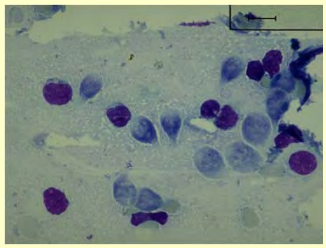
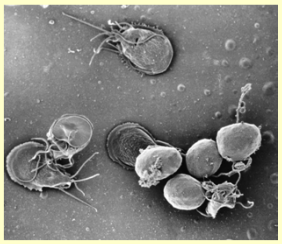
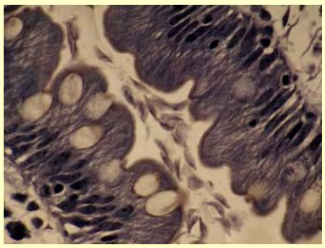
Giardia intestinalis Treatment: Where do we stand?

TropNet Workshop Prague - 06.10.2012

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Giardiasis - Epidemiology

- Most common intestinal protozoal infection
- Prevalence depending on level of hygiene
  - Highest prevalences in unsewered environments like slums of Asia, Africa, Latin America
  - children: up to 80 %, adults: up to 30 %
  - ≈ 1000 million cases at any one time


Rio de Janeiro


Jakarta

Molecular Characterization of Giardia intestinalis

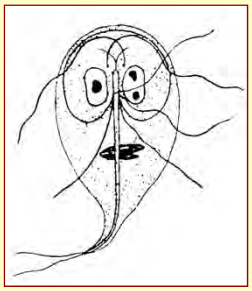
Assemblages	Some Species Commonly Infected
A-I	Humans and animals (cats, dogs, livestock, deer, muskrats, beavers, voles, guinea pigs, ferrets)
A-II	Humans (more common than A-I)
A-III and A-IV	Exclusively animals
B	Humans and animals (livestock, chinchillas, beavers, marmosets, rodents)
C and D	Dogs, coyotes
E	Alpacas, cattle, goats, pigs, sheep
F	Cats

Source: CDC

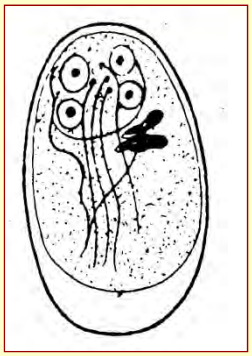
Most cases of Giardiasis are due to anthroponotic spread

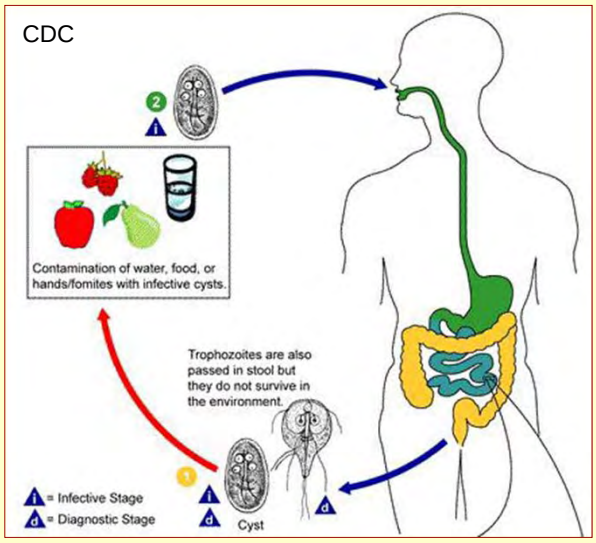
Giardia intestinalis

Life cycle



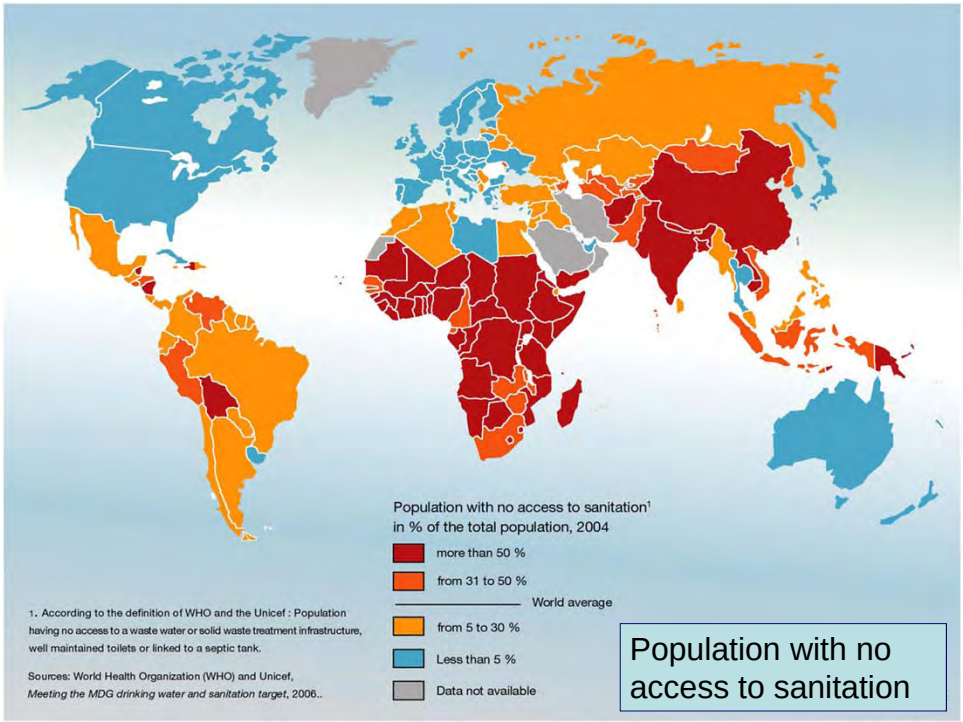
↑ Trophozoite, Cyst ↓





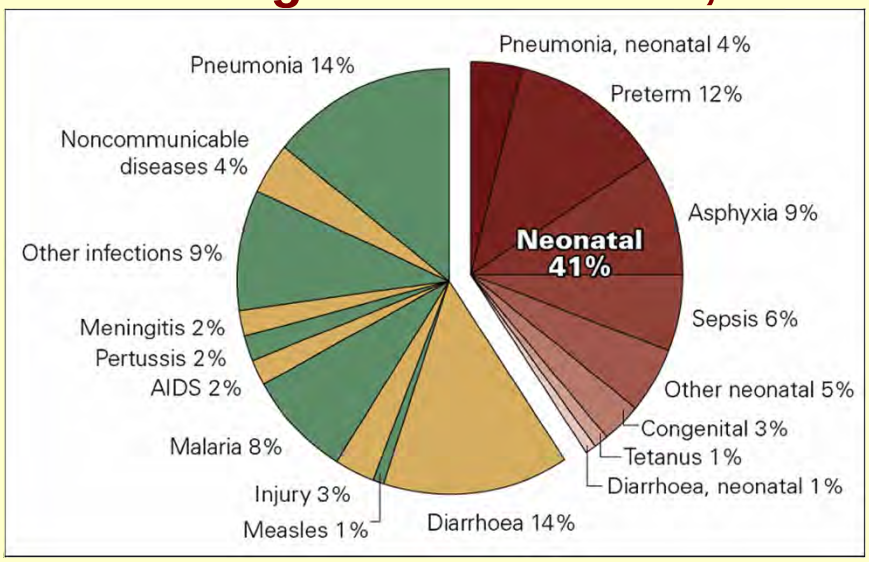
Giardia intestinalis - Mode of transmission

The faecal – oral route



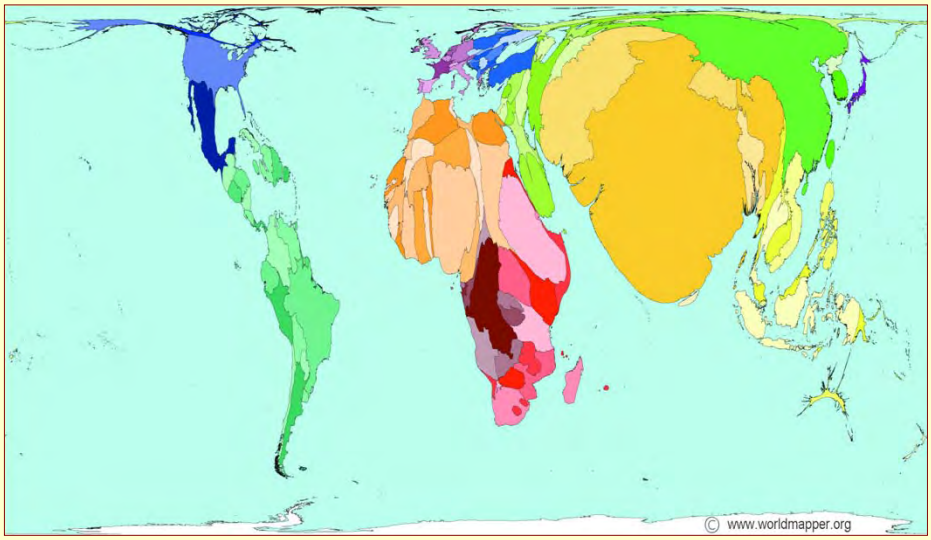
Population with no access to sanitation

Global causes of death among children ages 0–59 months, 2008



Undernutrition contributes to one-third of child deaths. Source: WHO

Childhood Diarrhea



Giardia intestinalis is an important pathogen contributing to childhood illness

www.worlmapper.org

Impact of protozoal infections in childhood

Study on 116 Children of a semi-urban slum in India with a past history of giardial diarrhoea showed

- a trend towards lower SQ (p=0.09)
- had significantly lower IQ (p=0.04)
- increased wasting (p=0.04).
- Cryptosporidial diarrhoea was not associated with poor IQ, SQ or physical growth.

(SQ = Social Quotient, IQ = Intelligence Quotient)

Ajjampur SS et al., Ann Trop Paediatr. 2011;31(3):205-12.

Impact of protozoal infections in childhood

Author‘s Conclusion:

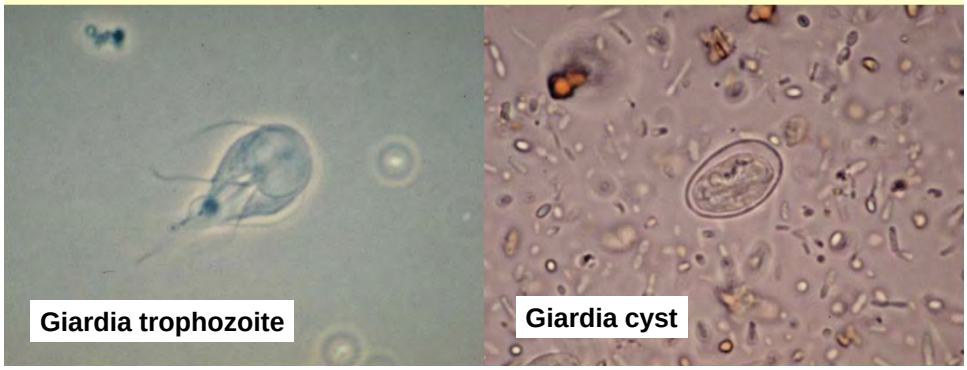
- study demonstrates the long-term effect of protozoan diarrhoea
- especially that caused by giardia,
- on both intelligence and physical growth in Indian children as early as 3 years of age
- re-inforces the need for early detection and prevention of early childhood protozoan diarrhoea.

Ajjampur SS et al., Ann Trop Paediatr. 2011;31(3):205-12.

Giardiasis – Course of infection

3 possibilities

- Spontaneous clearance of infection
- Asymptomatic excretion of cysts
- Long-lasting subacute or chronic infection



Giardiasis - Symptoms

	%	Range
Diarrhea	89	64 - 100
Feeling unwell	84	72 - 97
Flatulence	74	35 - 97
Offensive smelling, fatty stool	72	57 - 79
Abdominal repletion	69	59 - 79
Inappetence	64	41 - 82
Weight loss	64	56 - 73
Vomiting	27	17 - 36
Fever	13	0 - 21
Constipation	9	0 - 17
Urticaria	9	0 - 17

(R. Hill in: Principles and Practice of Infectious Diseaes, 1995)

Drugs commonly used for the treatment of Giardiasis

5-Nitroimadozole compounds

- Metronidazole
- Tinidazole
- Secnidazole

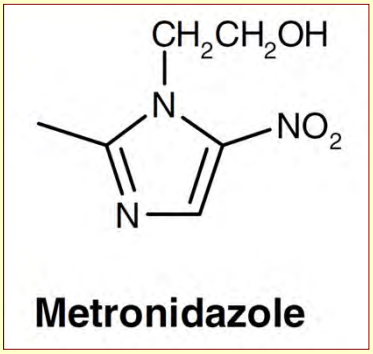
Benzimidazole drugs

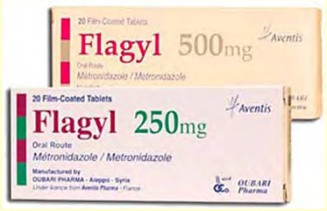
- Albendazole
- Mebendazole

- Paromomycin
- Nitazoxanide
- Quinacrine
- Furazolidone

Metronidazole

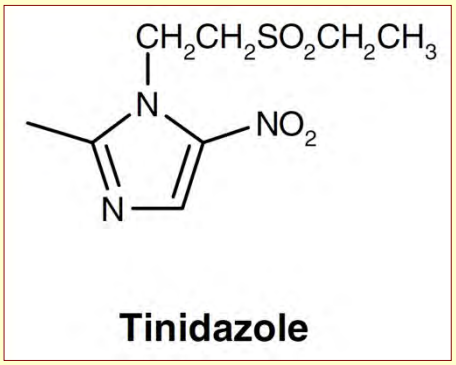
- Drug of choice in many guidelines
- Serum half life 7 h
- Side effects
  - Bitter, metallic taste
  - Nausea, fatigue, malaise
  - Disulfiram-like reaction
  - Genotoxic, causing DNA strand breaks, but no clear evidence of teratogenic risk in humans



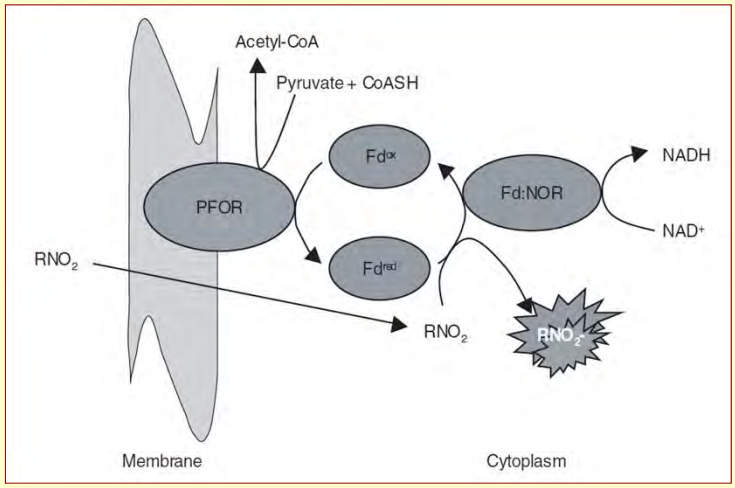


Tinidazole

- Advantage of 1 day treatment regimen
- T ½ 12 h
- Comparable efficacy to metronidazole
- Not available in Germany anymore



Activation of the Nitroimidazole drugs



- Adminestered orally in the inactive form
- Diffuses into the parasite
- Reduction via the parasite pyruvate:ferredoxin oxidoreductase (PFOR) → toxic nitro anion radicals (RNO2-)

Mechanism of parasite resistance to 5-nitromidazole drugs

- decrease in PFOR activity
- Reduced uptake
- Active efflux mechanisms

5-Nitroimazole drugs - Treatment schedule

Metronidazole

- Adults: 250 – 500mg tid / 5 – 7 days
- Children: 15mg/kg in 3 devided doses / 5 d

Tinidazole

- Adults: 2g once
- Children: 50mg/kg once

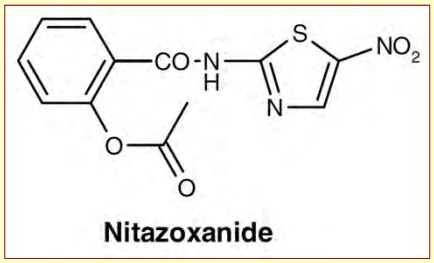
Secnidazole

- Adults: 2g once
- Children: 50mg/kg once

The medical letter 2008: drugs for parasitic infections

Nitazoxanide

- 5-nitrothiazolyl derivate
- Broad antiparasitic and antibacterial activity
  - Entamoeba histolytica
  - Blastocystis hominis
  - Isospora belli
  - Cryptosporidium
  - Enterobius, Ascaris, Trichuris
  - Hymenolepis nana



Nitazoxanide

Nitazoxanide

- first new drug developed for treating giardiasis in more than 20years
- In vitro 8x more effective against metronidazole – susceptible strains

Mode of action

- analogue to metronidazole involving PFOR pathway ?
- Cross resistance to metronidazole ?

Nitazoxanide

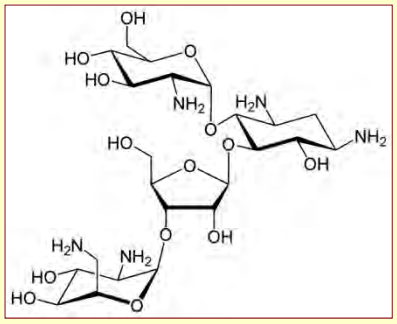
- FDA approved
- Not licensed in Germany
  - Comparably expensive

Dosage

- Adults: 500 mg bid x 3d
- Children: 1 - 3 yrs: 100 mg bid x 3d
- 4 - 11 yrs: 200 mg bid x 3d

Paromomycin

- Aminoglycosid antibiotic
- Minimally absorbed in the intestine
  - Use in pregnancy ?
- Usually well tolerated



Paromomycin

Mode of action:

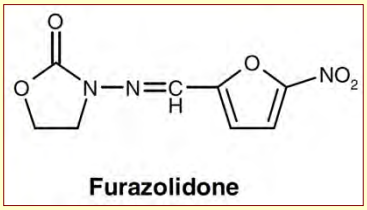
- binding to giardial ssRNA → inhibition of protein synthesis

Dosage:

- Adults: 25-35 mg/kg/d in 3 doses x 7d
- Children: 25-35 mg/kg/d in 3 doses x 7d
  - Capsules can be opened and substance mixed with food for paediatric application

Furazolidone

- Synthetic nitrofuran
- Antigiardial activity detected in 1960
- Minimally absorbed in the intestine
- Activated by parasite
- Mode of action: reduction to toxic nitro radicals by NADH oxidase
- Mechanism of resistance: membrane transporters?



