Acute schistosomiasis in a cluster of travellers from Rwanda: diagnostic contribution of schistosoma DNA detection in serum compared to parasitology and serology

Jan Clerinx [1], Emmanuel Bottieau [1], Dominic Wichmann [2,3], Egbert Tannich [2], Marjan Van Esbroeck [1]

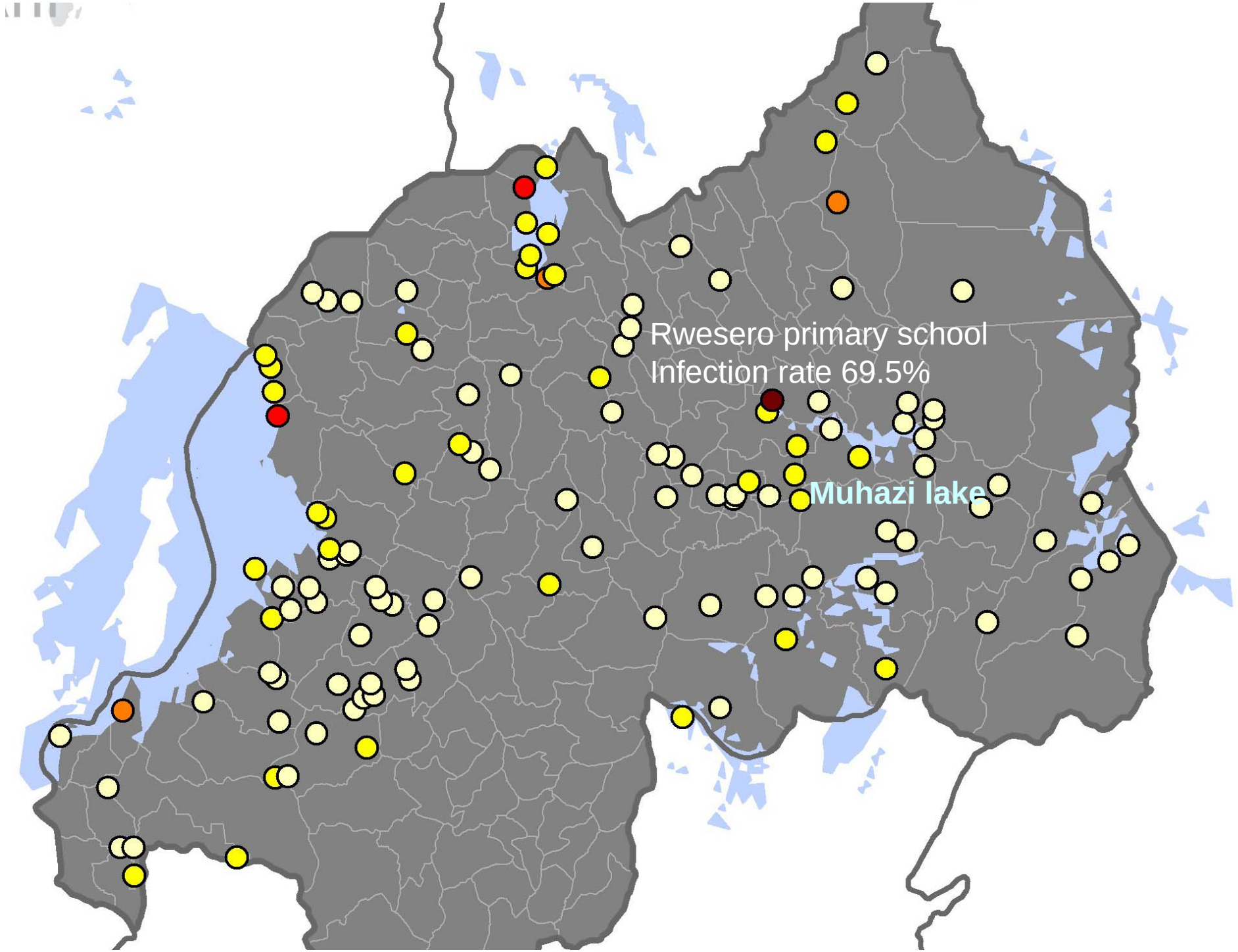
1. Department of Clinical Sciences, Institute for Tropical Medicine Antwerp
2. Bernhard-Nocht Institute for Tropical Medicine, Hamburg, Germany
3. University Hospital Hamburg-Eppendorf, Department of Intensive Care Medicine, Hamburg, Germany

