmed 100% specificity (n=19)

Ab positive selected cases (n=229)

- Travellers => 50-60% positive (all low CAA levels)
- Migrants => 60-80% positive (≈ 50% low CAA levels)
- All PCR/microscopy positives (n=12) => CAA positive

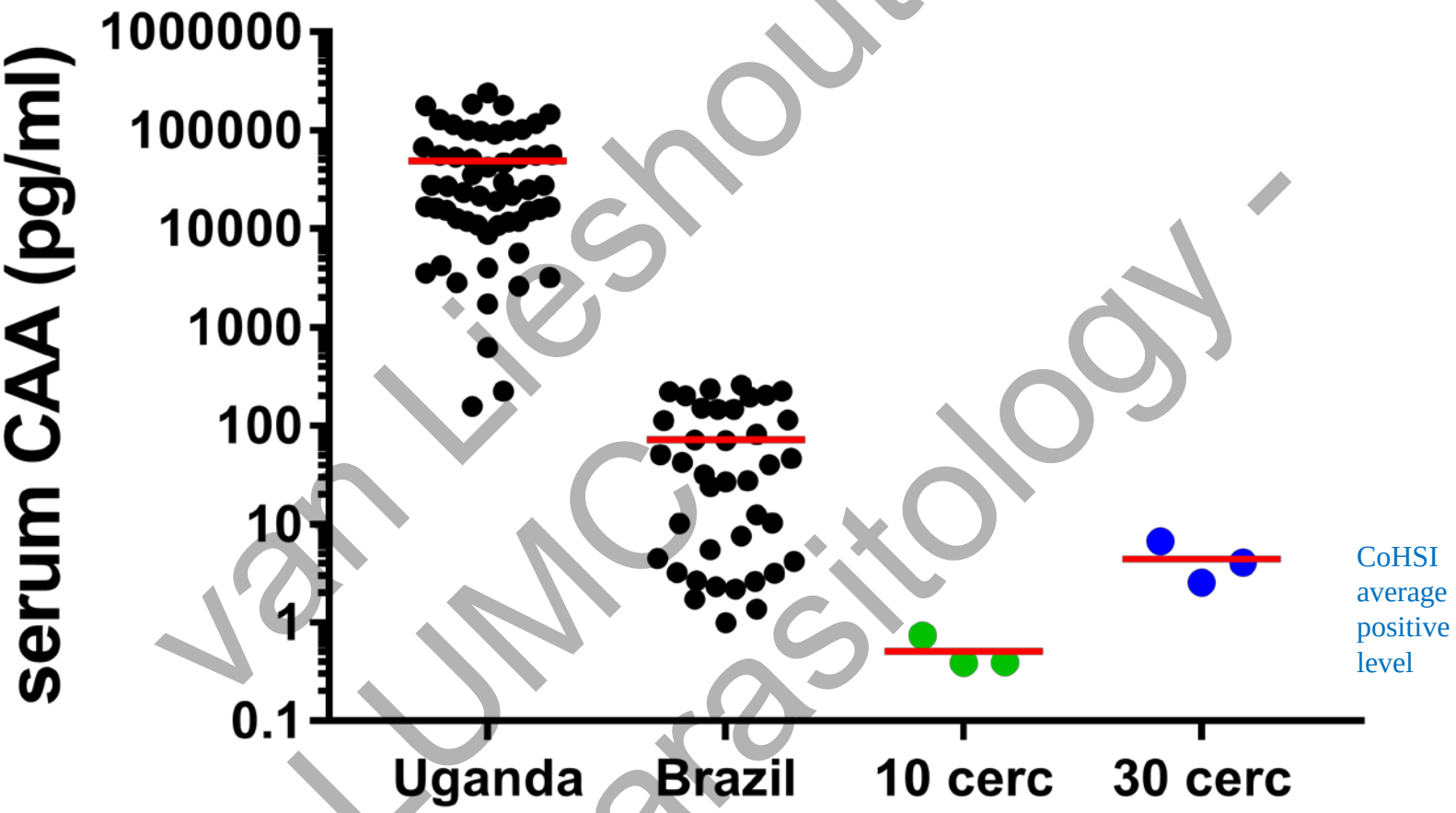
Basel study; screening of asymptomatic refugees Chernet et al., Clin Inf Dis (2017)

- N=107 => 47% positive (≈ 50% low CAA levels)