Furazolidone

Furazolidone

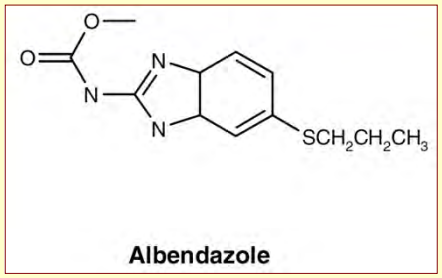
- Not licensed in Germany
  - Import via international pharmacy

Dosage

- Adults: 100 mg qid x 7-10d
- Children: 6 mg/kg/d in 4 doses x 7-10d

Benzimidazole drugs

- Introduced as anthelminthic in the 1960ies
  - Mebendazole, albendazole
- Bind to β-tubuline of some lower eukaryots
- giardia cytoskeleton contains 4 types of microtubule structures which might be the target



Albendazole

Albendazole

- Used in veterinary medicine
  - E.g. for giardiasis in dogs, 50mg/kg

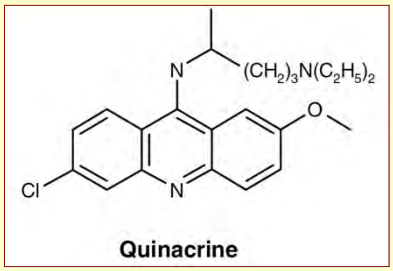
Dosage

- Adults: 400mg od / 5 d (bid / 5d)
- Children: 400mg od (2 yrs+)

Quinacrine

syn. Mepacrin, Atebrin®, Atabrine®

- acridine derivate
- detected 1930 by W. Kikuth as antimalarial
- Lost its importance with the introduction of chloroquine
- Used since 1941 for giardiasis
- only drug for giardiasis until 5-nitroimidazoles were introduced



Quinacrine

Quinacrine

- Mode of action not known
  - Binding to DNA?
  - Membrane fragility in giardia?
- Mechanism of resistance
  - Active exclusion of the drug from the parasite
- Advantage: kills cysts
- Side effects
  - bitter taste, nausea, vomiting, yellow discouloration of the skin,headache, dizziness, fever, toxic psychosis, seizures, CNS stimulation, crosses the placenta

Quinacrine

- Not licensed and not available in many countries
- Used as a 2nd line drug in therapy refractory cases

Dosage

- Adults: 100 mg tid x 5d
- Children: 2 mg/kg tid x 5d (max. 300 mg/d)

Alternative drugs with antigiardial activity

A variety of drugs shows **in-vitro** activity:

- Rifampicin
- Bithionol
- Pyrimethamine
- Chloroquine
- Mefloquine
- Doxycyclin
- Azithromycin (but ineffective in vivo)
- Ciprofloxacin – inhibits trophozoite attachment in-vitro, reduces viability of trophozoites

Cochrane analysis on drug therapy for Giardiasis

- Randomised / quasi-randomised trials
- 34 trials included
  - Only 1 without serious methodological flaws
  - 29 in developing countries / 5 in industrialized countries
- 12 different drugs investigated

Zaat, Mank, Assendelft. Drugs for treating giardiasis (Review) The Cochrane Collaboration 2007.

Cochrane analysis on drug therapy for Giardiasis

Great heterogenicity of studies:

- Study population
- Dose schedules
  - 28 studies with metronidazol – 27 different dose schedules,
  - dosage ranging from 33 – 500mg/kg if recalculated over the total period of treatment
- Parasitological laboratory techniques
  - Average „Parasitological Quality Index“ low 4,8 (range 0-8, max. 15)
- Follow up period between 7 days and 4 weeks
- Symptoms and side effects poorly described

Zaat, Mank, Assendelft. Drugs for treating giardiasis (Review) The Cochrane Collaboration 2007.

Cochrane analysis on drug therapy for Giardiasis

Author‘s conclusion:

- No difference in cure between single dose therapy and longer therapy
- No difference in parasitological cure rate between tinidazole and other short therapies
- A single dose of tinidazole appears to give the highest clinical cure rate for giardiasis with relatively few adverse effects.

Zaat, Mank, Assendelft. Drugs for treating giardiasis (Review) The Cochrane Collaboration 2007.

Mean rate of cure in randomised control clinical trials

	Drugs tested	Number of studies	Mean rate of cure % ± SD (CI)
1	Metronidazole	21	81.5 ± 18.6 (71.0–92.0)
2	Tinidazole	10	91.1 ± 6.3 (87.2–95.0)
3	Albendazole	9	73.4 ± 19.8 (58.7–88.1)
4	Mebendazole	8	65.6 ± 17.3 (50.4–80.8)
5	Ornidazole	3	97.6 ± 2.5 (95.4–99.8)
6	Nitazoxanide	3	79.7 ± 1.8 (77.2–82.2)

Busatti, Santos, Gomes. Biologics:Targets & Therapy 2009:3 273-287

More effective doses of drugs tested in randomized clinical trials

Drugs	Unit	Recommended doses
Metronidazole	mg/Kg/day	15–50 TID – 5 to 10 days
	mg	500–750 TID – 5 to 10 days
Tinidazole	mg	2 MID – One dose
	mg/Kg/day	50 MID – One dose
Albendazole	mg	400 MID – One day
	mg	400 MID – 5 days
	mg/Kg/day	10 MID – 5 days
Mebendazole	mg	200 TID – 5 days
Ornidazole	mg/Kg/day	20–40 MID – 1 to 5 days
Nitazoxanide	mg	500 MID – 3 days

TID = 3x/d, MID 1x/d

Busatti, Santos, Gomes. Biologics:Targets & Therapy 2009:3 273-287

Author‘ conclusion:

- Similar **efficacy** among the drugs currently used – none being better than the other
- Differences in **side effects** poorly investigated and documented in most studies
  - Apparantly similar in most studies
  - Milde to moderate and transient

Busatti, Santos, Gomes. Biologics:Targets & Therapy 2009:3 273-287

Therapy refractory giardiasis

- According to clinical observation an increasing problem
  - Often in infections aquired in India or SE Asia
- No generally accepted definition of „resistance“ or „therapy refractory“ in giardiasis
- No study data on the extend of the problem in Germany

Therapy refractory giardiasis

Clinically: recurrence of symptoms after therapy

- Resurgence ?
- Non-compliance with therapy ?
- True drug resistance ?
- Reinfection after cure ?
- Post-Giardia lactose intolerance?

Parasitologically: evidence of persistent infection after therapy

- Microscopy, AG-ELISA, PCR

Therapy refractory giardiasis

- Treatment failures reported with all common antigiardial drugs
- In-vitro resistance has been demonstrated to all recommended drugs
- Often prolonged courses of high doses of drugs result in cure - ↑ side effects ↑
- Instead: shorter combination therapies with unrelated drugs?

Therapy refractory giardiasis

Combination therapies

- Mechanism of action should be known
- Should involve different pathways in the parasite

E.g.

- Metronidazole + Albendazole
- Metronidazole + Paromomycin
- Metronidazole + Quinacrine

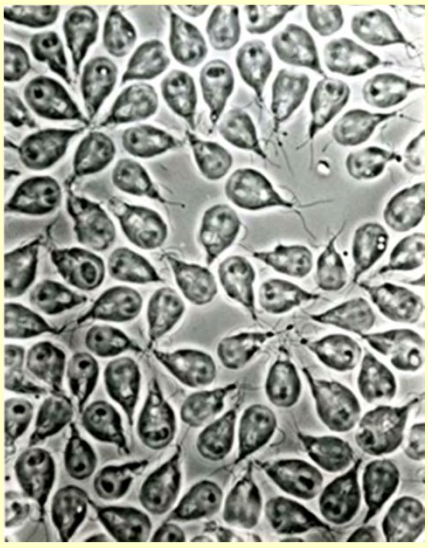
Possible causes of failure in the treatment of Giardia

- Wrong diagnosis ?
  - Evidence by reliable diagnostic method ?
- Re-colonisation of the small bowl from an anatomical site not reached by the antibiotic ?
  - Jejunum, Ileum ?
  - Gall bladder ?
  - Pancreatic duct ?
- Immunodeficiency ?
  - IgA-Deficiency, Hypogamaglobulinaemia (CVID), AIDS
- Drug resistance ?

Drug Susceptibility Testing for Giardia

Requirements

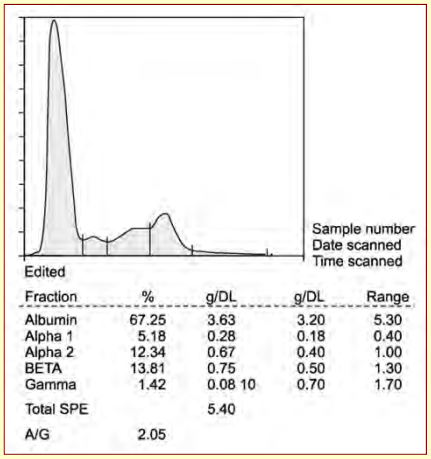
- Stable, axenic culture of the strain
  - Isolated cysts from faecal samples
  - Trophozoites from biopsies
- Exposure of the culture to different concentrations of the antibiotic
  - No standardized protocol
  - Not a routine !
- Animal Models ?
- So far genetic DST not possible



Giardia Culture, Source: CDC

Role of the host immune system

- None of the available drugs is capable of clearing Giardia infection in patients with certain immune defects, e.g. CVID – Syndrome, AIDS



Fraction	%	g/DL	g/DL	Range
Albumin	67.25	3.63	3.20	5.30
Alpha 1	5.18	0.28	0.18	0.40
Alpha 2	12.34	0.67	0.40	1.00
BETA	13.81	0.75	0.50	1.30
Gamma	1.42	0.08 10	0.70	1.70
Total SPE		5.40		
A/G	2.05			

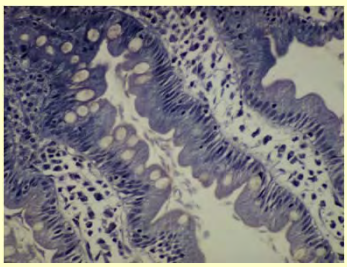
Electrophoresis of a patient with CVID: markedly decreased gamma globulin fraction

Tuberc Respir Dis. 2011 Oct;71(4):282-285

Summary

Despite

- high prevalence of Giardiasis
- impact on morbidity



Many questions remain to be answered

- Immunology / Immunopathology
- Mode of drug action
- Best 2nd line treatment / combination therapy for refractory cases

Study on therapy refractory giardiasis needed

Potential problem:

- Different assemblages may cause
- Different symptoms
- Response to treatment
- Spontaneous cure rate

  Should Genotyping be included?

Avoiding shortcomings of previous studies

- Precise description of study population
- Precise treatment protocol with limited no. of combinations
- Strict inclusion / exclusion criteria
- Clear definition of parasitological cure
  - follow up scheme / laboratory techniques

Thank you very much for your attention !

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Giardiasis is one of the most common causes of diarrheal disease worldwide, and the most frequent parasite isolated from stools of travellers with TD.

Prevalence of infection: 2–5% in the developed world and 20–30% in the low income countries .

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- The parasite exists in two forms: the infectious cyst and the disease-causing trophozoite.
- Transmission: by the oral uptake of cysts from contaminated water or food, or by person-to-person contact.
- Inoculum: 10 cysts are enough to initiate infection.

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- Upon passage through the stomach into the small intestine, the trophozoites emerge from the cysts and colonize the upper small intestine.
- Incubation period: 1–2 weeks
- Symptoms of giardiasis include abdominal cramps and pain, nausea, bloating, flatulence and diarrhea, which can lead to malabsorption.
- The acute phase of infection usually lasts several days. Most infections are self-limiting, but symptoms can persist and chronic infections are not uncommon.
- Re-infections are common in endemic areas.

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Antiparasitic therapies are not always effective and drug resistance occurs in vivo and in vitro. The review focuses on recent advances in the treatment of Giardiasis.

Treatment compliance is a key factor affecting the outcome of giardiasis

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CURRENT DRUGS against Giardia

- The most widely used drugs against giardiasis are 5-Nitroimidazoles compounds. This 5-NI are prodrugs that require activation by microbial reductases. Affects electron transport of the parasite.

Metronidazol

Ornidazol
Tinidazol
Secnidazol

All have similar activity profiles but longer serum half-lives

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CURRENT DRUGS against Giardia

Other Nitroderivatives.

- 5-Nitrofurans (furazolidone)
- 5-Nitrothiazoles (nitazoxanide)

- Probable action mechanism:

  Adduction and protein/DNA inactivation

Giardia lines resistant to metronidazole are highly cross-resistant to other 5-NI drugs, but to a lesser extent to nitazoxanide

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CURRENT DRUGS against Giardia

- Quinacrine, has potent antigiardial activity, but can have serious side-effects.

- Mechanism of action: probably inhibition of nucleic acid synthesis.

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CURRENT DRUGS against Giardia

Benzimidazoles.

- Albendazole and mebendazole, bind to tubulin and inhibit parasite attachment to solid substrates, which may interfere with continued colonization of the small intestine.
- A recent report comparing the efficacy of albendazole and metronidazole as treatments against giardiasis in humans showed similar effectiveness*

*Solaymani-Mohammadi S, et al. PLoS Negl Trop Dis 2010; 4:e682.

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CURRENT DRUGS against Giardia

- Chloroquine, is also active against Giardia.
- Paromomycin, an aminoglycoside antibiotic, appears to be less effective against giardiasis than metronidazole or albendazole*.

*Morch K, et al. J Infect 2008; 56:268–273.

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CURRENT DRUGS against Giardia

- Metronidazol (Tinidazol, Secnidazol, Ornidazol)
- Furazolidone
- Nitazoxanide
- Quinacrine
- Albendazole
- Chloroquine
- Paramomycin

Treatment preferences vary among clinicians and in different locations.

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Treatment

- Metronidazole is administered in doses of 250-500 mg /8h/ 5–7 d for adults and 15 mg/kg 3 times a day for 5–7 days in children.
- The effectiveness of albendazole, when given as a single dose of 400 mg/day for 5 days (80-95% = metronidazol)
- A single dose of tinidazole (2.0 g) has been shown to have a clinical efficacy of 80–100% in different clinical trials.

Treatment

Drugs	Doses (adults)	Efficacy
• Nitazoxanide	500 mgr/12h /3d	65%-85%
• Quinacrine	100 mgr/8h/5d	>90%
• Furazolidone	100 mgr/6 h/7-10d	80%-85%
• Paramomicina*	500 mgr/8h/7d	60-70%

*pregnant women

Drug Combinations:

Metronidazol + quinacrine

Metronidazol + albenzadole

Results of the Barcelona Study

Table 2. Efficacy rate of the different options for Giardiasis's therapy.

	Number of patients cured after the treatment				Global efficacy rate
	1st line (95)	2nd line (19)	3rd line (13)	4rd line (5)	
Nitroimidazole	74/95[1] (78%)				74/95 (78%)
Nitroimidazole retreatment		2/12 [2] (17%)	0/3 (0%)		2/15 (13%)
Paramomicin		0/3[2] (0%)			0/3 (0%)
Quinacrine		**1/1[2] (100%)**	**8/8 (100%)**	**5/5 (100%)**	**14/14 (100%)**
Albendazole			0/1 (0%)		0/1 (0%)
Total	95	19	13	5	

(%) Percentage of cured patients.

1: 2 patients lost to follow-up; 2: 1 patient lost to follow-up.

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Treatment Protocol in Barcelona

- First line:
  - Tinidazol 2 gr single dose
  - Control stools x 2 after 1 month

- Second line:
  - Quinacrine
  - HIV serology and IgA dosification

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TropNet
European Network for Tropical Medicine and Travel Health

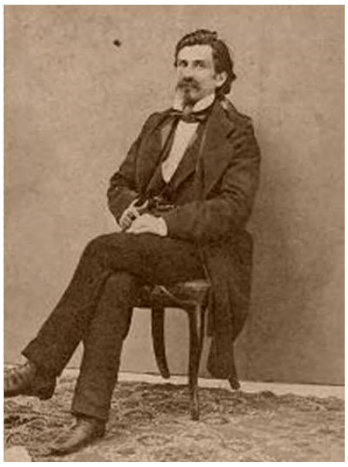
Swiss TPH
Swiss Tropical and Public Health Institute
Schweizerisches Tropen- und Public Health-Institut
Institut Tropical et de Santé Publique Suisse

TropNet study:

Evaluation of first- & second-line treatment of *Giardia lamblia*

TropNet
European Network for Tropical Medicine and Travel Health

Swiss TPH
Swiss Tropical and Public Health Institute
Schweizerisches Tropen- und Public Health-Institut
Institut Tropical et de Santé Publique Suisse



Vilém Dušan Lambl
Czech linguist & physician

remembered for his description of *"Cercomonas intestinalis" in 1859*

later renamed:
Lamblia intestinalis (1888)
Giardia lamblia (1915)

How do experts treat *Giardiasis* ?