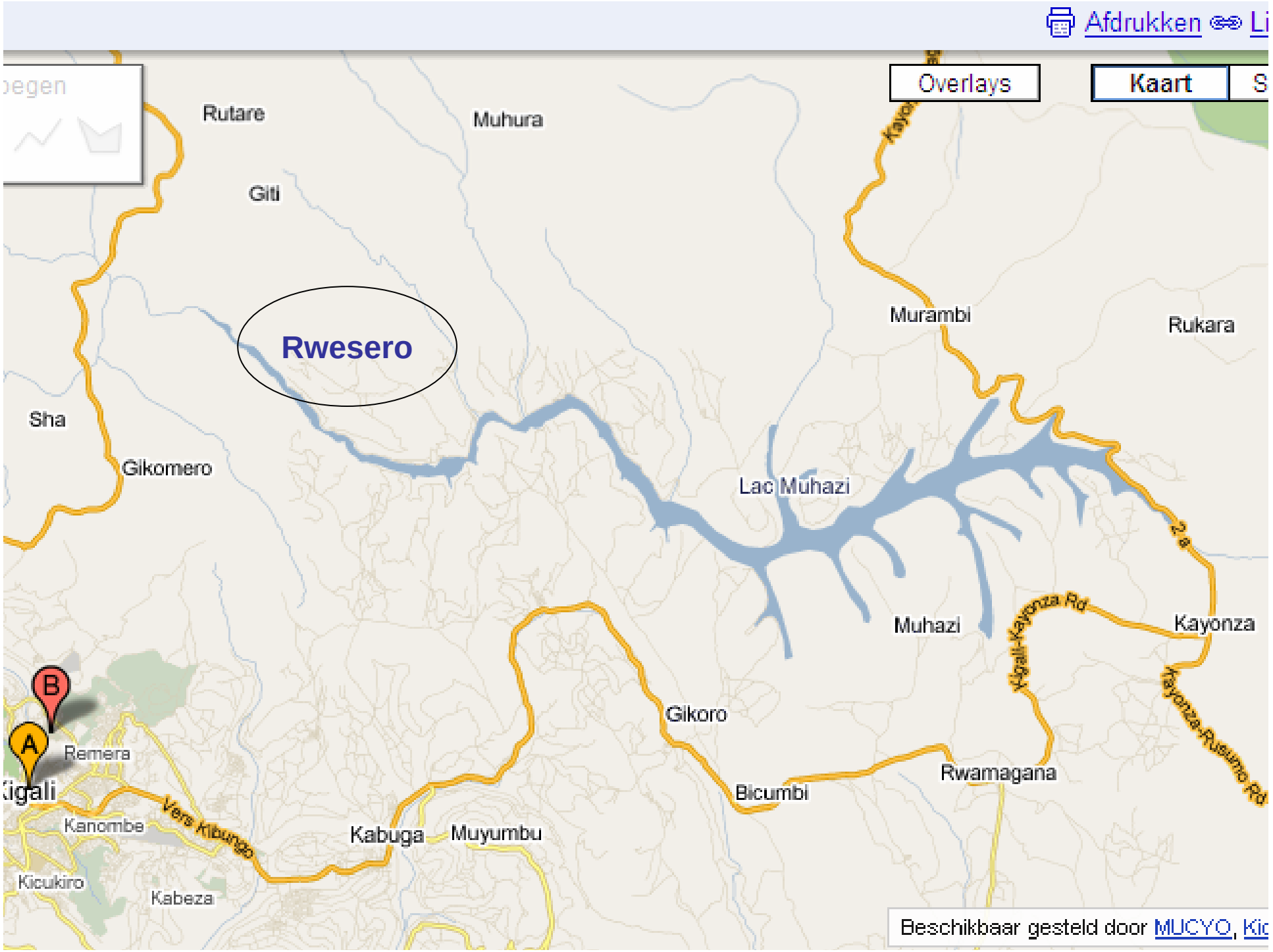
Setting

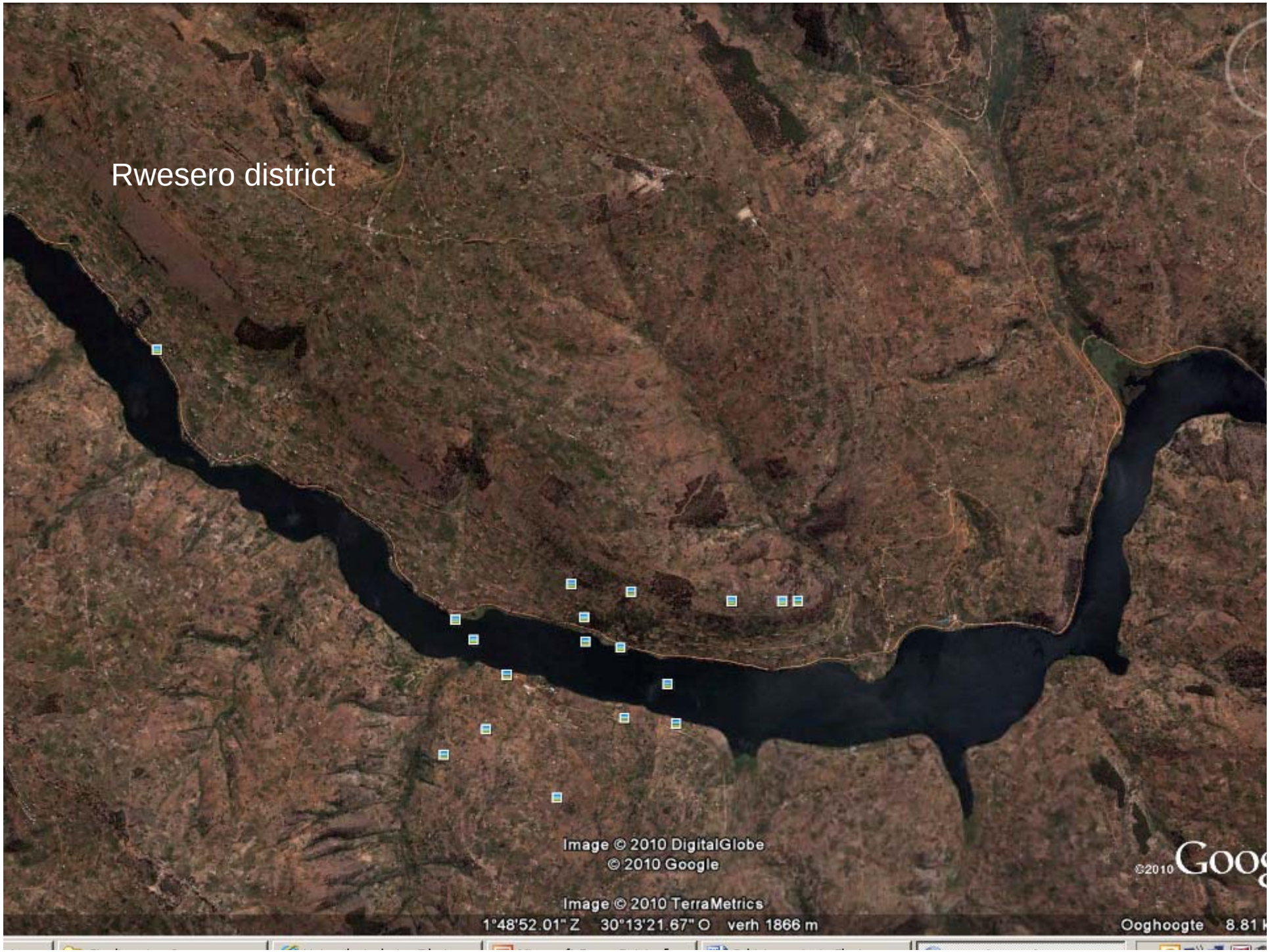
A group of 12 belgian boys (6 to 16 years) and their custodian held a summer camp at the Don Bosco Parish, Rwesero district, Rwanda, on the northwestern shore of Lake Muhazi.

