

Werkgroep klinische parasitologie  
“Wat heb je op je lever”

Lisette van Lieshout  
Parasitology  
LEIDEN UNIVERSITY MEDICAL CENTER

Met dank aan Dr. Jaap van Hellemond, Dr. Pieter Wismans, Dr. Ries Schouten  
en betrokken laboratoriumedewerkers van het KML



## Fasciola diagnosis LUMC

Serology (in-house IFA en EIA)

50-75 samples/year (↑)

*Fasciola* ≈1 positive case/year (19 serology positives 2001-2015)

- Some of them borderline/indecisive
- Some of them other Food-born Trematodes

2016

2 cases in December

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## Case 1 – as seen at LUMC

Dutch male, DOB 07-03-1939

Serum (ZKS Gelderse Vallei) 23-12-2016

=>*Fasciola* IFA 1:512, EIA 1:640

No other requests, only information:

“Micro-abscesses in liver”

No travel recently, never outside Europe

Lives in a rural area of NL

- 23 December hospitalized, night sweating, mild fever, liver dysfunction, eosinophilia (2.7 x10<sup>9</sup>/L)
- Faeces microscopy negative

Liver biopsies => AMC and Tilburg for PCR

30-12-2016 treatment started with Triclabendazole

2x 10 mg/kg in 24 hrs; Follow-up .....



## Case 2 – as seen at LUMC

Dutch Female, DOB 11-02-1988

Serum (olvg) 26-08-2016

Amoebiasis, echinococcosis negative, no information

<never rule out false-negative serology for these species>

Liver biopsy 26-08-2016 (olvg)

Liver abscess – travel to Bali

*E. histolytica/E. dispar* PCR- negative

Serum (EMC/HZK) 23-11-2016 => *Fasciola* IFA 1:1024, EIA 1:160

Stored serum (EMC/HZK) 04-10-2016 => *Fasciola* IFA 1:1024, EIA 1:160

Retrospectively serum (olvg) 26-08-2016 => *Fasciola* IFA 1:1024, EIA 1:160

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## Case 2 – clinical background

Dutch Female, DOB 11-02-1988

Via HZK/OLVG: Clinical letter 15-11-2016

Liver abscess after travel to Indonesia, positive amoebiasis serology (?? Tested in France???)

TFT negative

- Conclusie: abcederende afwijking in de lever. DD amoebie dan wel biogeneen bij vooralsnog onduidelijke focus blijft overeind.

Pragmatische behandeling zoals elders ingesteld zal naar alle waarschijnlijkheid tot volledige curatie leiden.

Progressive, painful, a.o. paromomycin, six weeks i.v. with meropenem

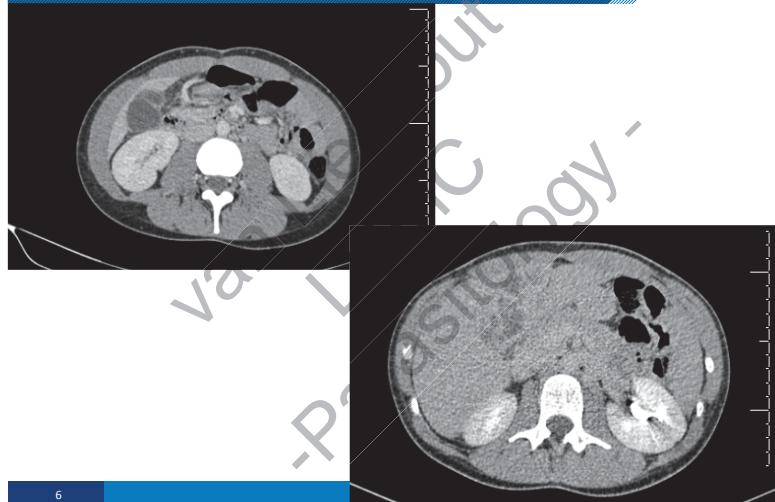
Referred to HZK

↑Liver enzymes, high eosinophilia

- 04-10-2016: Eosinophils count abs  $4.09 \times 10^9/L$  (40%)
- 23-11-2016: Eosinophils count abs  $1.62 \times 10^9/L$  (26%)

*Fasciola hepatica*? => serum to LUMC

22 August 2016



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## Case 2 – clinical background