TropNet survey on Giardia treatment

1st line	regimen	2nd line	regimen	3rd line	4th line
Metronidazole	400 mg TID for 5 days	Metronidazole	600 mg TID for 7 days	Paramomycin or Albendazole	-
Metronidazole	500 mg TID for 7 days	Tinidazole	2 g once daily, - about duration	-	-
Metronidazole	250 mg TID for 5-7 days	Tinidazole	2 g single dose	-	-
Metronidazole	500 mg TID, days ?	Tinidazole	-	-	-
Metronidazole	750 mg - 1g for 7 days	Tinidazole	2 g single dose	-	-
Metronidazole	250 mg TID for 5-7	Tinidazole	2 g once	-	-
Metronidazole	500 mg BID for 5 days	Tinidazole	2 g single dose	-	-
Metronidazole	-	Albendazole	-	-	-
Metronidazole	250 mg TID for 5 days	Albendazole	400 mg TID for 5 days	-	-
Metronidazole	-	Albendazole	-	-	-
Metronidazole	500 mg TID for 7 days	Albendazole	-	-	-
Metronidazole	500 mg TID for 7 days	Albendazole	400 mg TID for 7 days	-	-
Metronidazole	250 mg TID for 10 days	Albendazole	400 mg QID for 5 days	-	
Metronidazole	250 mg TID for 5 days	Albendazole	400 mg once daily for 5 days	-	-
Metronidazole	250 mg TID for 7 days	Albendazole or Mebendazole	-	-	-
Metronidazole	400 mg TID for 6 days	Paromomycin	500 mg TID for 9 days	Metronidazol + Paromomycin	-
Metronidazole	500 mg BID for 5 days	Paromomycin	500 mg TID for 7 days	-	-
Metronidazole	500 mg TID for 7 days	Paramomycin	500 mg TID for 10 days	-	-
Metronidazole	250 mg TID for 10 days	Nitazoxanide	Nitazoxanide 500 mg BID for 3 days	-	-
Metronidazole	2 g OD for 3 days or 250 mg TID for 5 days	Nitazoxanide	500 mg BID for 3 days	-	-
Metronidazole	400 mg TID for 7 days	Quinacrine	100 mg TID for 5 days	-	-
Metronidazole	400mg TID for 6 days	Metronidazole + Albendazole	Metronidazole: 400mg TID for 6 days + Albendazole: 400mg OD for 5-10 days	-	-
Metronidazole	400 mg TID for 7 days	Metronidazole + Albendazole	Metronidazol 250 mg BID + Albendazole 400 mg BID for 7 days	Paramomycin	Quinacrine + Metronidazole
Metronidazole	250mg TID for 5 days	Metronidazole + Paromomycin	Metronidazole 750mg TID for 7 days + Paromomycin 30mg/kg in 3 doses for 7 days	-	-
Metronidazole	500mg TID for 7 days	Metronidazole + Paromomycin	Metronidazol 2 g for 3 days followed by Paromomycin 25-35 mg/kgKG for 7-10 days	Nitazoxanid	-
Metronidazole	-	Combination therapy, no details	-	-	-
Tinidazole	2 g single dose	Tinidazole	2 g OD for 3 days	Albendazole	-
Tinidazole	2 g single dose	Tinidazole	2 g OD for 3 days	-	-
Tinidazole	2 g single dose	Metronidazole	250 mg TID for 5-7 days	-	-
Tinidazole	2 g single dose	Metronidazole	500 mg TID for 7 days	-	-
Tinidazole	-	Metronidazole	-	-	-
Tinidazole	2 g single dose, repeated after 5 days	Metronidazole	400 mg TID for 5 days (or 2g OD for 3 days)	-	-
Tinidazole	2 g single dose, repeated after 5 days	Metronidazole	400 mg TID for 5 days	-	-
Tinidazole	-	Metronidazole	-	-	-
Tinidazole	-	Albendazole	-	-	-
Tinidazole	-	Albendazole	-	-	-
Tinidazole	2 g single dose, repeated after 7 days	Albendazole	400 mg TID for 7 days	-	-
Tinidazole	2 g single dose	Albendazole	400 mg once daily for 5 days	-	-
Tinidazole	2g single dose	Quinacrine	100 mg TID for 5 days	-	-
Tinidazole	2 g single dose	Quinacrine	100 mg TID for 5 days	-	-
Tinidazole	-	Quinacrine	500 mg TID for 5 days	Albendazol + Metronidazole or + Paramomycin	-
Tinidazole	2 g single dose	Quinacrine	100 mg TID for 5 days	-	-
Tinidazole	-	Nitazoxanide	-	-	-
Tinidazole	2g single dose	Metronidazole + Albendazole	Metronidazole 400mg TID for 6 days + Albendazole 400mg OD for 5-10 days	Paromomycin or Quinacrine	-
Tinidazole	2 g single dose, repeated after 14 days	Metronidazole + Paromomycin	Metronidazole 750mg TID for 7 days ± Paromomycin 30mg/kg in 3 doses for 7 days	-	-
Tinidazole	2g once daily for 2 days	Albendazol + Paramomycin	Albendazole 400 mg BID + Paromomycin 750 mg TID for 5 days	-	-
Tinidazole	2 g single dose, repeated on day 10-14	Albendazole + Paromomycin	400 mg TID for 7 days followed by Paromomycin 500 mg TID for 7 days	-	-
Ornidazole	-	Metronidazole	-	-	-
Ornidazole	500 mg BID for 5 days	Albendazole	400 mg BID for 3 days	-	-
Ornidazole	500 mg BID for 7 days	Albendazole + Paromomycin	Paramomycin 500 mg TID + Albendazole 400 mg QID for 5 days	-	-
Albendazole	-	Metronidazole	-	-	-
Nitazoxanide	500 mg BID for 3 days	Quinacrine	100 mg TID for 5 days	-	-
Paramomycin	25-35 mg/kg/day in 3 doses for 5-10 days	Quinacrine	100 mg TID for 5 days	-	-

3

Drug regimen for treating *Giardia lamblia*

Survey among TropNet members :

53 centres: **39** different regimens using **7** drugs given alone or in combination in different dosage & duration

…expert opinions…

4

Survey on currently used treatment regimen for *G. lamblia* within TropNet (- = no data available).

TropNet site	1st line regimen		2nd line	regimen	3rd line	4th line
1	Metronidazole	400 mg TID for 5 days	Metronidazole	600 mg TID for 7 days	Paramomycin or Albendazole	-
2	Metronidazole	500 mg TID for 7 days	Tinidazole	2 g single dose	-	-
3	Metronidazole	250 mg TID for 5-7 days	Tinidazole	2 g single dose	-	-
4	Metronidazole	500 mg TID, days ?	Tinidazole	-	-	-
5	Metronidazole	750 mg - 1g for 7 days	Tinidazole	2 g single dose	-	-
6	Metronidazole	250 mg TID for 5-7	Tinidazole	2 g single dose	-	-
7	Metronidazole	500 mg BID for 5 days	Tinidazole	2 g single dose	-	-
8	Metronidazole	-	Albendazole	-	-	-
9	Metronidazole	250 mg TID for 5 days	Albendazole	400 mg TID for 5 days	-	-
10	Metronidazole	-	Albendazole	-	-	-
11	Metronidazole	500 mg TID for 7 days	Albendazole	-	-	-
12	Metronidazole	500 mg TID for 7 days	Albendazole	400 mg TID for 7 days	-	-
13	Metronidazole	250 mg TID for 10 days	Albendazole	400 mg QID for 5 days	-	-
14	Metronidazole	250 mg TID for 5 days	Albendazole	400 mg OD for 5 days	-	-
15	Metronidazole	250 mg TID for 7 days	Albendazole or Mebendazole	-	-	-
16	Metronidazole	400 mg TID for 6 days	Paromomycin	500 mg TID for 9 days	Metronidazol + Paromomycin	-
17	Metronidazole	500 mg BID for 5 days	Paromomycin	500 mg TID for 7 days	-	-
18	Metronidazole	500 mg TID for 7 days	Paramomycin	500 mg TID for 10 days	-	-
19	Metronidazole	250 mg TID for 10 days	Nitazoxanide	500 mg BID for 3 days	-	-
20	Metronidazole	2 g OD for 3 days or 250 mg TID for 5 days	Nitazoxanide	500 mg BID for 3 days	-	-
21	Metronidazole	400 mg TID for 7 days	Quinacrine	100 mg TID for 5 days	-	-
22	Metronidazole	400mg TID for 6 days	Metronidazole + Albendazole	Metronidazole: 400mg TID for 6 days + Albendazole: 400mg OD for 5-10 days	-	-
23	Metronidazole	400 mg TID for 7 days	Metronidazole + Albendazole	Metronidazol 250 mg BID + Albendazole 400 mg BID for 7 days	Paramomycin	Quinacrine + Metronidazole
24	Metronidazole	250mg TID for 5 days	Metronidazole + Paromomycin	Metronidazole 750mg TID for 7 days + Paromomycin 30mg/kg in 3 doses for 7 days	-	-
25	Metronidazole	500mg TID for 7 days	Metronidazole + Paromomycin	Metronidazol 2 g for 3 days followed by Paromomycin 25-35 mg/kgKG for 7-10 days	Nitazoxanid	-
26	Metronidazole	-	Combination therapy, no details	-	-	-
27	Tinidazole	2 g single dose	Tinidazole	2 g OD for 3 days	Albendazole	-
28	Tinidazole	2 g single dose	Tinidazole	2 g OD for 3 days	-	-
29	Tinidazole	2 g single dose	Metronidazole	250 mg TID for 5-7 days	-	-
30	Tinidazole	2 g single dose	Metronidazole	500 mg TID für 7 days	-	-
31	Tinidazole	-	Metronidazole	-	-	-
32	Tinidazole	2 g single dose, repeated after 5 days	Metronidazole	400 mg TID for 5 days (or 2g OD for 3 days)	-	-
33	Tinidazole	2 g single dose, repeated after 5 days	Metronidazole	400 mg TID for 5 days	-	-
34	Tinidazole	-	Metronidazole	-	-	-
35	Tinidazole	-	Albendazole	-	-	-
36	Tinidazole	-	Albendazole	-	-	-
37	Tinidazole	2 g single dose, repeated after 7 days	Albendazole	400 mg TID for 7 days	-	-
38	Tinidazole	2 g single dose	Albendazole	400 mg once daily for 5 days	-	-
39	Tinidazole	2 g single dose	Quinacrine	100 mg TID for 5 days	-	-
40	Tinidazole	2 g single dose	Quinacrine	100 mg TID for 5 days	-	-
41	Tinidazole	-	Quinacrine	100 mg TID for 5 days	Albendazol + Metronidazole or + Paramomycin	-
42	Tinidazole	2 g single dose	Quinacrine	100 mg TID for 5 days	-	-
43	Tinidazole	-	Nitazoxanide	-	-	-
44	Tinidazole	2 g single dose	Metronidazole + Albendazole	Metronidazole 400mg TID for 6 days + Albendazole 400mg OD for 5-10 days	Paromomycin or Quinacrine	-
45	Tinidazole	2 g single dose, repeated after 14 days	Metronidazole + Paromomycin	Metronidazole 750mg TID for 7 days ± Paromomycin 30mg/kg in 3 doses for 7 days	-	-
46	Tinidazole	2 g single dose for 2 days	Albendazol + Paramomycin	Albendazole 400 mg BID + Paromomycin 750 mg TID for 5 days	-	-
47	Tinidazole	2 g single dose, repeated on day 10-14	Albendazole + Paromomycin	400 mg TID for 7 days followed by Paromomycin 500 mg TID for 7 days	-	-
48	Ornidazole	-	Metronidazole	-	-	-
49	Ornidazole	500 mg BID for 5 days	Albendazole	400 mg BID for 3 days	-	-
50	Ornidazole	500 mg BID for 7 days	Albendazole + Paromomycin	Paramomycin 500 mg TID + Albendazole 400 mg QID for 5 days	-	-
51	Albendazole	-	Metronidazole	-	-	-
52	Nitaxozanide	500 mg BID for 3 days	Quinacrine	100 mg TID for 5 days	-	-
53	Paramomycin	25-35 mg/kg/day in 3 doses for 5-10 days	Quinacrine	100 mg TID for 5 days	-	-

Summary of the regimen:

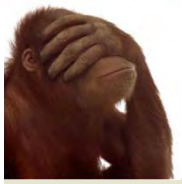
Metronidazole	250 mg TID for 5 days
	400mg TID for 6 days
	250 mg TID for 7 days
	250 mg TID for 10 days
	400 mg TID for 5 days
	400 mg TID for 6 days
	400 mg TID for 7 days
	500 mg BID for 5 days
	500 mg TID for 7 days
	600 mg TID for 7 days
	750 mg - 1g for 7 days
	2 g OD for 3 days
Tinidazole	2 g single dose
	2 g single dose, repeated after 5 days
	2 g single dose, repeated after 7 days
	2 g single dose, repeated after 10 days
	2 g single dose, repeated after 14 days
Ornidazole	500 mg BID for 5 days
	500 mg BID for 7 days

Nitaxozanide	500 mg BID for 3 days
Quinacrine	100 mg TID for 5 days
Albendazole	400 mg OD for 5 days
	400 mg BID for 3 days
	400 mg TID for 5 days
	400 mg TID for 7 days
	400 mg QID for 5 days
Paromomycin	500 mg TID for 9 days
Paromomycin	500 mg TID for 7 days
Paramomycin	500 mg TID for 10 days
Paramomycin	25-35 mg/kg/day in 3 doses for 5-10 days
Albendazole + Paromomycin	Paramomycin 500 mg TID + Albendazole 400 mg QID for 5 days
Metronidazole + Albendazole	Metronidazole 400mg TID for 6 days + Albendazole 400mg OD for 5-10 days
Metronidazole + Paromomycin	Metronidazole 750mg TID for 7 days ± Paromomycin 30mg/kg in 3 doses for 7 days
Albendazol + Paramomycin	Albendazole 400 mg BID + Paromomycin 750 mg TID for 5 days
Albendazole + Paromomycin	400 mg TID for 7 days followed by Paromomycin 500 mg TID for 7 days
Metronidazole + Albendazole	Metronidazole: 400mg TID for 6 days + Albendazole: 400mg OD for 5-10 days
Metronidazole + Albendazole	Metronidazol 250 mg BID + Albendazole 400 mg BID for 7 days
Metronidazole + Paromomycin	Metronidazole 750mg TID for 7 days + Paromomycin 30mg/kg in 3 doses for 7 days
Metronidazole + Paromomycin	Metronidazol 2 g for 3 days followed by Paromomycin 25-35 mg/kgKG for 7-10 days

Proposal of a TropNet multi-centre-study on the treatment of *Giardia lamblia* infections:

First of all: the study design is a challenge…

- too many expert opinions out there
- simplicity & feasibility of protocol
- drugs: local availability / national approval…
- multiple-national ethical clearances
- …



5

Proposal of a multi-centre TropNet-study on the treatment of *Giardia lamblia* infections:

Main objectives (ambitious):

1. Defining the most effective 1st-line treatment regimen for *G. lamblia*
2. Defining the most effective 2nd-line treatment regimen for *G. lamblia*
3. Assessing treatment failure rates of different regimens

6

Additional objectives

1. Collecting geographic data (continent/country of infection) ➔ regional treatment failures ?
   ➔ region-specific treatment recommendations ?

2. Collecting data on $2^{nd}/3^{rd}/X^{th}$-line treatment failure

3. Collecting data on adverse events linked to different drugs and treatment regimens

Study design

Prospective, observational, open-label, multi-centre study

Inclusion criteria

- Any person being tested positive for *G. lamblia* (by stool microscopy or stool antigen-test) [travellers & autochthonous infections]
- Referred cases for $2^{nd}/3^{rd}/X^{th}$-line therapy (definition of `treatment failure´: detection of *G. lamblia* in stool by microscopy or positive antigen test ≥4 week after completing a *G. lamblia* specific treatment regimen

Optional 1st-line regimens

Metronidazole	400 - 500mg* TID x 7 d
or	
Tinidazole	2g OD x 1 d
or	
Tinidazole	2g OD x 2 d

Why these regimen? → `common denominator´ of the TropNet survey

Optional 2nd- line regimens

Metronidazole	400 - 500mg* TID x 7 d
or	
Tinidazole	2g OD x 2 d
or	
Quinacrine	100mg TID x 5 d
or	
Albendazole	400mg BID x 5 d
or	
Nitazoxanide	500mg BID x 3 d
or	
Paromomycin	500mg TID x 7 d

or

any combination therapy (concomitant or sequential) with two of the listed 2nd-line regimens (but in identical dosage & duration)

9

Optional 3rd/Xth-line treatments

In case of a 2nd-line treatment failure any of the listed 2nd-line treatment regimens might be used (alone or in combination – as in 2nd-line therapy) for 3rd/Xth-line therapy.

Definition of endpoints

`*Treatment success*´:
2 negative stool samples (no detection of *G. lamblia* by microscopy or antigen test) ≥ 3 weeks after completing antimicrobial therapy with the assigned study regimen

`*Treatment failure*´:
Detection of *G. lamblia* by microscopy or by antigen test ≥ 3 weeks after completing antimicrobial therapy with the assigned study regimen

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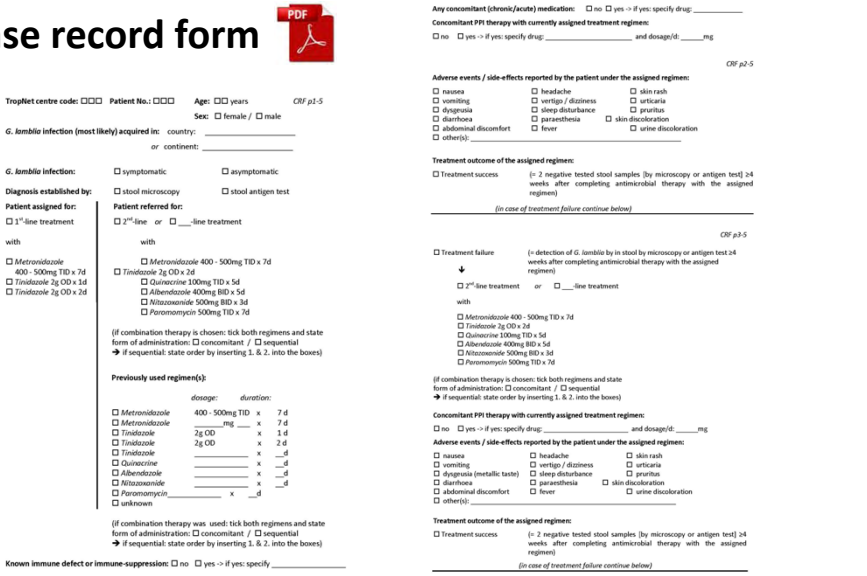
Institutional review board approval

- Due to the design as an observational, open-label study review and approval by the institutional review board / ethical committee of the participating TropNet sites will (in most cases) not be necessary as the treatment and follow-up are standard procedures.
- The site coordinator of the participating TropNet sites is responsible to check the necessity for study review/ approval at her/his site and to choose drug and treatment regimens, which (if applicable) comply with the national or local/institutional treatment guideline/recommendation.