Swimming, fishing and canoeing were almost daily activities for upto 14 days.

Four persons had been holidaying at the same spot two years before as well


Rwesero primary school
Infection rate 69.5%
Muhazi lake


Afdrukken
Overlays
Kaart
Rutare
Muhura
Giti
Rwesero
Murambi
Rukara
Sha
Gikomero
Lac Muhazi
Muhazi
Kayonza
Kigali-Kayonza Rd
Kayonza-Rusumo Rd
Gikoro
Rwamagana
Bicumbi
B
A
Remera
Kigali
Kanombe
Vers Kibungo
Kicukiro
Kabeza
Kabuga
Muyumbu
Beschikbaar gesteld door MUCYO


Rwesero district
Image © 2010 DigitalGlobe
© 2010 Google
Image © 2010 TerraMetrics
1°48'52.01" Z 30°13'21.67" O verh 1866 m
Ooghoogte 8.81

Lake Muhazi, 1450m

Acute schistosomiasis in a cluster of travellers from Rwanda

Clinical features

Presence of following symptoms

-incubation period
-symptom duration
-fever
-urticaria
-angio-oedema
-abdominal pain
-cough

Acute schistosomiasis in a cluster of travellers from Rwanda

Laboratory tests

At diagnosis and at 6 weeks posttreatment with PZQ sd

- eosinophil count
- serology for schistosoma using IFAT and HAI
- faeces concentration test using SAEX method
- RT-PCR using a 121 bp tandem repeat sequence of Sm genome as the target gene. Primers SRS2 en SRA1

to detect schistosoma sp. DNA in a 2 ml serum sample

How does a real time-PCR operate?

(Parasite) DNA-extraction from serum, whole blood, feces….
Phenol-chloroform extraction and centrifugation of DNA

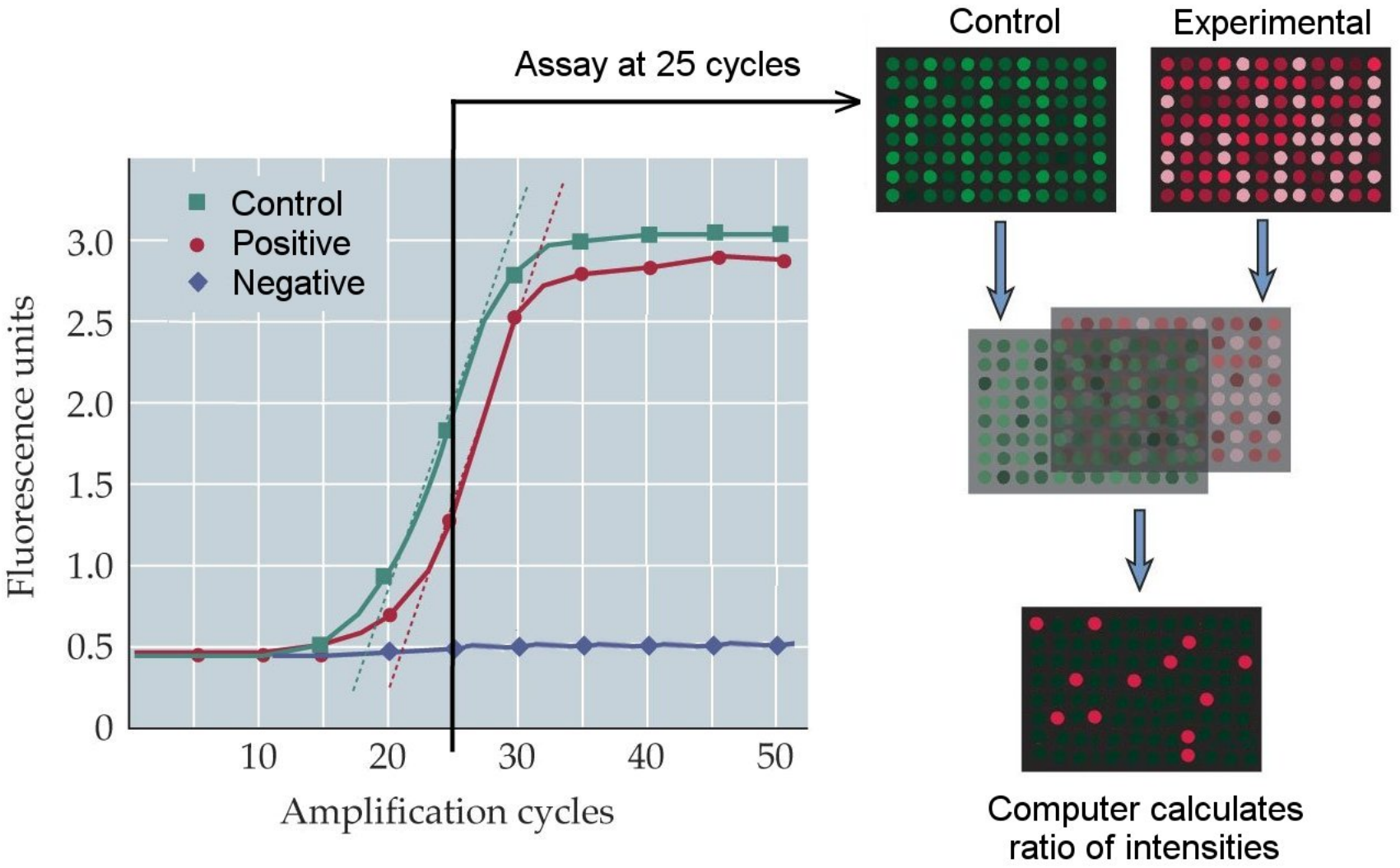
Primer: 121 bp tandem repeat sequence of the *S.mansoni* genome