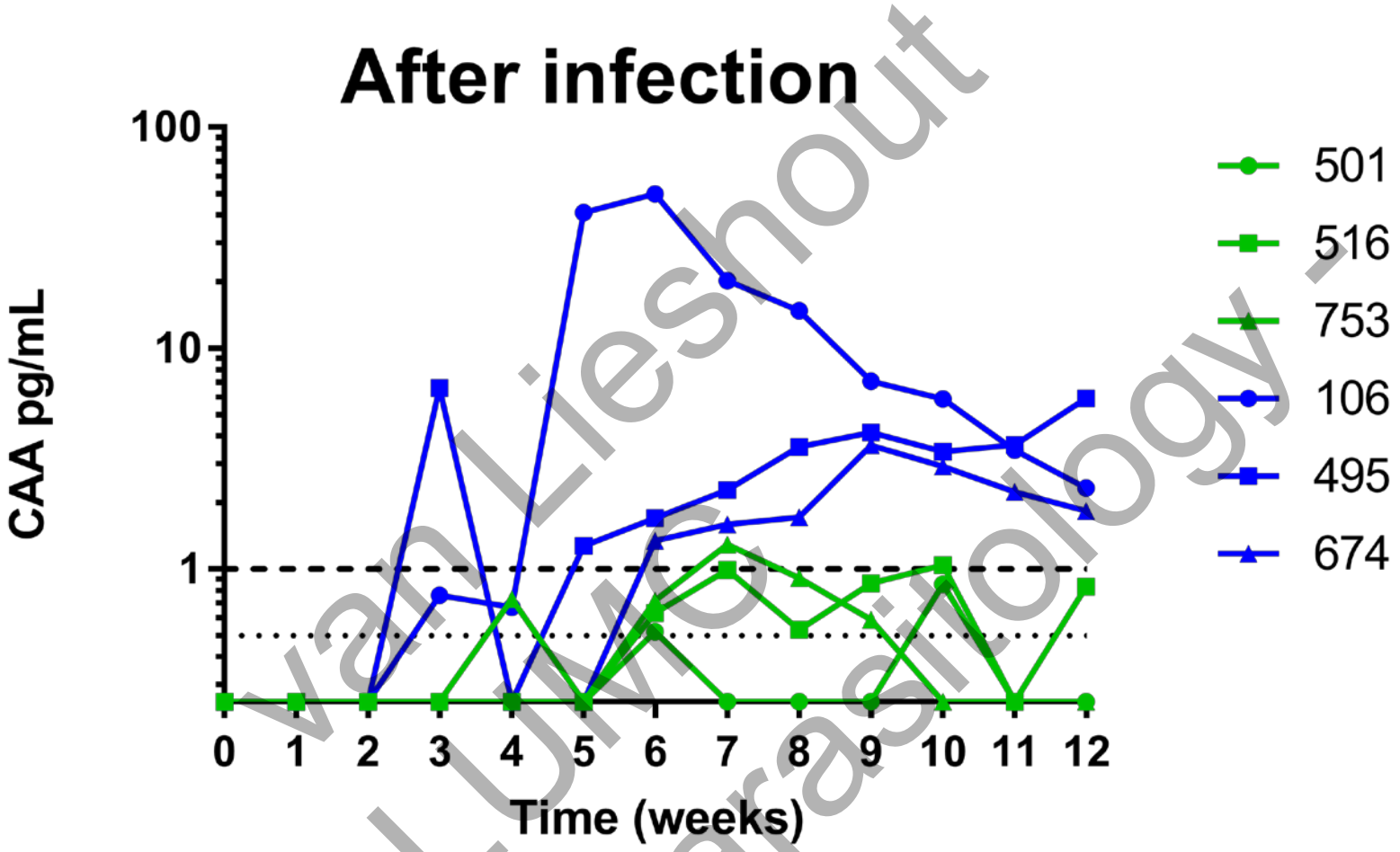
Clinical setting LUMC – prospective validation

a.o. CoHSI study

Serum CAA levels in CoHSI model comparable to low endemic area



Serum CAA levels in CoHSI model



Roestenberg, Van Dam, Corstjens, Van Lieshout *et al.,* (manuscript in preparation)

CAA levels in routine diagnostics
Prospective validation

1. antibody positives & > 0.5 mL serum
2. on special requests (serum or >4 mL urine)

Case 1 17Y (M) Somalia, haematuria, Ab neg, microscopy pos (Deventer)

- Only serum => CAA positive (low)

Case 2 21Y (M) SL, haematuria, Ab neg, PCR & microscopy pos (Maastricht)

- Serum => CAA positive (high)
- Urine=> CAA positive (moderate)

Case 3 25Y (M) Eritrea, obstipation, mild eosinophilia, feces PCR pos (LUMC)

- Serum => CAA positive (high)
- Urine=> CAA positive (moderate)

Schistosomiasis - conclusions

Microscopy: limited sensitivity

Non-microscopy: each test has it owns pro/con

Serology: tests differ in Sens/Spec

some excellent for travelers with 100% sero-conversion in 4-8 weeks

PCR: different targets**;** all good alternative for microscopy, sensitivity varies per target, feces/urine less suitable for travelers

Antigen detection:

POC-CCA: chronic infections/migrants; Sm only, simple to use

UCP-LF CAA: ultra-sensitive; all species; 3-5 weeks after exposure

Schistosomiasis - future

- More post-treatment follow-up studies

- Further standardization of POC-CCA reading
- More user-friendly CAA detecting format
- Implementation as routine diagnostic test

Store:

1 mL serum

5 mL urine

Contact us…. lvanlieshout@lumc.nl