Case record form



TropNet centre code: ☐☐☐ Patient No.: ☐☐☐ Age: ☐☐ years CRF p1-5
Sex: ☐ female / ☐ male

G. lamblia infection (most likely) acquired in: country: _____
or continent: _____

G. lamblia infection: ☐ symptomatic ☐ asymptomatic

Diagnosis established by: ☐ stool microscopy ☐ stool antigen test

Patient assigned for:
☐ 1st-line treatment
with
☐ *Metronidazole* 400 - 500mg TID x 7d
☐ *Tinidazole* 2g OD x 1d
☐ *Tinidazole* 2g OD x 2d

Patient referred for:
☐ 2nd-line or ☐ ___-line treatment
with
☐ *Metronidazole* 400 - 500mg TID x 7d
☐ *Tinidazole* 2g OD x 2d
☐ *Quinacrine* 100mg TID x 5d
☐ *Albendazole* 400mg BID x 5d
☐ *Nitazoxanide* 500mg BID x 3d
☐ *Paromomycin* 500mg TID x 7d

(if combination therapy is chosen: tick both regimens and state form of administration: ☐ concomitant / ☐ sequential
➔ if sequential: state order by inserting 1. & 2. into the boxes)

Previously used regimen(s):

	dosage:	duration:
☐ *Metronidazole*	400 - 500mg TID	x 7 d
☐ *Metronidazole*	____mg ___	x 7 d
☐ *Tinidazole*	2g OD	x 1 d
☐ *Tinidazole*	2g OD	x 2 d
☐ *Tinidazole*	_____	x __d
☐ *Quinacrine*	_____	x __d
☐ *Albendazole*	_____	x __d
☐ *Nitazoxanide*	_____	x __d
☐ *Paromomycin*	_____	x __d
☐ unknown		

(if combination therapy was used: tick both regimens and state form of administration: ☐ concomitant / ☐ sequential
➔ if sequential: state order by inserting 1. & 2. into the boxes)

Known immune defect or immune-suppression: ☐ no ☐ yes -> if yes: specify _____

Any concomitant (chronic/acute) medication: ☐ no ☐ yes -> if yes: specify drug: _____

Concomitant PPI therapy with currently assigned treatment regimen:
☐ no ☐ yes -> if yes: specify drug: _____ and dosage/d: _____mg

CRF p2-5

Adverse events / side-effects reported by the patient under the assigned regimen:

☐ nausea	☐ headache	☐ skin rash
☐ vomiting	☐ vertigo / dizziness	☐ urticaria
☐ dysgeusia	☐ sleep disturbance	☐ pruritus
☐ diarrhoea	☐ paraesthesia	☐ skin discoloration
☐ abdominal discomfort	☐ fever	☐ urine discoloration

☐ other(s): _____

Treatment outcome of the assigned regimen:
☐ Treatment success (= 2 negative tested stool samples (by microscopy or antigen test) ≥4 weeks after completing antimicrobial therapy with the assigned regimen)

(in case of treatment failure continue below)

CRF p3-5

☐ Treatment failure (= detection of *G. lamblia* by in stool by microscopy or antigen test ≥4 weeks after completing antimicrobial therapy with the assigned regimen)
↓
☐ 2nd-line treatment or ☐ ___-line treatment
with
☐ *Metronidazole* 400 - 500mg TID x 7d
☐ *Tinidazole* 2g OD x 2d
☐ *Quinacrine* 100mg TID x 5d
☐ *Albendazole* 400mg BID x 5d
☐ *Nitazoxanide* 500mg BID x 3d
☐ *Paromomycin* 500mg TID x 7d

(if combination therapy is chosen: tick both regimens and state form of administration: ☐ concomitant / ☐ sequential
➔ if sequential: state order by inserting 1. & 2. into the boxes)

Concomitant PPI therapy with currently assigned treatment regimen:
☐ no ☐ yes -> if yes: specify drug: _____ and dosage/d: _____mg

Adverse events / side-effects reported by the patient under the assigned regimen:

☐ nausea	☐ headache	☐ skin rash
☐ vomiting	☐ vertigo / dizziness	☐ urticaria
☐ dysgeusia (metallic taste)	☐ sleep disturbance	☐ pruritus
☐ diarrhoea	☐ paraesthesia	☐ skin discoloration
☐ abdominal discomfort	☐ fever	☐ urine discoloration

☐ other(s): _____

Treatment outcome of the assigned regimen:
☐ Treatment success (= 2 negative tested stool samples (by microscopy or antigen test) ≥4 weeks after completing antimicrobial therapy with the assigned regimen)

(in case of treatment failure continue below)

TropNet centre code: ☐☐☐ **Patient No.:** ☐☐☐ **Age:** ☐☐ years

Sex: ☐ female / ☐ male

***G. lamblia* infection (most likely) acquired in:** country: _____

or continent: _____

***G. lamblia* infection:** ☐ symptomatic ☐ asymptomatic

Diagnosis established by: ☐ stool microscopy ☐ stool antigen test

Patient assigned for:

☐ 1st-line treatment

with

☐ *Metronidazole* 400 - 500mg TID x 7d
☐ *Tinidazole* 2g OD x 1d
☐ *Tinidazole* 2g OD x 2d

Patient referred for:

☐ 2nd-line *or* ☐ ___-line treatment

with

☐ *Metronidazole* 400 - 500mg TID x 7d
☐ *Tinidazole* 2g OD x 2d
☐ *Quinacrine* 100mg TID x 5d
☐ *Albendazole* 400mg BID x 5d
☐ *Nitazoxanide* 500mg BID x 3d
☐ *Paromomycin* 500mg TID x 7d

(if combination therapy is chosen: tick both regimens and state form of administration: ☐ concomitant / ☐ sequential
➔ if sequential: state order by inserting 1. & 2. into the boxes)

Previously used regimen(s):

	dosage:		*duration:*
☐ *Metronidazole*	400 - 500mg TID	x	7 d
☐ *Metronidazole*	_____mg ___	x	7 d
☐ *Tinidazole*	2g OD	x	1 d
☐ *Tinidazole*	2g OD	x	2 d
☐ *Tinidazole*	_____	x	__d
☐ *Quinacrine*	_____	x	__d
☐ *Albendazole*	_____	x	__d
☐ *Nitazoxanide*	_____	x	__d
☐ *Paromomycin*	_____	x	__d

☐ unknown

(if combination therapy was used: tick both regimens and state form of administration: ☐ concomitant / ☐ sequential
➔ if sequential: state order by inserting 1. & 2. into the boxes)

Known immune defect or immune-suppression: ☐ no ☐ yes -> if yes: specify _____

Any concomitant (chronic/acute) medication: ☐ no ☐ yes -> if yes: specify drug: _____

Concomitant PPI therapy with currently assigned treatment regimen:

☐ no ☐ yes -> if yes: specify drug: _____ and dosage/d: _____mg

Adverse events / side-effects reported by the patient under the assigned regimen:

☐ nausea	☐ headache	☐ skin rash
☐ vomiting	☐ vertigo / dizziness	☐ urticaria
☐ dysgeusia	☐ sleep disturbance	☐ pruritus
☐ diarrhoea	☐ paraesthesia	☐ skin discoloration
☐ abdominal discomfort	☐ fever	☐ urine discoloration

☐ other(s): _____

Treatment outcome of the assigned regimen:

☐ Treatment success (= 2 negative tested stool samples [by microscopy or antigen test] ≥4 weeks after completing antimicrobial therapy with the assigned regimen)

(in case of treatment failure continue below)

☐ Treatment failure (= detection of *G. lamblia* by in stool by microscopy or antigen test ≥4 weeks after completing antimicrobial therapy with the assigned regimen)

↓

☐ 2nd-line treatment *or* ☐ ___-line treatment

with

☐ *Metronidazole* 400 - 500mg TID x 7d
☐ *Tinidazole* 2g OD x 2d
☐ *Quinacrine* 100mg TID x 5d
☐ *Albendazole* 400mg BID x 5d
☐ *Nitazoxanide* 500mg BID x 3d
☐ *Paromomycin* 500mg TID x 7d

(if combination therapy is chosen: tick both regimens and state form of administration: ☐ concomitant / ☐ sequential
➔ if sequential: state order by inserting 1. & 2. into the boxes)

Concomitant PPI therapy with currently assigned treatment regimen:

☐ no ☐ yes -> if yes: specify drug: _____ and dosage/d: _____mg

Adverse events / side-effects reported by the patient under the assigned regimen:

☐ nausea	☐ headache	☐ skin rash
☐ vomiting	☐ vertigo / dizziness	☐ urticaria
☐ dysgeusia (metallic taste)	☐ sleep disturbance	☐ pruritus
☐ diarrhoea	☐ paraesthesia	☐ skin discoloration
☐ abdominal discomfort	☐ fever	☐ urine discoloration

☐ other(s): _____

Treatment outcome of the assigned regimen:

☐ Treatment success (= 2 negative tested stool samples [by microscopy or antigen test] ≥4 weeks after completing antimicrobial therapy with the assigned regimen)

(in case of treatment failure continue below)

☐ Treatment failure (= detection of *G. lamblia* by in stool by microscopy or antigen test ≥4 weeks after completing antimicrobial therapy with the assigned regimen)

↓

☐ 3rd-line treatment *or* ☐ ___-line treatment

with

☐ *Metronidazole* 400 - 500mg TID x 7d
☐ *Tinidazole* 2g OD x 2d
☐ *Quinacrine* 100mg TID x 5d
☐ *Albendazole* 400mg BID x 5d
☐ *Nitazoxanide* 500mg BID x 3d
☐ *Paromomycin* 500mg TID x 7d

(if combination therapy is chosen: tick both regimens and state form of administration: ☐ concomitant / ☐ sequential
➔ if sequential: state order by inserting 1. & 2. into the boxes)

Concomitant PPI therapy with currently assigned treatment regimen:

☐ no ☐ yes -> if yes: specify drug: _____ and dosage/d: _____mg

Adverse events / side-effects reported by the patient under the assigned regimen:

☐ nausea	☐ headache	☐ skin rash
☐ vomiting	☐ vertigo / dizziness	☐ urticaria
☐ dysgeusia (metallic taste)	☐ sleep disturbance	☐ pruritus
☐ diarrhoea	☐ paraesthesia	☐ skin discoloration
☐ abdominal discomfort	☐ fever	☐ urine discoloration

☐ other(s): _____

Treatment outcome of the assigned regimen:

☐ Treatment success (= 2 negative tested stool samples [by microscopy or antigen test] ≥4 weeks after completing antimicrobial therapy with the assigned regimen)

(in case of treatment failure continue below)

☐ Treatment failure (= detection of *G. lamblia* by in stool by microscopy or antigen test ≥4 weeks after completing antimicrobial therapy with the assigned regimen)

↓

___-line treatment

with

☐ *Metronidazole* 400 - 500mg TID x 7d
☐ *Tinidazole* 2g OD x 2d
☐ *Quinacrine* 100mg TID x 5d
☐ *Albendazole* 400mg BID x 5d
☐ *Nitazoxanide* 500mg BID x 3d
☐ *Paromomycin* 500mg TID x 7d

(if combination therapy is chosen: tick both regimens and state form of administration: ☐ concomitant / ☐ sequential
➔ if sequential: state order by inserting 1. & 2. into the boxes)

Concomitant PPI therapy with currently assigned treatment regimen:

☐ no ☐ yes -> if yes: specify drug: _____ and dosage/d: _____mg

Adverse events / side-effects reported by the patient under the assigned regimen:

☐ nausea	☐ headache	☐ skin rash
☐ vomiting	☐ vertigo / dizziness	☐ urticaria
☐ dysgeusia (metallic taste)	☐ sleep disturbance	☐ pruritus
☐ diarrhoea	☐ paraesthesia	☐ skin discoloration
☐ abdominal discomfort	☐ fever	☐ urine discoloration

☐ other(s): _____

Treatment outcome of the assigned regimen:

☐ Treatment success (= 2 negative tested stool samples [by microscopy or antigen test] ≥4 weeks after completing antimicrobial therapy with the assigned regimen)

(in case of treatment failure continue below)

(a copy of this page might be used to record further

treatment attempts in case of continuing failure)

Pros & Cons

- Giardia = common problem, no consensus on standard Tx (yet)
- High study participation will be needed
- Giardia is a frequently seen parasite at most sites. Simple study design facilitates broad participation
- Various regimens need to be used to compare outcomes
- Participation of different sites with preferences for different regimen will make sure data on varies regimen will be available
- Too many options for combination treatment?
- In reality not any possible combination will be used in the end…
- The study will not reach scientific gold standard… RCT
- The study will be a good filter to identify suitable regimen for further focused studies and help members to gain & exchange experience

Not the ”egg-laying milk-giving wool-pig” (*Eierlegende Wollmilchsau*)…

[Bavarian saying refering to something having only advantages and satifying all expectations & needs…]

…but a feasible & probably realistic approach.

Introduction

Challenges of diagnosis in travelers:

- low parasite load

- imperfect serologic detection of latent infection

- late (and imperfect) etiologic confirmation in acute schistosomiasis

What is the added value of a (semi)quantitive test?

Definitions in acute schistosomiasis

Prepatent period (6 to 12 weeks)
= time lapse between first exposure and parasitologic diagnosis (oviposition)

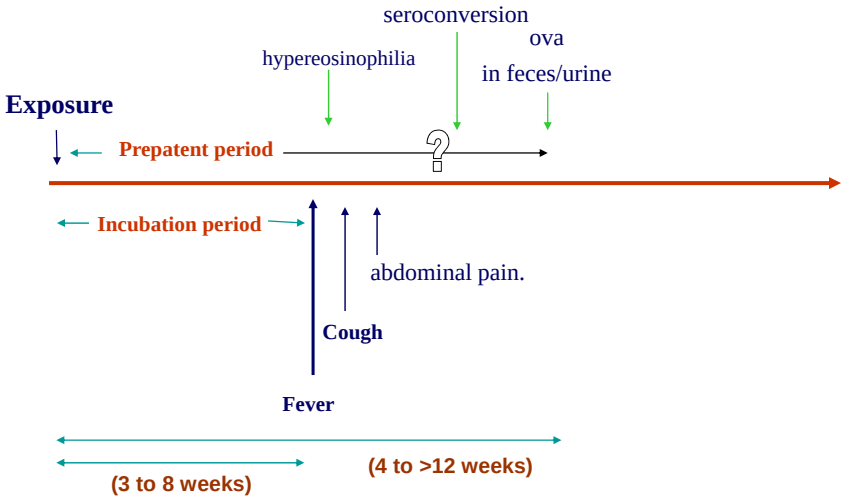
Incubation period (3 to 8 weeks)
= time lapse between first exposure and appearance of symptoms

Confirmation of diagnosis may take 3 to 9 weeks after symptom appearance

Problems:
Definition of prepatent period may vary according to diagnostic standard: ova detection, serum antibody conversion, serum antigen appearance…

What if parasitologic diagnosis = negative?

Timeline in acute schistosomiasis



Definitions in acute schistosomiasis

Prepatent period: expanded definition

= time lapse between first exposure and *first evidence of schistosome infection*

based on at least one of the following criteria:

- ***Ova*** *in faeces, urine, rectal snips, other tissue*
- *Positive schistosome serum* ***antibody test*** *in hitherto negative patients*
- *Positive schistosome faeces and urine* ***antigen test****: CAA, CCA*

and/or

- *Positive schistosome serum/faeces/urine* ***DNA test****: PCR using specific markers for human schistosomiasis*

Acute schistosomiasis : laboratory tests

Current situation

Prepatent period (asymptomatic patients)	**Time lapse (weeks)**
• raised eosinophil count	3 - 8
• serology for *Schistosoma sp*. (IFAT, ELISA, HAI, using adult worm or egg antigen)	>6
Patent period	
• faeces and/or urine microscopy using a concentration test	>6
• rectal snips microscopy for ova (Sm & Sh)	>5?
Incubation period (symptomatic patients)	
• raised eosinophil count *	>3?

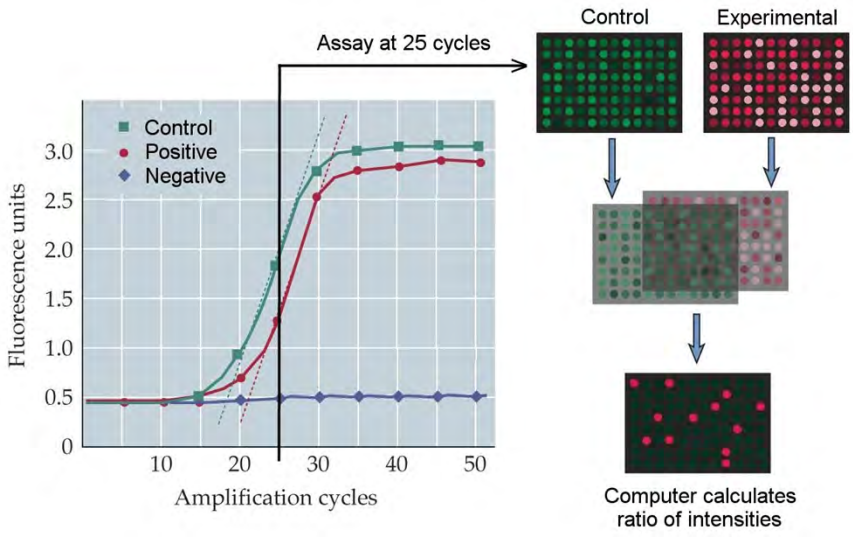
* Probably not raised in the first days of symptom appearance.

Acute schistosomiasis : laboratory tests

Diagnostic tools now and later

	Time lapse (weeks)
• New serology (ELISA using schistosomula antigen)	?
• New schistosome urine antigen tests	?
• RT-PCR for schistosome antigen in feces, urine	>5?
• RT-PCR serum	?

Real Time PCR diagnosis



Overview of molecular techniques for diagnosis of schistosomiasis in endemic and non-endemic settings.

Author	Sample type	Origin	Detection method	Target gene	Species
Pontes	Feces/Serum	Brazil	Conventional PCR	121-bp tandem repeat	Sm
Pontes	Feces (n=194)	Brazil	Conventional PCR	121-bp tandem repeat	Sm
Sandoval	Urine (n=18)	Spain	Conventional PCR	28S rDNA	S.spp
Ten Hove	Feces (n=176)	Senegal	Triplex real-time PCR	COX B DNA	Sm/Sh
0beng	Urine (n=153)	Ghana	Duplex real-time PCR	ITS2gB DNA	Sm/Sh/S.interc.
Wichmann	Plasma(n=52)	Germany	Real-time PCR	121-bp tandem repeat	Sm
Allam	Feces(n=995)	Egypt	Conventional PCR	121-bp tandem repeat	Sm
Gomes	Feces (n=67)	Brasil	Conventional PCR	121-bp tandem repeat	Sm
Kjetland	Vaginal lavage(n=83)	Zimbabwe	Duplex real-time PCR	ITS2*Cox*1*Cox*1gB DNA	Sh/Sm
Huyse	Urine/Feces (n=575)	Senegal	Duplex conventional PCR	ITS rDNA*Cox*1 mtDNA	Sh/Sm
Oliveira	Feces (n=102)	Brazil	Conventional PCR	121-bp tandem repeat	
				28S rDNA	Sm
Gomes	Feces (n=206)	Brasil	PCR-ELISA	121-bp tandem repeat	S.spp
Ibironke	Urine (n=149)	Niger	Conventional PCR	121-bp *Dra* 1 tandem rep.	Sh
Cnops	Feces/Urine	Belgium	Duplex real-time PCR	28S rDNA	S.spp

Table 2. Patients with Katayama syndrome. Schistosome 121bp tandem repeat sequence

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

10ml plasma sample

…but no species identification…

Diagnostic parameters of schistosomiasis in a cluster of patients exposed to *S.mansoni* infection at diagnosis (W8-W14)

	N	eosino/µl	ELISA	HAI	fEPG	RT-PCR	Ct RT-PCR
Primary infection	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
Repeat infection	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

RT-PCR, 121bp tandem repeat sequence in 2ml serum sample

PCR results for schistosomiasis in a cluster of patients exposed to *S.haematobium* infection (n= 9) (Cnops et al.)

Sample	*Target gene*	*Pretreatment N pos /n*	*Posttreatment N pos /n*
Serum	121bp trs	5/9	2/9
Serum	Sh specific	9/9	4/9
Urine	28S	1/3*	2/8
Faeces	28S	7/9**	0/9

* the one PCR positive with ova in urine

** all 7 PCR positive with ova in feces

Diagnosis of (acute) schistosomiasis by PCR in feces and urine (Cnops et al. 2012)

PCR analysis (28S) on 149 faecal samples* in comparison to parasitological examination by microscopy

	microscopy		
PCR	pos	neg	tot
pos	67	9	76
neg	0	73	73
tot	67	82	149

* 70 faecal samples c.pos. microscopy: *S. mansoni* (n = 60), *S haematobium* (n = 9), *S. mekongi* (n = 1)
82 faecal samples c.neg. microscopy

Diagnosis of (acute) schistosomiasis by PCR in feces and urine (Cnops et al. 2012)

PCR analysis on 32 urine samples* in comparison to parasitological examination by microscopy

	microscopy		
PCR	pos	neg	tot
pos	4	1	5
neg	0	27	27
tot	4	28	32

* 4 urine samples c.pos. microscopy: *S. haematobium* (n = 4)
28 urine samples c.neg. Microscopy (including 5 with *S.mansoni*)

***What* can we learn from all this?**

RT-PCR diagnosis of schistosomiasis: a new routine tool?

•Maybe so but there is no common probe that fits all!