A real time PCR uses fluo-labeled probes (primers)
in complementary 5’ and 3’ sequences

Amplification: at every cycle: denaturation extracted schisto DNA, binding with probes, incubation with nucleotides, messenger RNA and Taq thermostable RNA polymerase (Taq = Thermus aquaticus)

N **cycles** is set at maximum 40. This produces an exponential amplification of the target sequence at every cycle

Ct value = n amplification cycles needed to provide the minimum preset fluorescence intensity, compared to a standard assay with a predefined probe and with a negative control


Assay at 25 cycles
Control
Positive
Negative
Fluorescence units
0
0.5
1.0
1.5
2.0
2.5
3.0
10
20
30
40
50
Amplification cycles
Control
Experimental
Computer calculates
ratio of intensities

Clinical features of schistosomiasis in a cluster of patients recently exposed to S.mansoni infection

N	exposure	Katayama	T from onset	fever	cough	angio-oed.	urticaria.	abdom.	diarrhea
1	primary	yes	5	**yes**	**yes**	no	no	**yes**	**yes**
2	primary	yes	14	**yes**	**yes**	**yes**	**yes**	**yes**	**yes**
3	primary	yes	7	no	no	**yes**	no	**yes**	no
4	primary	yes	28	no	**yes**	**yes**	no	**yes**	no
5	primary	yes	5	no	no	**yes**	no	**yes**	**yes**
6	primary	yes	3	no	**yes**	no	no	no	no
7	primary	yes	n/a	no	no	**yes**	no	no	no
8	primary	no	n/app						
9	primary	no	n/app						
10	prior	no	n/app						
11	prior	no	n/app						
12	prior	no	n/app						
13	prior	no	n/app						

Legend:
T from onset: symptom onset prior to diagnosis (days); angio: angio-oedema; urticar: urticarial rash; abd: abdominal pain;
diarh: diarrhea; n/a: not available; n/app: not applicable; primary: recently exposed for the first time;
prior: recently exposed but also exposed two years prior.