•Qualitative diagnostic test only

In serum:

• RT-PCR targeting the **121bp tandem repeat sequence** common to human schistosomes only performs well in S.mansoni infection

• RT-PCR targeting the **gene sequence specific of *S.haematobium*** is sensitive (and specific for *S.haematobium infection)*

• RT-PCR targeting the **28S gene sequence** common to human schistosomes does not work (data not shown)

What can we learn from all this?

RT-PCR diagnosis of schistosomiasis: a new routine tool?

In faeces:

RT-PCR targeting the **28S sequence** common to human schistosomes only performs well in S.mansoni infection

In urine:

RT-PCR targeting the *S.haematobium* specific sequence performs well in *S.haematobium* infection


Thank You for Your attention!

StaphTrav -

European network on imported

Staphylococcus aureus

Philipp G. Zanger
Institute of Tropical Medicine and Parasitology, Tübingen, Germany

Global emergence of MRSA

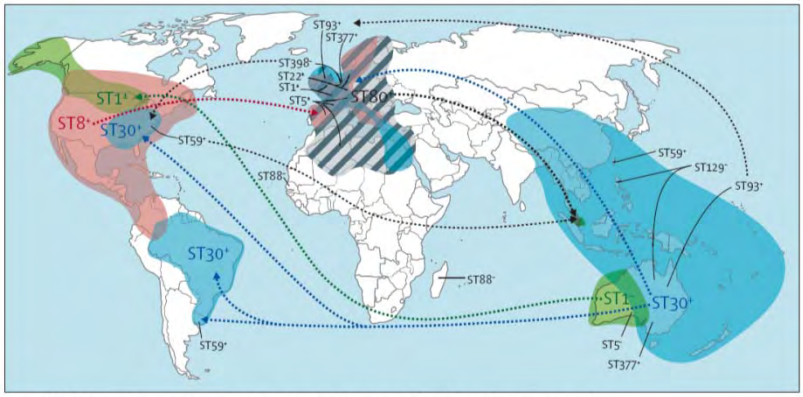
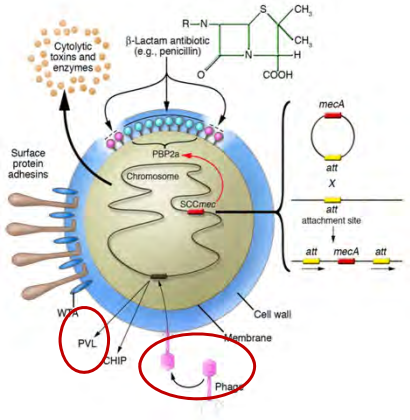


Figure 1: Global distribution of community-associated meticillin-resistant *Staphylococcus aureus* (CA-MRSA) by multilocus sequence type (ST)

DeLeo, Lancet 2010

Global spread of *S. aureus*

Many virulence factors of *S. aureus* are mobile.



mecA= gene coding for methicillin resistance

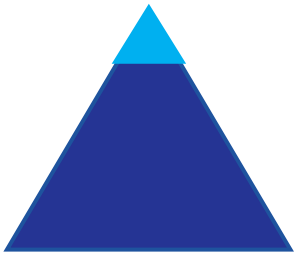
PVL= Panton-Valentine leukocidin

Foster, J Clin Invest 2004

3

MRSA – the tip of the *S. aureus* iceberg

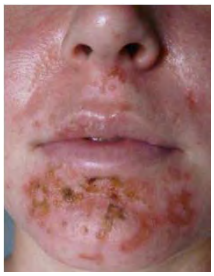
Methicillin Resistant *S. aureus* (MRSA)

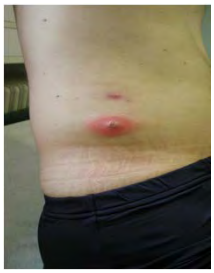


Methicillin Susceptible *S. aureus* (MSSA)

4

S. aureus skin & soft tissue infection (SSTI)





Common:

- 1-3 % of returnees

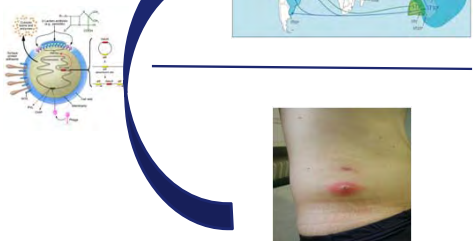
Often complicated:

- deep seated abscesses
- recurrent episodes
- spread to close contacts

5

Travel & the global *S. aureus* epidemic

PVL⁺ MRSA

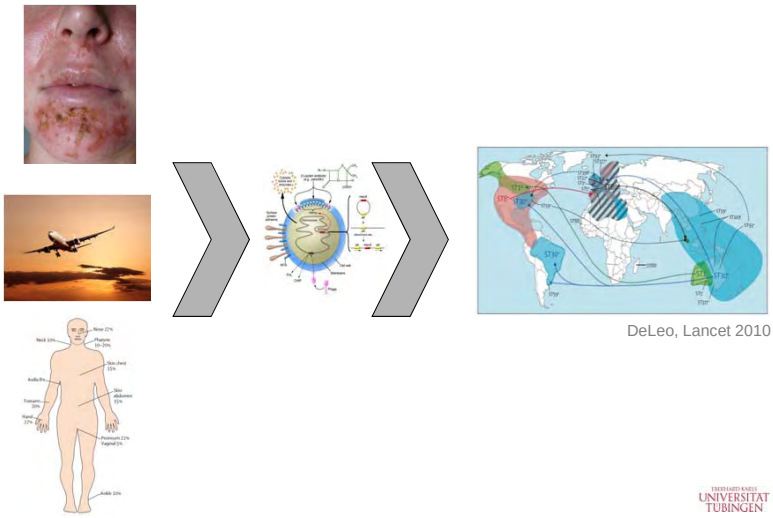


PVL⁺ MSSA

Figures : DeLeo, Lancet 2010 & Foster, J Clin Invest 2004 & ITM Tübingen, Germany

6

Potential drivers of the MRSA/MSSA epidemic



DeLeo, Lancet 2010

Pilot study on travel and *S. aureus* import

MAJOR ARTICLE

Import and Spread of Panton-Valentine Leukocidin–Positive *Staphylococcus aureus* Through Nasal Carriage and Skin Infections in Travelers Returning From the Tropics and Subtropics

Conclusions. Geographic variation in the risk of SSTIs in travelers supports a globally heterogeneous distribution of virulent *S. aureus*. Complicated SSTIs in returnees from nontemperate climates are associated with PVL^+ *S. aureus* and promote the emergence and spread of virulent and antibiotic-resistant strains. We propose a network for the surveillance of imported *S. aureus* (www.staphtrav.eu).

CID 2012:54 (15 February)

StaphTrav: 9 submitting sites by 10/2012

9

StaphTrav: Case definition

- Patient has **pus producing skin infection** on the day of the clinic visit
- **Onset** of skin infections **while abroad** or **within 30 days after return** to home country from a trip outside the EU

10

StaphTrav: Preliminary results

Period:	23.05.2011 – 20.08.2012	
Submissions:	130	
S. aureus:	73/130	(56.2%)
PVL:	45/73	(61.6%)
MRSA:	5/73	(6.9%)

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Travel destination in *S. aureus* SSTI

Destination	Freq.	Percent	Cum.
.	3	4.11	4.11
Pacific	4	5.48	9.59
South Am.	3	4.11	13.70
S.E.Asia	25	34.25	47.95
South Asia	16	21.92	69.86
SS-Africa	22	30.14	100.00
Total	73	100.00	

12

Purpose of travel & *S. aureus* SSTI

	Freq.	Percent	Cum.
missing	4	5.48	5.48
leisure	40	54.79	60.27
business	5	6.85	67.12
VFR	6	8.22	75.34
volunteer	16	21.92	97.26
migration	2	2.74	100.00
Total	73	100.00	

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PVL by destination

Destin.	PVL 0	PVL 1	Total
S.-E.Asia	12	13	25
	48.00	52.00	100.00
South Asia	6	10	16
	37.50	62.50	100.00
SS-Africa	7	**15**	22
	31.82	**68.18**	100.00

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PVL & use of systemic antibiotics

Systemic AB	PVL 0	1	Total
0	19	12	31
	61.29	38.71	100.00
1	8	29	37
	21.62	78.38	100.00
Total	27	41	68
	39.71	60.29	100.00

Pearson chi2(1) = 11.0872 **Pr = 0.001**

OR = 5.7 , 95% CI 2.0 - 16.7

15

PVL & surgical incision

Surgical Incision	PVL 0	1	Total
0	25	27	52
	48.08	51.92	100.00
1	2	14	16
	12.50	87.50	100.00
Total	27	41	68
	39.71	60.29	100.00

Pearson chi2(1) = 6.4688 Pr = **0.011**

OR = 6.5 , 95% CI 1.3 – 31.4

16

PVL & recurrent skin infections

Recurr.	PVL 0	1	Total
0	20	25	45
	44.44	55.56	100.00
1	6	19	25
	24.00	76.00	100.00
Total	26	44	70
	37.14	62.86	100.00

Pearson chi2(1) = 2.8772 Pr = **0.090**

OR = 2.5 , 95% CI 0.9 – 7.5

17

Destination and antibiotic resistance of *S. aureus*

Dest.	Ciprofloxacin Res.	Sens.	Total
Pacific	0	4	4
	0.00	100.00	100.00
South AM	0	3	3
	0.00	100.00	100.00
S.E.Asia	1	24	25
	4.00	96.00	100.00
South Asia	**6**		
	37.50		
SS-Africa	0	22	22
	0.00	100.00	100.00
Total	7	66	73
	9.59	90.41	100.00

NONE of these 6 is MRSA!

Pearson chi2(5) = 18.6719 **Pr = 0.002**

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Destination & antibiotic resistance of *S. aureus*

Seven *S. aureus* **Ciprofloxacin** resistant

- **6** from South-Asia, all MSSA
- **1** from South-East Asia (MRSA)
- none from the other destinations

Destination & antibiotic resistance of *S. aureus*

	TRIMETHOPRIM		
dest_code	Res.	Sens.	Total
Pacific	2	2	4
	50.00	50.00	100.00
South AM	1	2	3
	33.33	66.67	100.00
S.E.Asia	4	21	25
	16.00	84.00	100.00
South Asia	11	5	16
	68.75	31.25	100.00
SS-Africa	12	10	22
	54.55	45.45	100.00
Total	31	42	73
	42.47	57.53	100.00

Pearson chi2(5) = 13.3030 Pr = 0.021

PVL & antibiotic resistance

PVL	Ciprofloxacin Res	Sens	Total
0	0	28	28
1	**7**	38	45
Total	7	66	73

Pearson chi2(1) = 4.8175 Pr = **0.028**

PVL & antibiotic resistance

PVL	Trimethoprim Res.	Sens.	Total
0	7	21	28
	22.58	50.00	38.36
1	24	21	45
	77.42	50.00	61.64
Total	31	42	73
	100.00	100.00	100.00

Pearson chi2(1) = 5.6712 Pr = **0.017**

OR = 3.4 , 95% CI 1.2 – 10.2

PVL & antibiotic resistance

PVL	Tetracycline Res	Sens	Total
0	6	22	28
	31.58	40.74	38.36
1	13	32	45
	68.42	59.26	61.64
Total	19	54	73
	100.00	100.00	100.00

Pearson chi2(1) = 0.4990 Pr = **0.480**

23

Molecular epidemiology

ID 30: 53y., male, Papua Neuguinea

Phenotype: MSSA, PVL-negative, TMP, TET

spa-type: t7210

spa-server: One entry, MRSA from New Zealand 2010

24

Molecular epidemiology

ID 26: 68y., male, Haiti

- recurrent infection
- transmission to contacts

Phenotype: MSSA, PVL-positive, TET

spa-type: t5468

spa-server: first entry from Martinique 2009

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StaphTrav: Structure & Aims

- studies the contribution of **travel related import** towards the **emergence** of new more **virulent** and **antibiotic resistant** *S. aureus* strains
- is a **collaborative multicentre study** of travel clinics in Europe

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StaphTrav: How does it work?

Who can participate?

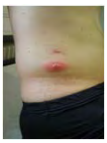
Anyone who sees travellers with skin infections in the EU or affiliated countries!

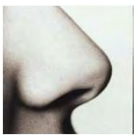
StaphTrav: How does it work?

How does it work?

Participating center's input

1.





2.

3.

sure we can

29

Study centre output

Study centre output:

Submitting centres receive …	Time frame
culture results (*S. aureus* positive or negative)	5-7 working days after arrival of swab
Results of methicillin resistance testing of *S. aureus*	10-12 working days after arrival of swab
summary report of submitted strains including own and other centres	once per year

Note: For research use only!

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StaphTrav: Ethical issues

- **Pseudonymized submission** of patient data
- **Ethical approval** granted in Tübingen
- Participating centres should obtain **local ethical clearance**
- **Documents** available for **download**

Cost

Study centre in Tübingen covers …

- infrastructure
- lab investigations (culture, genotyping, etc.)
- postage via TNT (according to UN 3133)

Participating institution covers …

- ethical clearance
- swabs, packaging material

StaphTrav: joint publication

- **first phase**: 12-24 months
- **estimated** ~ 150-200 *S. aureus* isolates
- **~ 10-20** isolates <u>per</u> named author
- **maximum** two authors per participating center

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Join us and become a star!

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StaphTrav: homepage & contact

www.staphtrav.eu

info@staphtrav.eu

Dr. Philipp Zanger, Institut für Tropenmedizin, Eberhard Karls Universität Tübingen, Germany

Babesiosis an exceptional imported infection in Europe

M Develoux (Paris, France)

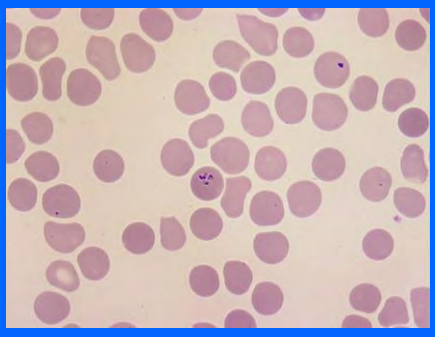
Case report

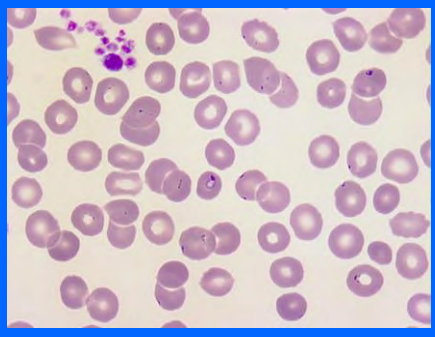
- Woman aged 68, french, retired, living alternately in France and in USA in the Hudson valley
- Tick bite 26 of July leg erythema, asthenia
- Treatment with amoxicillin for suspicion of Lyme confirmed with serology in France (Ig M+ IgG)
- About 3 weeks after the tick-bite she presented with fever in a teaching hospital of Paris
- Fever for three days, chills, headache, asthenia+++
- Clinical examination was normal

Laboratory findings and treatment

- 4340 leukocytes/µL 1300 pn 2260 lymp
- Hb: 10,1 g/dl thrombopenia: 62000 platelets/mm^3
- Liver function tests normal
- Pear forms and trophozoites of intraerythrocytic parasites (parasitaemia 0,25%)
- Dick stick antigen test result was negative
- Babesiosis confirmed, we are waiting for PCR results
- Treatment: atovaquone + azithromycine
- Hospitalisation 3 days, rapid disparition of fever

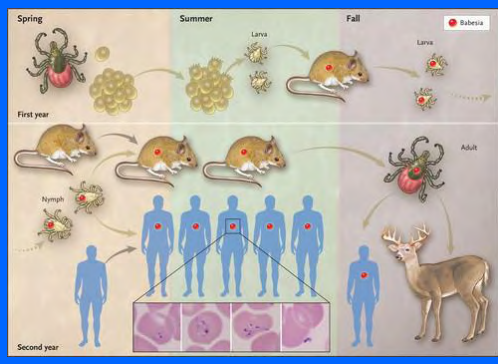
Peripherical blood smears



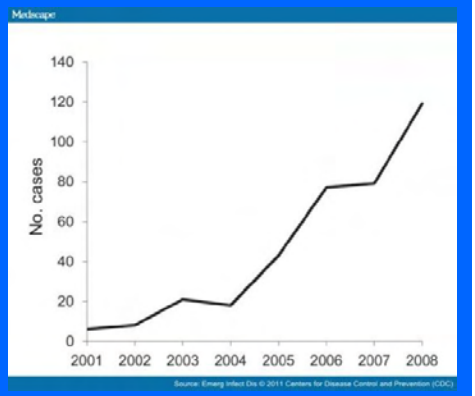


Transmission of *Babesia microti* by the *Ixodes scapularis* tick (N Eng j Med 2012;366:2397-407), north-east USA

- Primary reservoir is the white-footed mouse
- Transmission to human by *I scapularis*
- Other modes of transmission: transfusion, vertical



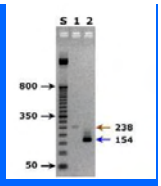
Recent emergence of Babesiosis in lower Hudson valley



Clinical caracteristics

- Incubation period:1 to 3 weeks
- Asymptomatic infection: 20%, may persit for months or years, blood donors at risk
- Mild to moderate illness: intermittent fever and ± chills, sweats, headache, arthralgia, myalgia, anorexia, cough
- Severe illness: HIV, malignancy, splenectomy >50 years…jaudnice, petechiae. Complications : acute respiratory failure, congestive heart failure, renal failure…
- Mortality: 4%, 21% in immunocompromised
- Coinfected (Lyme) may be more symptomatic and have longer disease duration+++

Diagnosis and treatment



- Blood smears : pleomorphic ring forms
- Parasitemia level from less 1% to 80%
- PCR real-time technology
- Indirect immunofluorescence immunoblot essay
- Xenodiagnosis
- Treatment 7 to 10 days
- Clindamycin 600 mg every 6 h + quinine 650 mg every 8h
- Azithromycin 500-600 mg d 1 and 250-600 mg on subsequent days + atovaquone 750 mg every 12 h

Recent data concerning Babesiosis in USA

- Babesiosis is an emerging infection in northeastern USA
- National surveillance began in 2011
- 1124 cases were reported, 97% in 7 states (Connecticut, Massachusetts, Minnesota, New Jersey, New York, Rhode Island, Wisconsin)
- 57% of human cases observed in persons ≥ 60 years
- 82% had symptom onset during June-August
- Fever: 85% chills: 66% myalgia: 64%
- 46% hospitalised medium age: 68, non hospi: 58
- 24 patients asplenic, 4 deaths, 3 due to babesiosis

Imported Babesiosis cases in Europe

- Loutan L et al. Imported babesiosis diagnosed as malaria. Lancet 1993;342:749.
- Humiczewska M et al. A case of imported babesiosis in poland. Wiad Parazytol 1997;43:227-9.
- Nohynková E et al. A case of *Babesia microti* imported in Czech Republic from the USA. Cas lek Cesk 2003;142:377-81.
- Van Vugt M et al. New England Souvenirs. J Travel Med 2011;105:617-23.