Diagnostic parameters of schistosomiasis at diagnosis

N	eosino/µl	ELISA	HAI	fEPG	RT-PCR	Ct RT-PCR
1	5400	p	320	30	p	28
2	2090	n	320	120	p	27
3	2640	**n**	**n**	**10**	p	30
4	14150	p	640	40	p	29
5	1150	**n**	**n**	**n**	p	35
6	2860	p	n	20	p	31
7	14270	p	320	60	p	30
8	11120	p	160	10	p	29
9	1960	p	320	n	p	32
10	1290	**n**	**n**	**n**	p	36
11	1210	p	n	10	p	32
12	2120	p	n	n	p	27
13	1700	p	320	10	p	35

Legend:

ELISA: schistosoma ELISA qualitative antibody test; IHA: schistosoma indirect hemagglutination inhibition test, in 1/titer;
Ct RT-PCR: schistosoma DNA detection by real-time PCR in serum sample, in Ct-values;
fEPG: eggs per gram feces; n/a not available; p: positive; n: negative

Evolution of diagnostic parameters of schistosomiasis before (D0) and 6 weeks (D42) after treatment with praziquantel

N	eosinophils/µl		ELISA		HAI		Ct RT-PCR	
	D0	D42	D0	D42	D0	D42	D0	D42
1	5400	570	p	p	320	160	28	28
2	2090	470	n	p	320	160	27	26
3	2640	290	**n**	**n**	**n**	**n**	30	28
4	14150	1960	p	p	640	160	29	29
5	1150	1210	**n**	**n**	**n**	**n**	35	35
6	2860	n/a	p	n/a	n	n/a	31	n/a
7	14270	870	p	p	320	160	30	34
8	11120	740	p	p	160	160	29	23
9	1960	1120	p	p	320	160	32	30
10	1290	390	**n**	**n**	**n**	**n**	36	33
11	1210	800	p	p	n	n	32	31
12	2120	1890	p	p	n	320	27	28
13	1700	1280	p	p	320	640	35	28

Legend:

ELISA: schistosoma ELISA qualitative antibody test;

HAI: schistosoma indirect hemagglutination inhibition test, in 1/titer;

Ct RT-PCR: schistosoma DNA detection by PCR, in Ct-values;

What can we learn from this?

Katayama syndrome > Katayama fever

New proposed definition of (presumed) Katayama syndrome

---

primary (potential) exposure to schistosomiasis < 3months
and eosinophil count > 1000/µl
and **at least one** of the following symptoms*

- urticaria
- angio-oedema
- abdominal pain
- diarrhea
- cough
- fever

---

* not attributable to another disease or condition

What can we learn from all this?

Katayama syndrome: is grading of intensity required?

Useful,

when consequential for symptomatic treatment

or to prevent exacerbation of symptoms posttreatment

or if correlated with parasite load

What’s the evidence?

Acute schistosomiasis in a cluster of travellers from Rwanda

Exacerbation of clinical symtoms after treatment with PZQ

N	Exposure	Katayama	Fever pré PZQ	Symptoms post PZQ	Treatment	Symptom duration (d)
1	primary	yes	yes	no		
2	primary	yes	yes	fever	csteroid sd*	1
3	primary	yes	no	fever, angio-oedema	csteroid sd	2
4	primary	yes	no	fever	csteroid sd	1
5	primary	yes	no	abdominal pain	none	2
6	primary	yes	no	no		
7	primary	yes	no	no		
8	primary	no	no	no		
9	primary	no	no	no		
10	prior	no	no	no		
11	prior	no	no	n/a		
12	prior	no	no	abdominal pain	none	2
13	prior	no	no	no		

- Methylprednisolon 8mg/15kg bodyweight, single dose once
- n/a not (yet) administered?

What can we learn from all this?

RT-PCR diagnosis of schistosomiasis in serum: an new routine tool?

RT-PCR using a schistosoma sp. specific probe is at least as
sensitive than serology and parasitology
to detect ongoing schistosoma infection.

As a qualitative test it seems to outperform both serology
and parasitology in acute schistosomiasis by a useful margin

As a semiquantitative test it cannot be used to assess
parasite load reduction as an early marker

The future of RT-PCR in schistosomiasis?