The European babesiosis

- *Babesia divergens* (70%) *Babesia* EU-1
- Vector: Ixodidae Reservoir: bovines
- Human disease rare ~ 40 cases
- Most cases described in France and UK
- Splenectomised in 84 % of cases
- Intravascular haemolysis
- Can occur in healthy persons, probably underestimated
- Lethality about 40%

Conclusion

- Babesiosis is rare in western Europe (France, UK), it is an emerging infection in northeastern USA
- Babesiosis can also be an imported disease in travellers who visited endemic american areas
- Babesiosis and Lyme disease can co-exist
- Tick-borne infectious diseases emergent in travellers ?

TREK study

Behrens; Cramer; Jelinek; Shaw; von Sonnenburg; Dewasthaly; Stablein; DuPont; Steffen; Chatterjee; Westrichnig et.al

Efficacy, Safety & Immunogenicity trial in target population

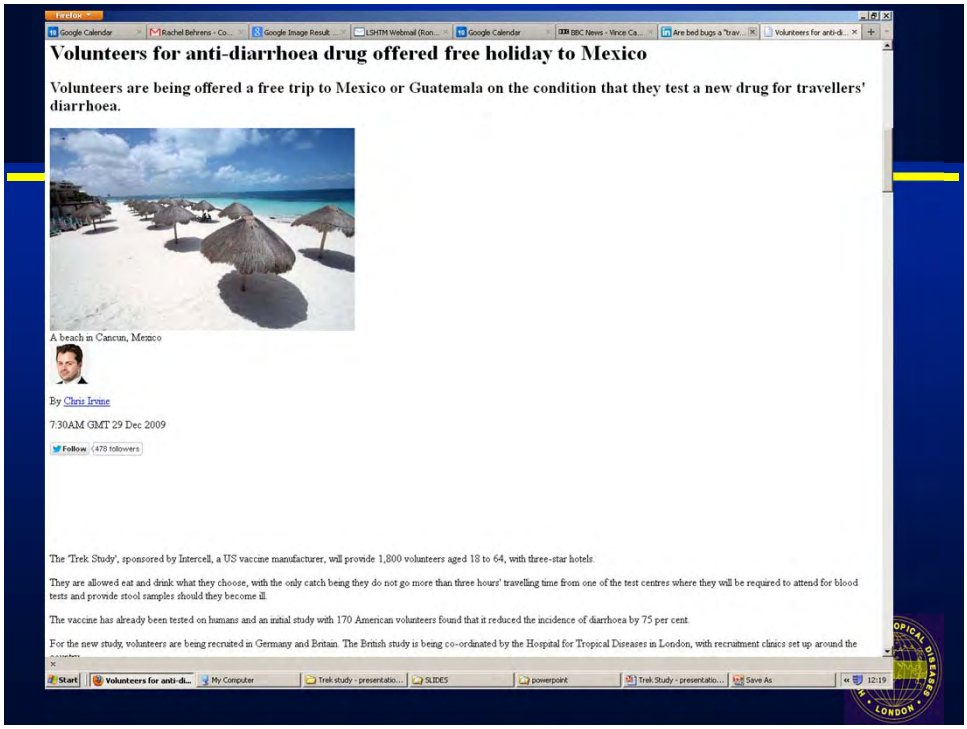
TREK STUDIES (Oct 2009-April 2011)

Randomised, double-blind, placebo controlled studies

Target population for vaccine (Travellers)

Challenge design

- Monitored during & post travel to:
  - Mexico & Guatemala
  - India



Volunteers for anti-diarrhoea drug offered free holiday to Mexico

Volunteers are being offered a free trip to Mexico or Guatemala on the condition that they test a new drug for travellers' diarrhoea.

A beach in Cancun, Mexico

By Chris Irvine

7:30AM GMT 29 Dec 2009

The 'Trek Study', sponsored by Intercell, a US vaccine manufacturer, will provide 1,800 volunteers aged 18 to 64, with three-star hotels.

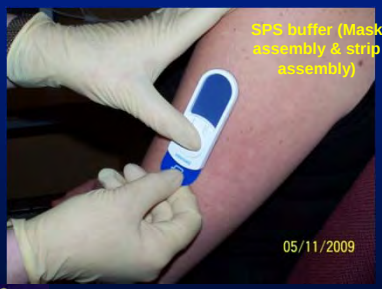
They are allowed eat and drink what they choose, with the only catch being they do not go more than three hours' travelling time from one of the test centres where they will be required to attend for blood tests and provide stool samples should they become ill.

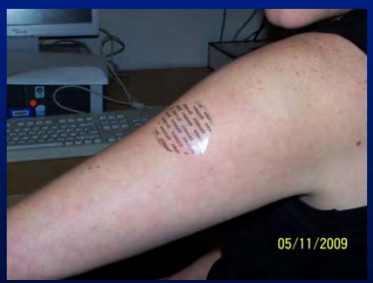
The vaccine has already been tested on humans and an initial study with 170 American volunteers found that it reduced the incidence of diarrhoea by 75 per cent.

For the new study, volunteers are being recruited in Germany and Britain. The British study is being co-ordinated by the Hospital for Tropical Diseases in London, with recruitment clinics set up around the

LT vaccine: TD Vaccine System

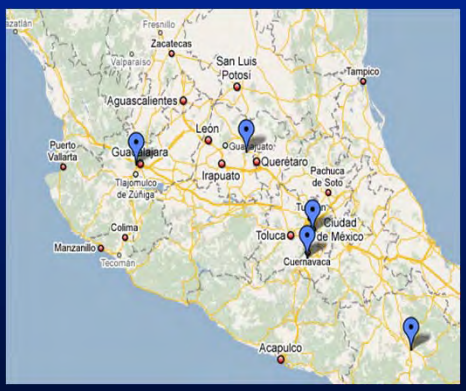
- LT enterotoxin of *e.coli* applied through a patch on arm over 6hr period and repeated on alternative arm after 2 weeks





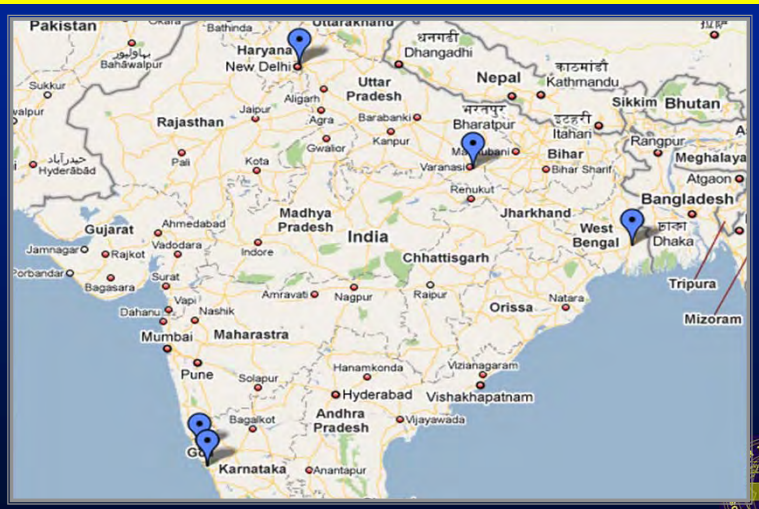
Destination Country study sites Latin America

Mexico



Guatemala

Destination Country India



Follows on from Phase II study Numbers 59 and 111 in each arm

- Main Finding
  - VE 75% all-cause moderate-to-severe diarrhoea
  - VE 84% all-cause severe diarrhoea
  - No protective efficacy against ETEC diarrhoea

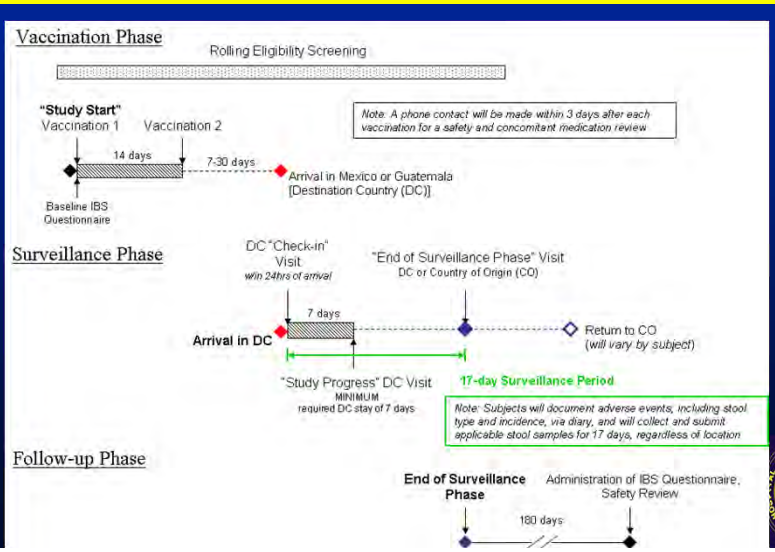
Frech SA, DuPont HL, Bourgeois AL. Lancet 2008

TREK Studies-Objectives

Primary objective

- Efficacy of the TD vaccine system to prevent moderate to severe ETEC disease among travellers in a field setting
- Safety
- Immunogenicity

Protocol



Protocol

APPENDIX 1 – ELT209 Study Procedures Schedule

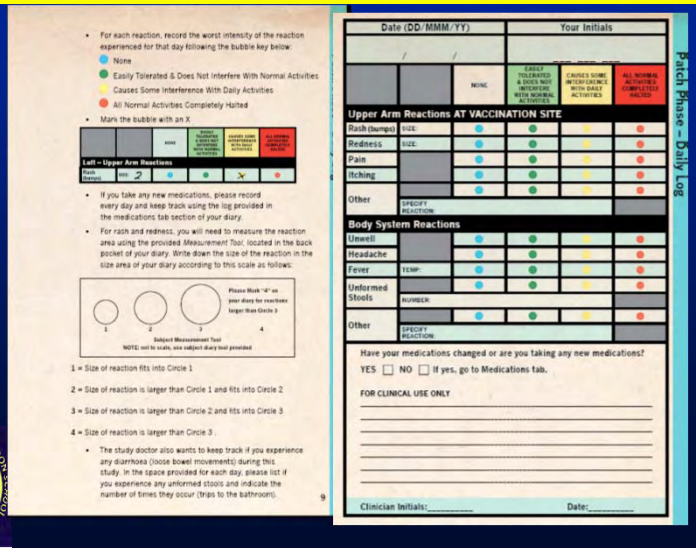
	Vac 1 Visit (CO Visit #1) Study Start	Post Vac Call (CO Call #1) [3 (2-3) days post Vac 1]	Vac 2 Visit (CO Visit #2) [14 (±2) days post Vac 1]	Post Vac Call (CO Call #2) [3 (2-3) days post Vac 2]	DC Visit #1 (Day 0) [1] [7-30 days after Vac 2]	DC Visit #2 (Day 7) [window 6-10 days]	DC or CO final surveillance phase visit (Day 17) [window 17-20 days]	CO or DC Final Visit (6 months post final surveillance phase visit) [window 180-210 days]
ICF/Addendum[2]	X				X			
Inclusion/Exclusion Criteria Review	X		X					
Physical Inspection	X							
Medical History	X		X					
Oral Temperature	X		X					
Pregnancy Test[3]	X		X					
Concomitant Medications Review	X	X	X	X	X	X	X	X
LT IgG, IgA, and TNA blood draw	X				X			X
Baseline IBS Questionnaire	X							
Clinician Completed Vaccination	X		X					
30 minute check	X		X					
Adverse Event review		X	X	X	X	X	X	X
Photograph of Vaccination site(s)	X		X		X	X	X	X
Vaccination site check			X		X	X	X	X
Diary Distribution	X				X			
Vaccination Phase Diary Review		X	X	X	X			
Vaccination Phase Diary Collection					X			
Surveillance Phase Diary Review						X	X	
Surveillance Phase Diary Collection							X	
Stool Collection Kit Distribution					X	X		
Follow-up IBS Questionnaire								X

[1] First destination country (DC) visit should be meet with 24 hours of arrival in DC (window of 0-2 days)
[2] If a subject visits multiple DC clinics during the surveillance phase, the subject will need to complete the informed consent addendum process (which contains PI information for that site) for each DC clinic that they may visit.
[3] Only female subjects of child bearing potential.

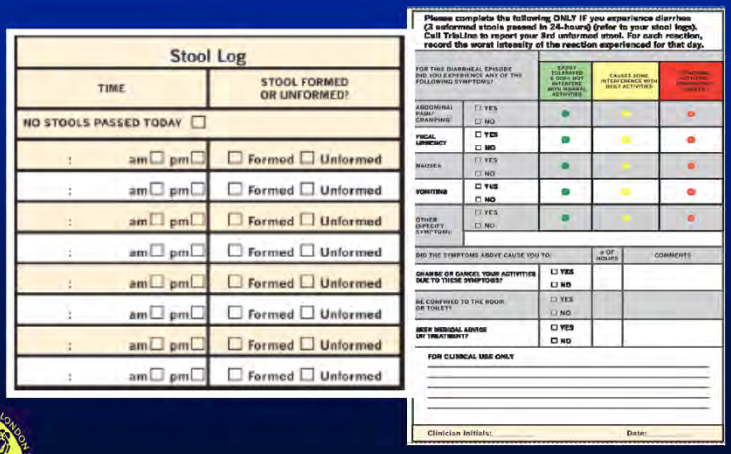
Study populations (Total)

	India n (%)	L.America n (%)
Randomised	723	2036
V1	723 (100)	2034 (>99)
V2	692 (96)	1941 (95)
DC check-in Visit	673 (93)	1833 (90)
Study progress visit (Day 7)	672 (93)	1812 (89)
End of surveillance visit (Day 17)	652 (90)	1754 (86)
Safety follow up visit	633 (88)	1691 (83)

Patch phase diary



Travel phase diary – stool log



Stool Log

TIME	STOOL FORMED OR UNFORMED?
NO STOOLS PASSED TODAY ☐	
: am☐ pm☐	☐ Formed ☐ Unformed
: am☐ pm☐	☐ Formed ☐ Unformed
: am☐ pm☐	☐ Formed ☐ Unformed
: am☐ pm☐	☐ Formed ☐ Unformed
: am☐ pm☐	☐ Formed ☐ Unformed
: am☐ pm☐	☐ Formed ☐ Unformed
: am☐ pm☐	☐ Formed ☐ Unformed
: am☐ pm☐	☐ Formed ☐ Unformed

Please complete the following ONLY IF you experience diarrhea (3 unformed stools passed in 24-hours) (refer to your stool log). Call TrisLine to report your 3rd unformed stool. For each reaction, record the worst intensity of the reaction experienced for that day.

FOR THIS DIARRHEAL EPISODE DID YOU EXPERIENCE ANY OF THE FOLLOWING SYMPTOMS?		EVEN TOLERATED & DOES NOT INTERFERE WITH NORMAL ACTIVITIES	CAUSES SOME INTERFERENCE WITH MOST ACTIVITIES	
ABDOMINAL PAIN/ CRAMPING	☐ YES ☐ NO	●	●	●
FECAL URGENCY	☐ YES ☐ NO	●	●	●
NAUSEA	☐ YES ☐ NO	●	●	●
VOMITING	☐ YES ☐ NO	●	●	●
OTHER (SPECIFY SYMPTOM):	☐ YES ☐ NO:	●	●	●

DID THE SYMPTOMS ABOVE CAUSE YOU TO:		# OF HOURS	COMMENTS
CHANGE OR CANCEL YOUR ACTIVITIES DUE TO THESE SYMPTOMS?	☐ YES ☐ NO		
BE CONFINED TO THE ROOM OR TOILET?	☐ YES ☐ NO		
SEEK MEDICAL ADVICE OR TREATMENT?	☐ YES ☐ NO		

FOR CLINICAL USE ONLY

Clinician Initials: Date:

Safety Assessment

Adverse events

- Documented in subject diary daily
  - Local reactions daily recording
  - Size of the reaction (rash & redness)
  - Systemic reactions for 7 days post vaccine
  - Worst intensity of reaction
- Assessed by clinician at each clinic visit
- Vaccination sites photographed at clinic visits

Con-meds

- Documented in subject diary daily

Results: Efficacy Duration

		LT Patch	Placebo	VE%	P value
L.Am	Total duration (days) of all cause diarrhoeal episodes regardless of severity	**2.2 (mean)**	**3.0 (mean)**	Diff 0.8	0.0080
India	Total duration (days) of all cause diarrhoeal episodes regardless of severity	2.8 (mean)	3.0 (mean)	Diff 0.2	0.4894
Frech	Total duration (days) of all cause diarrhoeal episodes regardless of severity	0.5 (mean)	2.1 (mean)	Diff 1.6	0.0006

Results: Efficacy (L.Am & India)

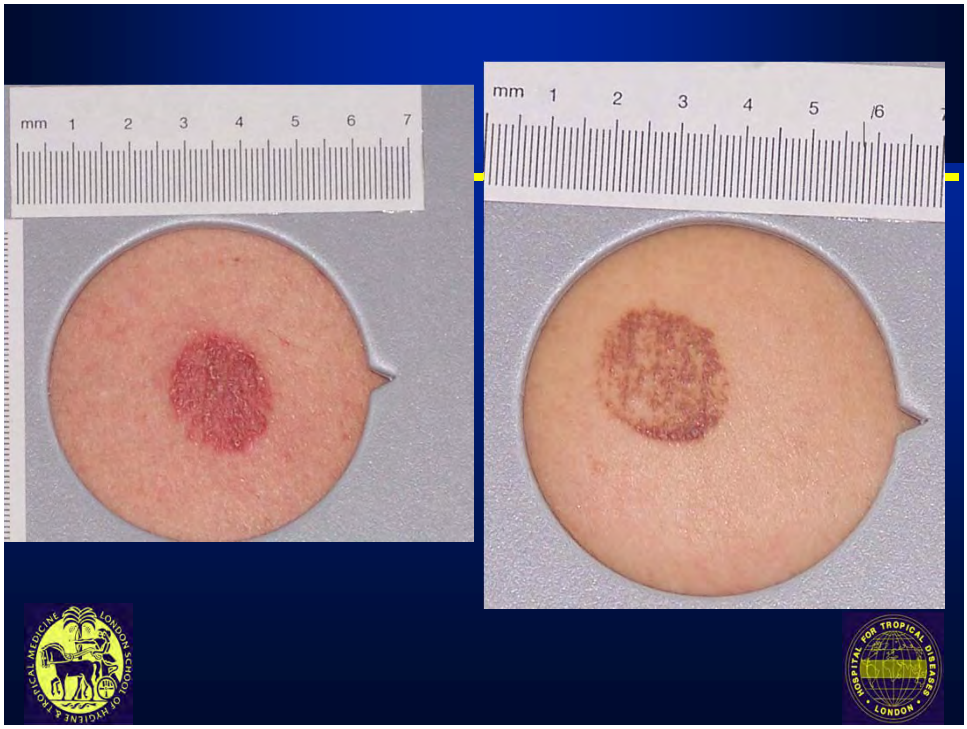
		LT Patch n (%)	Placebo n (%)	VE %	P value
L.Am	All cause diarrhoea /all severity				
L/Am	Moderate/severe ETEC diarrhoea	30 (3.7)	46 (5.6)	34.60	0.0621
L.Am	ETEC diarrhoea (with or without co-pathogen & irrespective of severity)	6.9%	8.3%		
L.Am	Moderate/severe all cause Diarrhoea	102 (12.4)	101 (12.3)	-1.24	0.9404
L.Am	LT+ alone ETEC	0.9%	2.2%	61%	0.04