As a qualitative test:

- Early diagnosis after infection
- Alternative test to serologic diagnosis in asymptomatic persons
- Development and testing of a species-specific probe to determine the infecting schistosoma species in persons with undetectable egg load in faeces and urine
- Evaluation of posttreatment parasite clearance as a late marker

As a semiquantitative test:

- Determining the parasite load? Serum vs feces/urine sample?
- Evolution of parasite load in function of treatment and reinfection

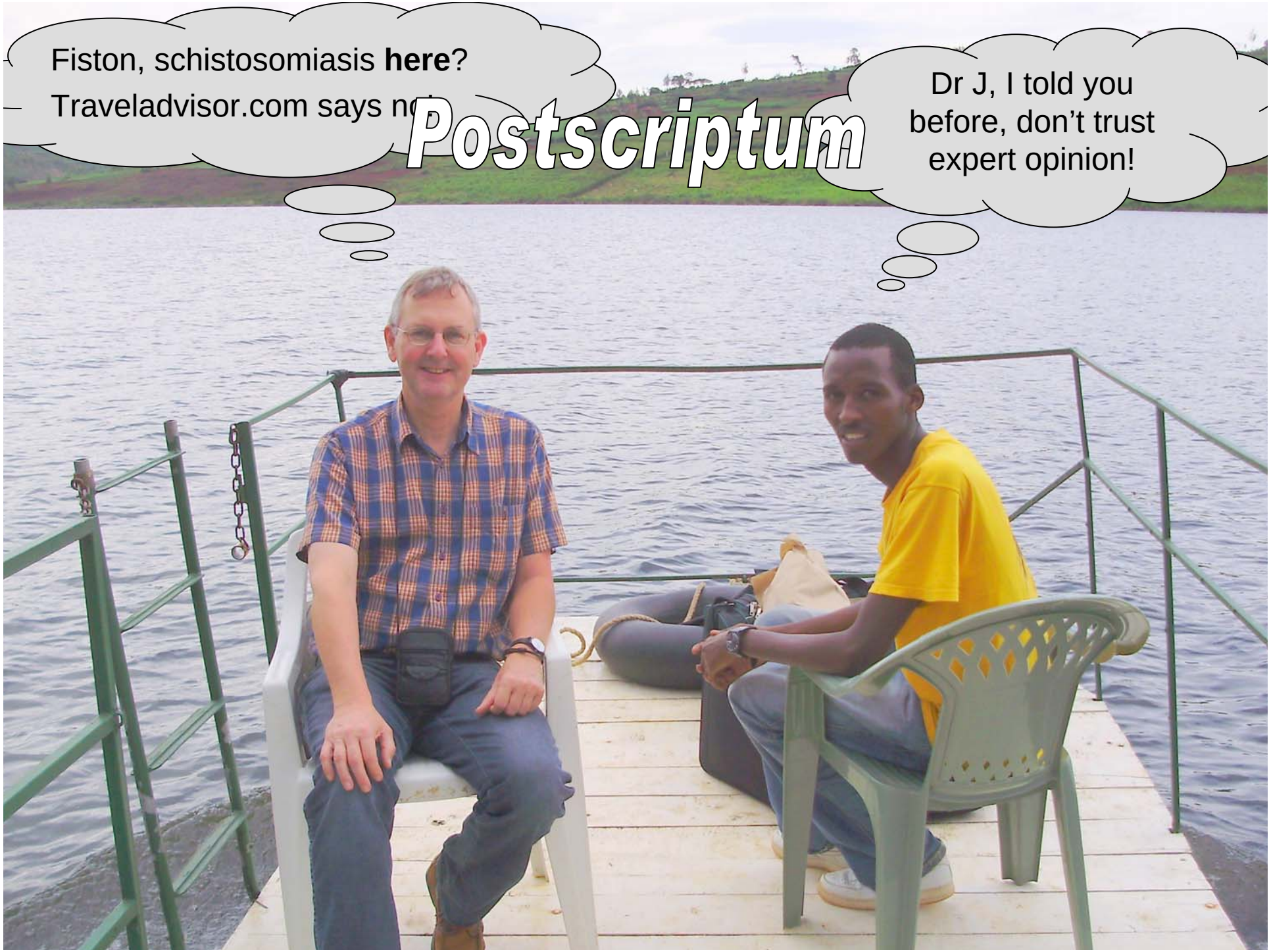
The future of RT-PCR in schistosomiasis?