Results: India study – ETEC attack rates combined groups

ETEC overall	10.1%
LT-secreting	1.3%
ST-secreting	4.0%
LT & ST	4.8%

Results: India study

Diarrhoeal episodes	LT Patch	Placebo	(P Value)	VE
Vaccine Preventable diseases incidence	6%	5.9%	1.0000	1.34%
All cause diarrhoea	21.1%	20.1%		
Incidence of moderate to severe all cause TD	12.7%	16.4%	0.2303	21.4%
ETEC present	11%	9.2%		
Moderate to severe ETEC with or without co-pathogen	8%	8.2%		
LT alone ETEC	1.3%	1.3%		
ST alone ETEC	3.7%	4.3%		
LT & ST ETEC	6.0%	3.6%		
Mean stool frequency - all cause TD	10.7%	13.0%	0.0779	
Loss of days	11.4%	14.8%	0.2287	23.18%
antibiotic treatment - all cause TD	3.7%	7.9%	0.0355	53.40%
Unscheduled clinic visits for treatment TD	1.3%	3.9%	0.0731	66.11%



Recruitment/ enrolment

- Germany - recruit only those with pre-planned travel to sites
- Intensive recruitment drive
- On line application & assessment by Inclinix USA
- Postponed – Swine flu Mexico
- Screen fails
- 1/3rd Subjects DNA'd at 1st visit
- Recruitment halted – low attack rate of TD
- Majority of travel in wet season (April-Oct)

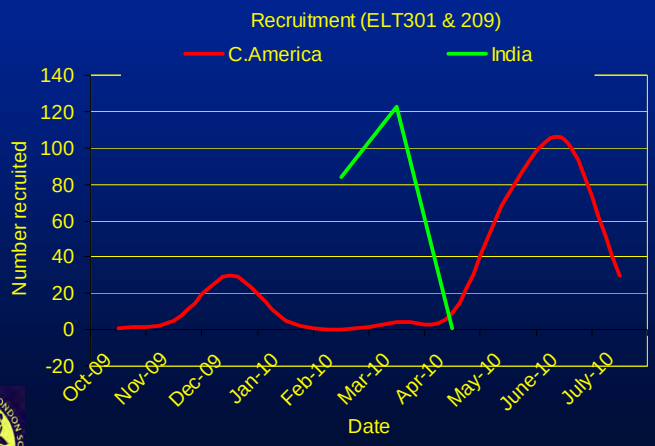
HTD Participant scheduling & follow up

~ 480 participants

- 2 vaccine visits (window ±2 *days)*
- 2 safety calls (window 2-3 days)
- Day 17 visit (window 17-20 days)
- 6 month visit (

Customised participant scheduler programme

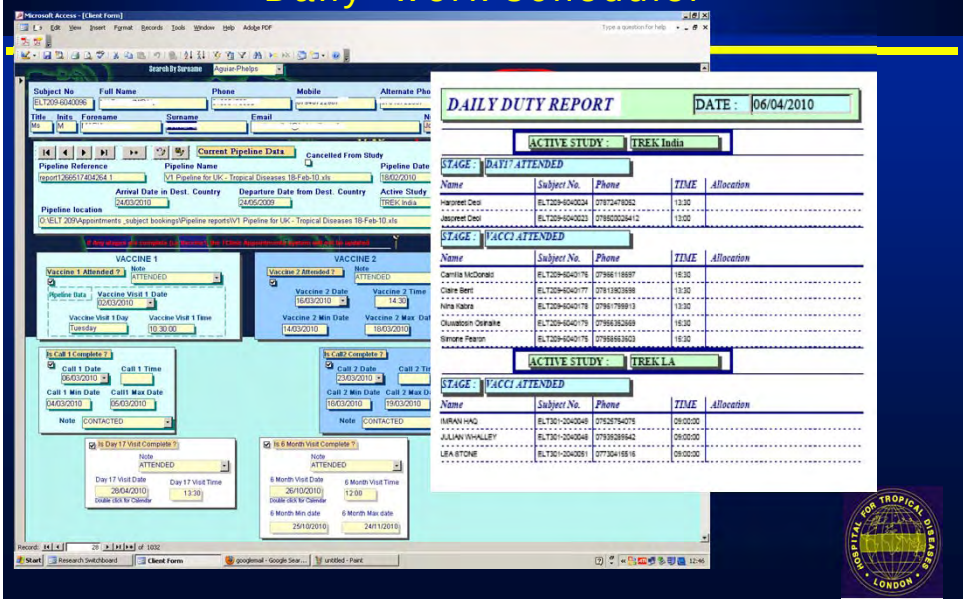
Recruitment HTD



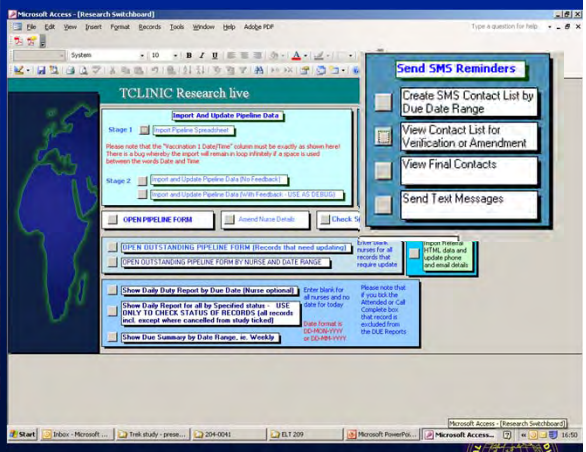
challenges

- Recruitment & travel plans management
  - Company based in USA (subject allocation to site)
    - Time difference made interaction difficult
    - Front face of study
  -)

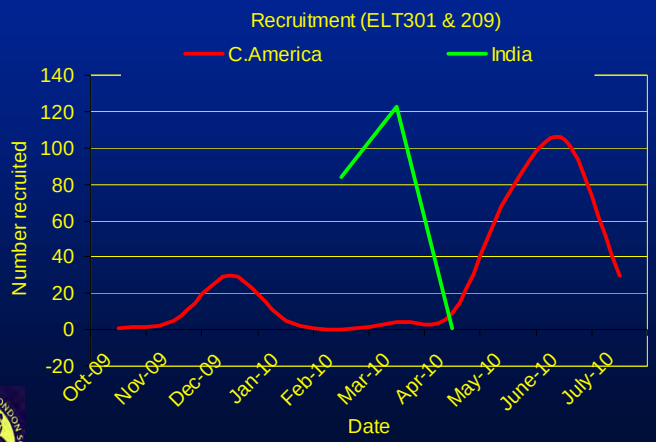
Daily work scheduler



Visit reminders automated SMS via NHS mail



Recruitment HTD



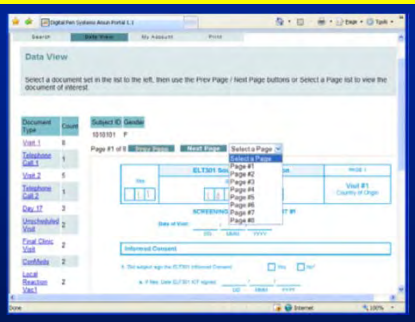
Participant Issues

- Travel plans
- Visa's
- Compensation
- Lost passports
- Lost luggage

Technology

- Site
  - Digital pen records
  - Digital camera
  - Electronic CRF
- Subject
  - Mobile phones
  - Debit cards

Digital pen recording

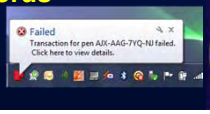


Installation

Uploading

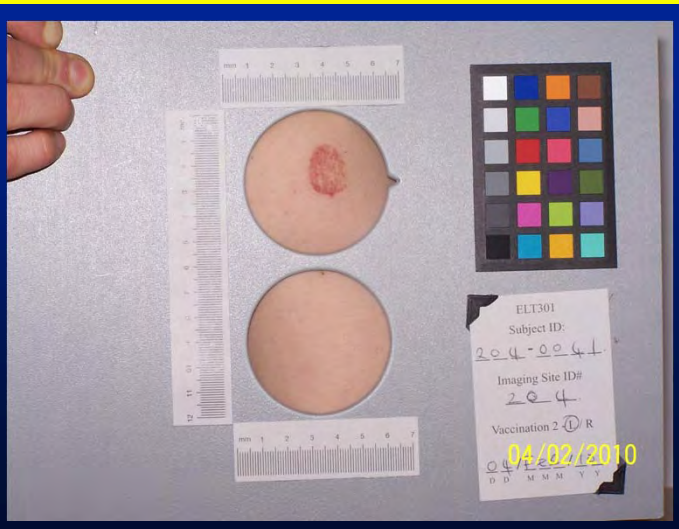
Accessing DC records

Troubleshooting



Vaccine site photographs (pre & post vaccination each visit)

Photographs



Archived files

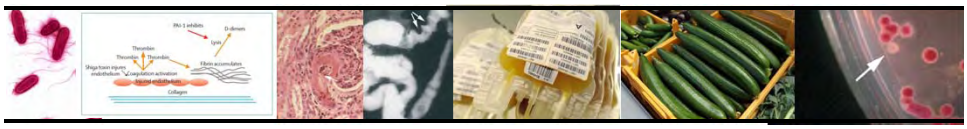
BERNHARD-NOCHT-INSTITUT
FÜR TROPENMEDIZIN

Universitätsklinikum
Hamburg-Eppendorf

EHEC: after the outbreak in Germany in 2011

Jakob P. Cramer

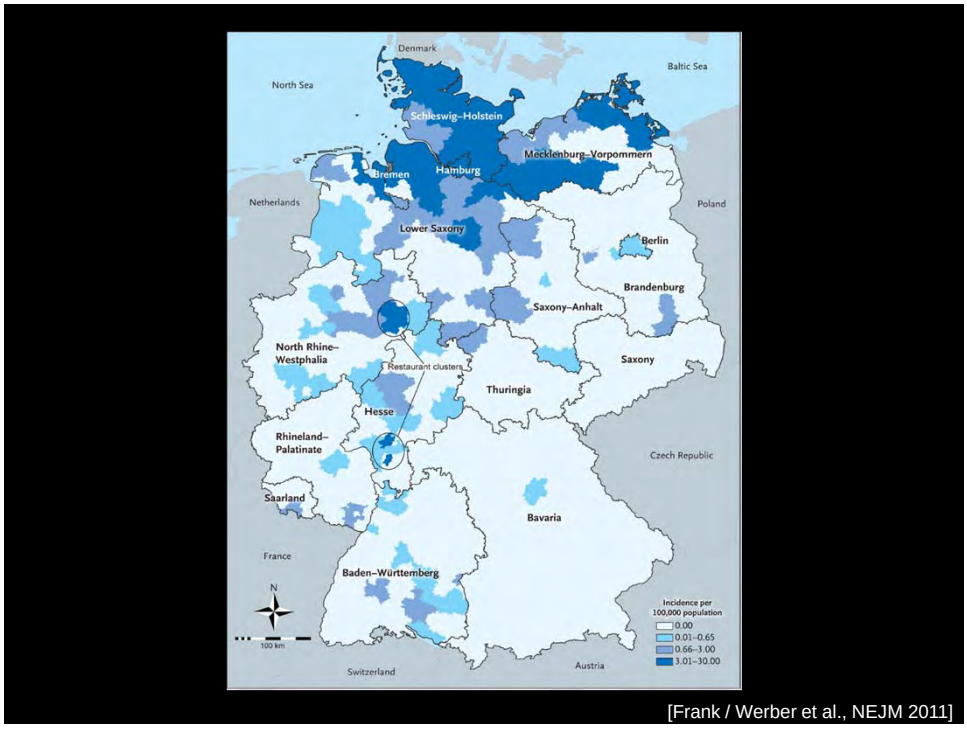
Prague, 06/Oct/2012



EHEC O104:H4 outbreak northern Germany 2011

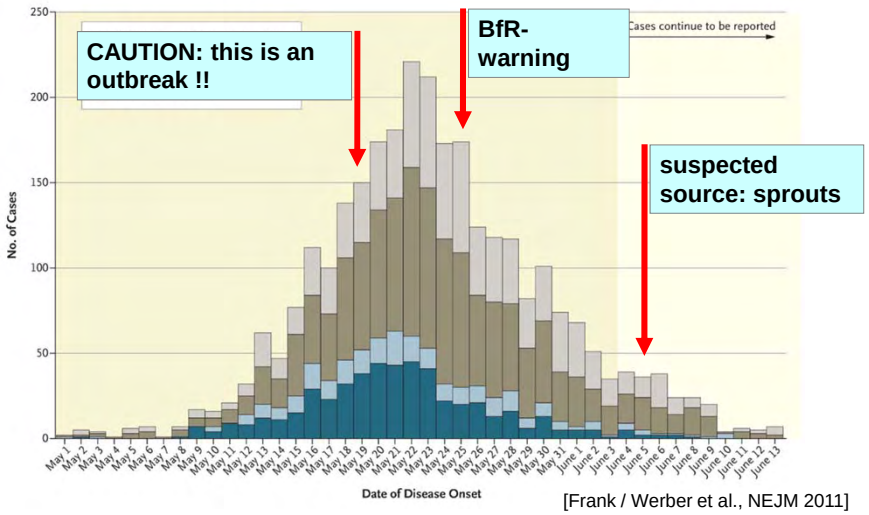

Stade (vendor)
Mölln (vendor)
Bienenbüttel
Point Source
Vendor


Stade (vendor)
Lübeck
Mölln (vendor)
Rotenburg
Lüneburg
Bienenbüttel
Bochum
Point Source
Vendor
Restaurant



[Frank / Werber et al., NEJM 2011]

EHEC O104:H4 Outbreak
Epidemiologic Course



[Frank / Werber et al., NEJM 2011]

Source ???

- early case control-studies: *n* was low !
- Federal Institute for Risk Assessment (BfR) 25. May 2011:
  - cucumber, tomato, salad: „do not eat raw“

Stealth-Bomber / Ham Sandwich

→ **„stealth food“**

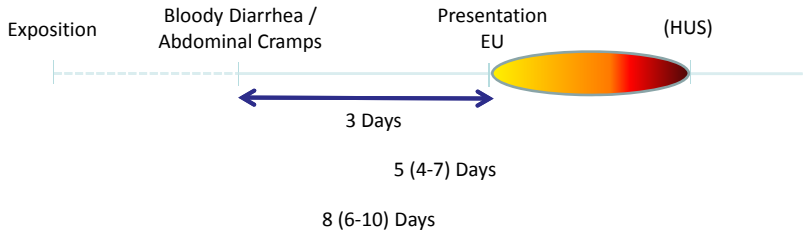
Sprout Production

temp. 35°C, high humidity

Clinical Manifestation / Course of Disease

EHEC – Clinical Manifestation

- incubation period 4 (3 – 11) days, (hours??)
- initially uncomplicated diarrhea, followed by bloody diarrhea
- abdominal pain / -cramps, often nausea, some vomiting



- typically no fever
- unspecific signs, malaise, reduced appetite

Table 1. Demographic and Clinical Characteristics and Laboratory Test Values of Patients Positive for Shiga-Toxin–Producing *E. coli* at First Presentation.*

Variable	Total (N=141)	Adults (N=124)		P Value†	Children (N=17)		P Value†
		Without HUS	With HUS		Without HUS	With HUS	
Age — yr				0.08			0.80
Mean	37	40	49		12	11	
Range	2–87	20–84	22–87		2–17	4–15	
Male sex — no./total no. (%)	49/141 (35)	14/110 (13)	0/11	0.02	3/5 (60)	5/11 (45)	1.00
Bloody diarrhea — no./total no. (%)	124/136 (91)	101/106 (95)	11/11 (100)	1.00	3/5 (60)	6/11 (55)	1.00
Abdominal pain — no./total no. (%)	117/131 (89)	95/105 (90)	9/11 (82)	0.30	5/5 (100)	7/8 (88)	1.00
Nausea — no./total no. (%)	33/97 (34)	23/77 (30)	4/8 (50)	0.30	3/4 (75)	2/5 (40)	0.50
Vomiting — no./total no. (%)	29/111 (26)	14/87 (16)	5/9 (56)	0.01	4/5 (80)	5/7 (71)	1.00
Temperature — °C	36.7±0.5	36.6±0.5	36.9±0.5	0.12	36.9±0.5	36.9±0.3	0.98
Hemoglobin — g/dl	13.5±2.1	14.2±1.4	10.8±2.4	<0.001	14.1±1.6	10.1±2.1	0.001
Leukocytes — billions/liter	11.5±4.2	11.1±3.5	13.2±7.2	0.08	13.3±5.2	12.2±5.8	0.73
Platelets — billions/liter	215.4±88.4	245.3±52.5	88.3±92.8	<0.001	297.4±39.4	51.3±36.7	<0.001
Creatinine — mg/dl	1.4±1.9	0.8±0.2	2.6±1.9	<0.001	0.7±0.3	4.9±4.8	0.08
Bilirubin — mg/dl	0.9±0.6	0.8±0.5	1.9±0.9	<0.001	0.8±0.5	1.4±0.8	0.19
Lactate dehydrogenase — U/liter	410±569	196±95	890±433	<0.001	230±42	1835±873	0.001
C-reactive protein — mg/liter	18.2±30.0	15.0±27.0	30.2±32.2	0.06	46.0±65.7	19.3±20.8	0.20

* Plus–minus values are means ±SD. Data are for patients who presented to the Hamburg University Medical Center between May 19 and June 1, 2011. To covert the values for creatinine to micromoles per liter, multiply by 88.4. To convert the values for bilirubin to micromoles per liter, multiply by 17.1. HUS denotes the hemolytic–uremic syndrome.
† P values are for the presence versus the absence of the hemolytic–uremic syndrome within subgroups of children and adults.