Issues:

Cost of test

Interpretation of test results:

- Earliest postexposure time lapse for a positive result?
- Interindividual variability of semiquantitative result?
- Sequential interpretation within the same patient?
- Posttreatment time lapse to negative test result, and its significance?


Fiston, schistosomiasis **here**?
Traveladvisor.com says no!
Postscriptum
Dr J, I told you before, don't trust expert opinion!


Evidence based travel medicine…
And I tested negative…
…but no PCR done!
The proof of the pudding is in the swimming!
The End

Table 2. Patients with Katayama syndrome.

Patient	Destination	Purpose	Visit	DPE[a]	DPO[b]	DPT[c]	LEUK[d]	EO[e]	EIA[f]	Cell-free DNA co
1	Mozambique	Professional	First	42	14		13.6	20.9	+	57227.85
			Second	210	195	156	5.3	3.6	+	21.42
2	Ethiopia	Professional	First	42	15		10.2	26	−	27930.64
			Second	135	120	79	7.3	4.1	+	1584.80
3	Uganda	Professional	First	56	18		10.1	19	+	21.42
			Second	270	250	200	4.9	2.6	+	5.10
4	Uganda	Professional	First	12	20		7.3	22	+	10.45
			Second	460	445	434	6.9	5.0	+	2.49
5	Malawi	Tourist	First	20	2		6.2	6.5	+	10.45
			Second	750	740	716	5.2	0.8	+	−
6	Mozambique	Tourist	First	56	21		50.7	65	−	13631.84
7	Jemen	Tourist	First	54	14		6.7	23	+	773.48
8	Malawi	Tourist	First	35	8		4.9	19.7	−	184.24

[a]Days post exposure with fresh water (most likely event).
[b]Days post onset of symptoms.
[c]Days post treatment for second visits.
[d]Leukocyte count (n per nL). Average leukocyte count in patients 1 to 5: first visit, 9.48 cells/nl; second visit, 5.92 cells/nl ($p<0.0017$).
[e]Percent eosinophiles in total leukocytes. Average eosinophile fraction in patients 1 to 5: first visit, 18.88%; second visit: 3.2% ($p<0.033$).
[f]Enzyme immunoassay.
[g]Note that 10 mL of plasma were processed. 1 copy per mL = 1.67 copies per PCR vial.
doi:10.1371/journal.pntd.0000422.t002

Table 1. Patients with chronic disease.

Patient	Suspected origin of infection	Country of residence	Residence status	EIA	*Schistosoma* species	Sample in which eggs were detected	Cell-free DNA cop/mL[a]
1	West Africa	No data available	Refugee	+	*S. haematobium*	Bladder biopsy	27930.64
2	Mozambique	Germany	NGO worker	+	*S. mansoni*	Rectum biopsy	27930.64
3	Nigeria	Nigeria	Immigrant	+	*S. mansoni*	Rectum biopsy	3247.14
4	Egypt	Egypt	Immigrant	+	*S. mansoni*	Rectum biopsy	1584.80
5	Egypt	Egypt	Immigrant	+	*S. mansoni*	Rectum biopsy	1584.80
6	Philippines	Germany	Expatriate	+	*S. japonicum*	Rectum biopsy	1584.80
7	Egypt	Egypt	Immigrant	+	*S. mansoni*	Rectum biopsy	773.48
8	Zambia	Germany	NGO worker	+	*S. mansoni*	Rectum biopsy	377.50
9	West Africa	No data available	Refugee	+	*S. mansoni*	Rectum biopsy	184.24
10	Ghana	Ghana	Immigrant	+	*S. mansoni*	Rectum biopsy	184.24
11	Gambia/Senegal	Gambia/Senegal	Immigrant	+	*S. mansoni*	Rectum biopsy	21.42
12	West Africa	West Africa	Immigrant	+	*S. haematobium*	Urine	21.42
13	Uganda	Uganda	Immigrant	+	*S. mansoni*	Stool	2.49
14	Egypt	Egypt	Immigrant	+	*S. mansoni*	Rectum biopsy	1.22

[a]Note that 10 mL of plasma were processed. 1 copy per mL = 1.67 copies per PCR vial.

doi:10.1371/journal.pntd.0000422.t001