[Frank / Werber et al., NEJM 2011]

Clinical Course

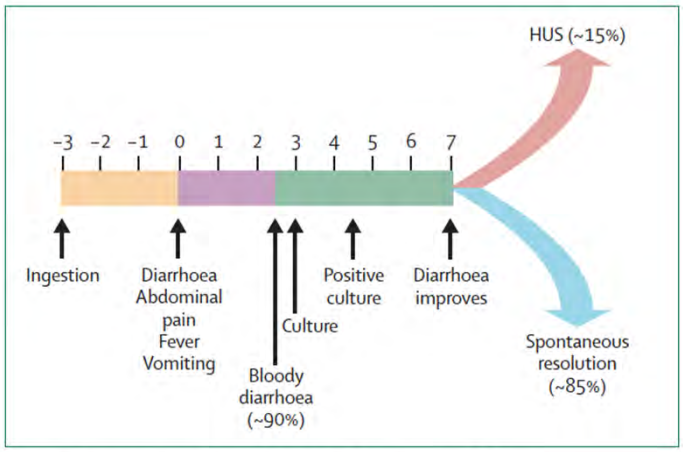


Figure 3: Progression of *E coli* O157:H7 infections in children

[Tarr et al., Lancet 2005]

Case: , 29 Years old

		Befundtyp	Klinische	Klinische	Klinische	Klinische
		Abnahme-Datum	30.05.11	29.05.11	28.05.11	27.05.11
		Abnahme-Zeit	11:30	11:05	10:17	09:15
		Anfordernde Stelle	69210	69210	69210	69210
Hämatologie						
Hämoglobin	14.0 - 17.5	g/dl	2) 16.5	2) 16.6	2) 15.9	2) 16.6
Hämatokrit	36 - 48	%	3) 46.3	3) 46.9	3) 45.7	3) **48.1***
Erythrozyten	4.50 - 5.90	Mrd/ml	5.20	5.20	5.14	5.42
MCV	80.0 - 94.0	fl	89.0	90.2	89.0	88.6
MCH	26.0 - 34.0	pg	31.8	32.0	31.0	30.7
MCHC	31.5 - 37.0	g/dl	35.7	35.4	34.8	34.6
EVB	11.5 - 14.5	%	11.8	12.2	11.8	11.8
Leukozyten	3.8 - 11.0	Mrd/l	10.6	**11.8***	8.5	10.2
Thrombozyten	150 - 400	Mrd/l	218	281	273	285
Klinische Chemie, Plasma						
Natrium	135 - 145	mmol/l	**146***	140	140	140
Kalium	3.5 - 5.0	mmol/l	3.6	**3.3***	4.1	4.2
Bilirubin gesamt	<1.2	mg/dl	1.2	0.9	0.7	0.9
Glucose	60 - 110	mg/dl	88	**115***	90	86
Harnstoff-N	9 - 21	mg/dl	16	13	9	10
Kreatinin	0.6 - 1.2	mg/dl	1.20	0.99	1.00	1.04
GFR (Berechnung nach M	> 60	ml/min				
AST (GOT)	10 - 50	U/l	25	22	15	26
ALT (GPT)	10 - 50	U/l	19	26	26	40
CK gesamt	- 173	U/l	68	66	82	76
C-reaktives Prot.	- 5	mg/l	**40***	**27***	**15***	**8***
LDH	135 - 225	U/l	**230***	165	187	176

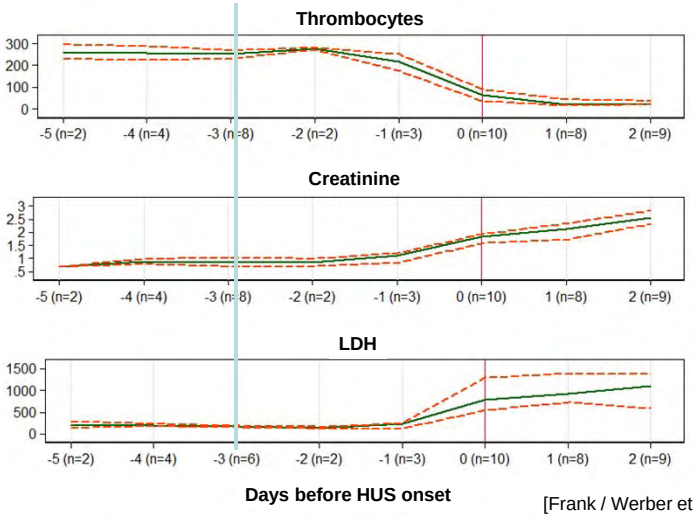
Case: , 29 Years old

		Befundtyp	Klinische	Klinische	Klinische	Klinische	Klinische
		Abnahme-Datum	31.05.11	30.05.11	29.05.11	28.05.11	27.05.11
		Abnahme-Zeit	11:00	11:30	11:05	10:17	09:15
		Anfordernde Stelle	69210	69210	69210	69210	69210
Hämatologie							
Hämoglobin	14.0 - 17.5	g/dl	2) 15.1	2) 16.5	2) 16.6	2) 15.9	2) 16.6
Hämatokrit	36 - 48	%	3) 42.1	3) 46.3	3) 46.9	3) 45.7	3) **48.1***
Erythrozyten	4.50 - 5.90	Mrd/ml	4.71	5.20	5.20	5.14	5.42
MCV	80.0 - 94.0	fl	89.4	89.0	90.2	89.0	88.6
MCH	26.0 - 34.0	pg	32.1	31.8	32.0	31.0	30.7
MCHC	31.5 - 37.0	g/dl	35.9	35.7	35.4	34.8	34.6
EVB	11.5 - 14.5	%	12.6	11.8	12.2	11.8	11.8
Leukozyten	3.8 - 11.0	Mrd/l	10.0	10.6	**11.8***	8.5	10.2
Thrombozyten	150 - 400	Mrd/l	4) **72***	218	281	273	285
Klinische Chemie, Plasma							
Natrium	135 - 145	mmol/l	145	**146***	140	140	140
Kalium	3.5 - 5.0	mmol/l	4.0	3.6	**3.3***	4.1	4.2
Bilirubin gesamt	<1.2	mg/dl	**2.7***	1.2	0.9	0.7	0.9
Glucose	60 - 110	mg/dl	84	88	**115***	90	86
Harnstoff-N	9 - 21	mg/dl	**24***	16	13	9	10
Kreatinin	0.6 - 1.2	mg/dl	**1.84***	1.20	0.99	1.00	1.04
GFR (Berechnung nach M	> 60	ml/min					
AST (GOT)	10 - 50	U/l	47	25	22	15	26
ALT (GPT)	10 - 50	U/l	19	19	26	26	40
CK gesamt	- 173	U/l	103	68	66	82	76
C-reaktives Prot.	- 5	mg/l	**26***	**40***	**27***	**15***	**8***
LDH	135 - 225	U/l	**676***	**230***	165	187	176

Case: , 29 Years old

		Befundtyp	Klinische	Klinische	Klinische	Klinische	Klinische	Klinische
		Abnahme-Datum	09.06.11	31.05.11	30.05.11	29.05.11	28.05.11	27.05.11
		Abnahme-Zeit	16:00	11:00	11:30	11:05	10:17	09:15
		Anfordernde Stelle	07100	69210	69210	69210	69210	69210
Hämatologie								
Hämoglobin	14.0 - 17.5	g/dl	1) **7.1***	2) 15.1	2) 16.5	2) 16.6	2) 15.9	2) 16.6
Hämatokrit	36 - 48	%	3) **20.3***	3) 42.1	3) 46.3	3) 46.9	3) 45.7	3) **48.1***
Erythrozyten	4.50 - 5.90	Mrd/ml	**2.18***	4.71	5.20	5.20	5.14	5.42
MCV	80.0 - 94.0	fl	92.9	89.4	89.0	90.2	89.0	88.6
MCH	26.0 - 34.0	pg	32.4	32.1	31.8	32.0	31.0	30.7
MCHC	31.5 - 37.0	g/dl	34.9	35.9	35.7	35.4	34.8	34.6
EVB	11.5 - 14.5	%	**20.0***	12.6	11.8	12.2	11.8	11.8
Leukozyten	3.8 - 11.0	Mrd/l	8.7	10.0	10.6	**11.8***	8.5	10.2
Thrombozyten	150 - 400	Mrd/l	4) **54***	4) **72***	218	281	273	285
Klinische Chemie, Plasma								
Natrium	135 - 145	mmol/l	145	145	**146***	140	140	140
Kalium	3.5 - 5.0	mmol/l	4.5	4.0	3.6	**3.3***	4.1	4.2
Bilirubin gesamt	<1.2	mg/dl	**4.2***	**2.7***	1.2	0.9	0.7	0.9
Glucose	60 - 110	mg/dl	**117***	84	88	**115***	90	86
Harnstoff-N	9 - 21	mg/dl	**59***	**24***	16	13	9	10
Kreatinin	0.6 - 1.2	mg/dl	**2.79***	**1.84***	1.20	0.99	1.00	1.04
GFR (Berechnung nach M	> 60	ml/min	**28.8***					
AST (GOT)	10 - 50	U/l	**84***	47	25	22	15	26
ALT (GPT)	10 - 50	U/l	**52***	19	19	26	26	40
CK gesamt	- 173	U/l	**304***	103	68	66	82	76
C-reaktives Prot.	- 5	mg/l	< 5	**26***	**40***	**27***	**15***	**8***
LDH	135 - 225	U/l	4) **1357***	**676***	**230***	165	187	176

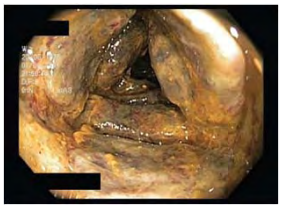
HUS: Rapid Decompensation



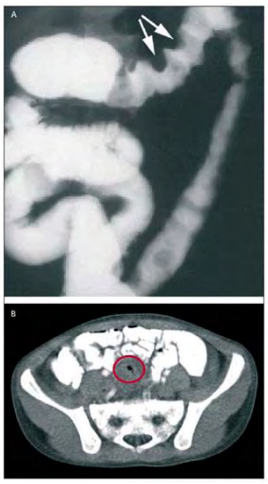
[Frank / Werber et al., NEJM 2011]

Early Complications

- HUS (22%)
  - hemolytic anemia
  - thrombocytopenia >150.000/µl
  - renal failure
- neurologic symptoms (ca. 50% of HUS cases)
- gastrointestinal complications (megacolon, severe haemorrhage, perforation)
- death (1%)



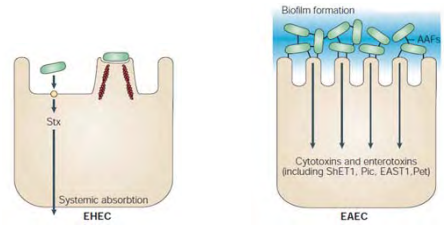
[Dücker et al., Dtsch Med Wochenschr]



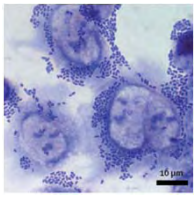
[Tarr et al., Lancet 2005]

What have we learnt ?

Ruminants: Mixing vessels
O104:H4



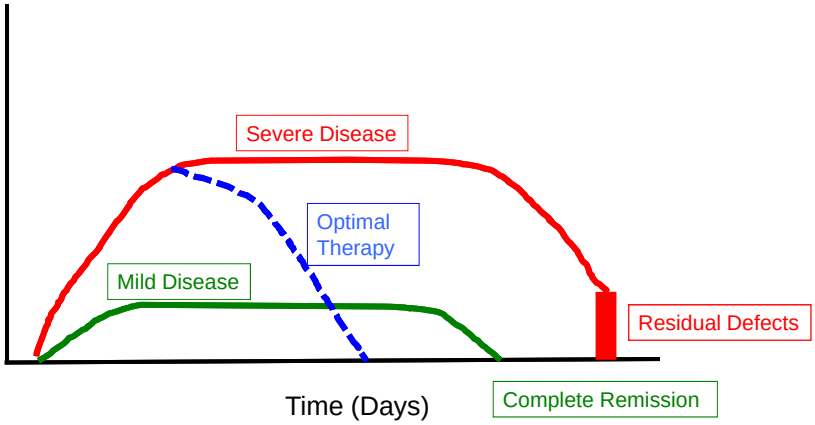
[Kaper et al., Nature Rev 2004]



[Bielaszewska et al., Lancet Infect Dis 2011]

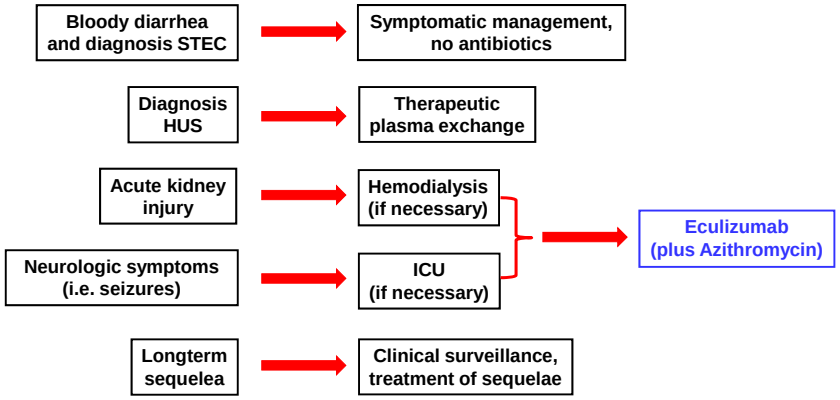
EHEC/HUS: Natural Course

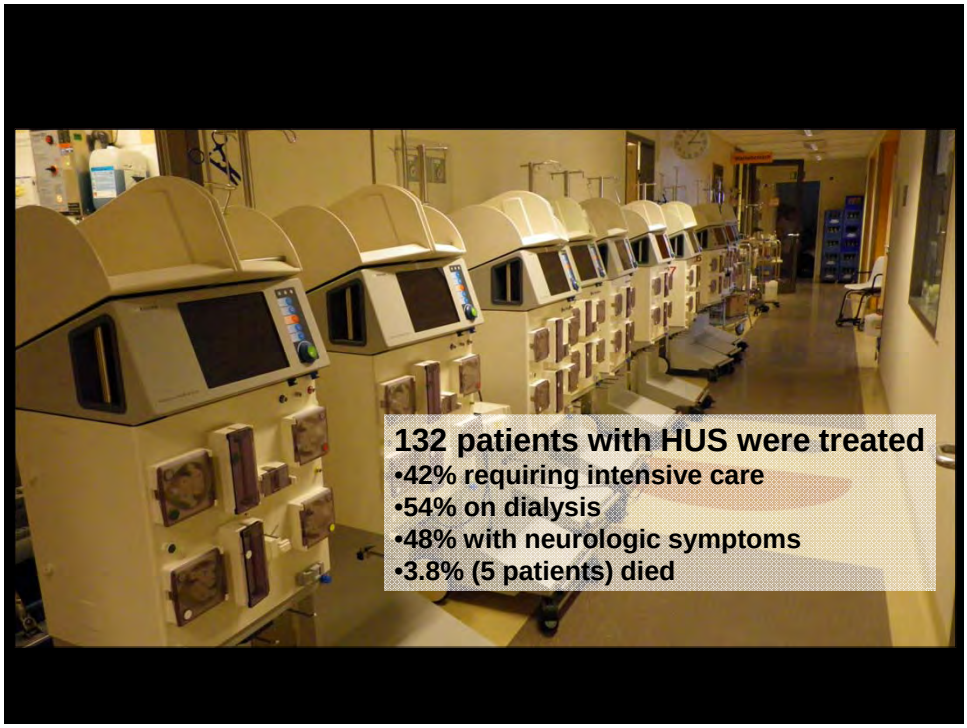
Severity of Disease



Clinical Management EHEC/HUS
UKE / Hamburg

Clinical status and resulting therapeutic intervention:





Role of Plasma Exchange ?

Cons:

- Stx never identified in the circulation
- EHEC density / Stx concentration in stool decreases before / as HUS occurs
- vascular injury present before clinical complications / HUS
- animal models: short half life of Stx
- some observations indicate no / detrimental effects (?)

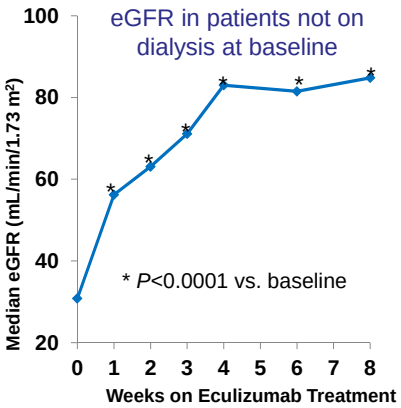
Pros:

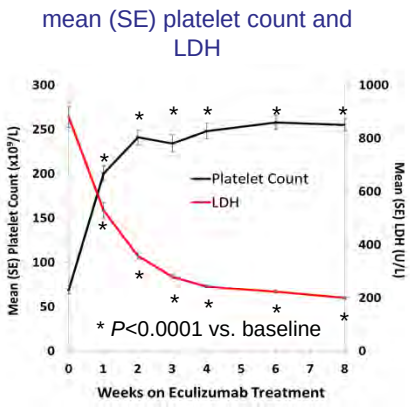
- what else to do ?
- some observations indicate beneficial effects (?)

⇒ in our experience: no beneficial effect

[Menne et al.,BMJ 2012]

Renal- / TMA- Efficacy Endpoints
8 weeks FU



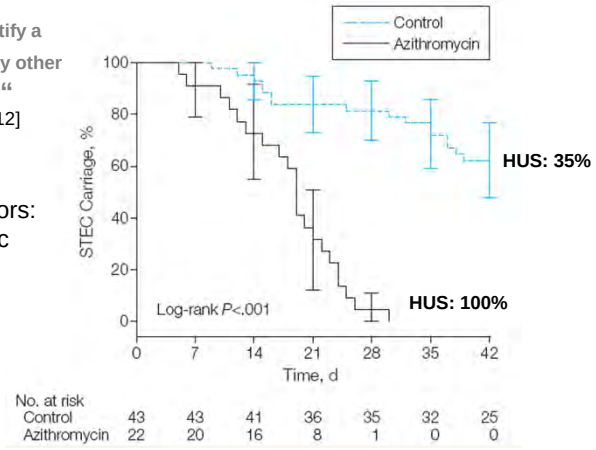


[Stahl R et., unpublished 2012]

EHEC: Azithromycin
effect on shedding

„…existing data do not justify a role for azithromycin (or any other antibiotic) early in illness…“
[Seifert / Tarr, Nature Rev 2012]

possibly relevant factors:
- selection of antibiotic
- timing
- combination
- patient selection

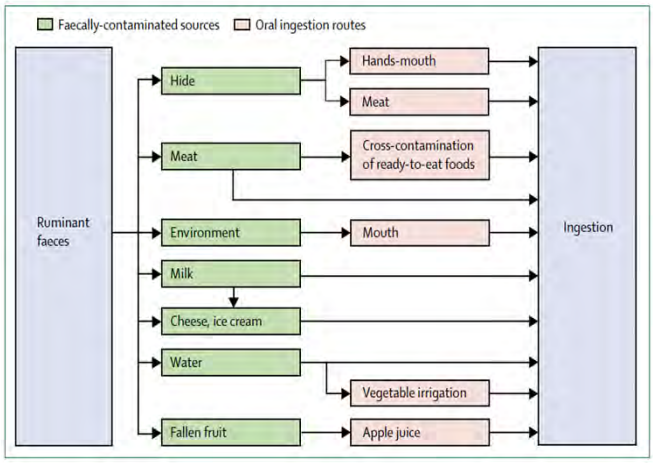


[Nitschke et al., JAMA 2012]

EHEC-Outbreaks: Sources
Industrialized Countries mostly O157:H7

reservoir: ruminants
(cattle, sheep, goats, …)

low infection dose
(min. ~100 bacteria)



[Pennington, Lancet Infect Dis 2010]

Concluding Remarks

What has been contributed to the evidence by the EHEC O104:H4 outbreak?

- we are back at a situation of perfect *clinical equipoisie*
- this is the optimal basis for RCTs
- these should be designed for continuous international recruitment
- this may, however, require new regulatory / ethical / legal guidance
- or may be impossible ???

BERNHARD-NOCHT-INSTITUT FÜR TROPENMEDIZIN

Universitätsklinikum Hamburg-Eppendorf