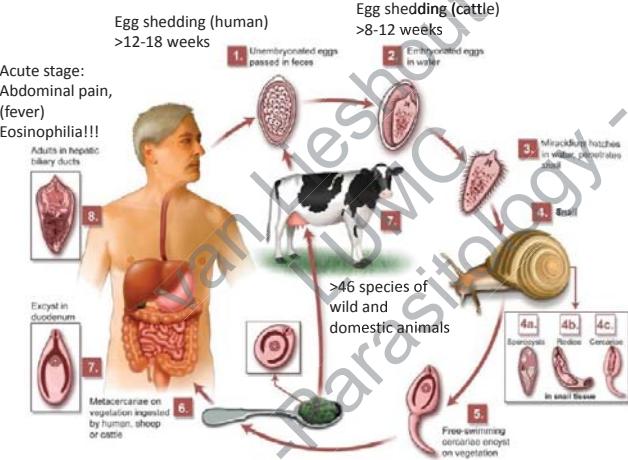
Dutch Female, DOB 11-02-1988

02-12-2016 treatment started with Triclabendazole

2x 12 mg/kg in 24 hrs

Follow-up .....

## *Fasciola hepatica / F. gigantica*



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<http://www.md-health.com/Fasciola-Hepatica-Life-Cycle.html>

## Fasciola hepatica / F. gigantica

Chronic stage:

Adult worm (3.5 cm/7.5 cm) => bile ducts

Latent => cholecystitis and cholangitis

Liver fibrosis

Possibly liver cancer

Machicado et al., 2016 PLoSNTD



2-17 million?

WHO: 2.4 M

>75 countries

(Fg only Africa, Asia)



- NTD
- EID

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## Food-borne trematode infections: >100 species infect humans

**Table 2** Habitat, infection sources, number of infections, population at risk, and treatment of choice of major food-borne trematodes and their underlying diseases

Species	Habitat	Source of infection	Number of infections ( $10^6$ )	At-risk population ( $10^6$ ) <sup>a</sup>	Treatment (dose)
<i>Clonorchis sinensis</i>	Liver	Freshwater fish	601	601	Praziquantel (3 x 25 mg/kg for 2 days or single dose of 40 mg/kg)
<i>Opisthorchis</i> spp.	Liver	Freshwater fish	11.2	79.8	Praziquantel (3 x 25 mg/kg for 2 days or single dose of 40 mg/kg)
<i>Paragonimus</i> spp.	Lung	Freshwater crabs, crayfish, wild boar meat	20.7	292.8	Praziquantel (3 x 25 mg/kg for 2 days)
Echinostomatidae	Intestine	Freshwater fish, frogs, mussels, snails, tadpoles	Not known	Not known	Praziquantel (single dose of 25 mg/kg)
<i>Gymnophalloides secolii</i>	Intestine	Oysters	Not known	Not known	Praziquantel (single dose of 10 mg/kg)
Heterophyidae	Intestine	Freshwater fish	Not known	Not known	Praziquantel (single dose of 25 mg/kg)
<i>Fasciola</i> spp.	Liver	Freshwater vegetables, contaminated water	2.4-17	2.4-17	Triclabendazole (single dose of 10 mg/kg or 20 mg/kg in two split doses within 12-24 h)

Toledo et al (2011) – Current status of food-borne trematode infections  
Eur J Clin Microbiol Infect Dis

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## Treatment of fascioliasis

### Triclabendazole

- Resistance described in sheep (o.a.Gordon et al., (2014); Veterinary Record)
- Several case reports of human failure, o.a. Netherlands

#### Key Figure

Global Distribution of Reports of Triclabendazole Resistance (TCBZ-R) in Livestock and Humans 1995–2015



Trends in Parasitology, June 2016, Vol. 32, No. 6

Figure 1. Grey, 1 reported case of TCBZ-R; purple, 2–4 reported cases; yellow, >5 reported cases of TCBZ-R. Hatched lines indicate reported human cases of TCBZ-R.

## Treatment of fascioliasis

### Triclabendazole

- Resistance described in sheep (o.a.Gordon et al., (2014); Veterinary Record)
- Several case reports of human failure, o.a. Netherlands

### Alternatives

Not well documented and/or not registered for human use

- Albendazole for >12 weeks adult worms
- Artesunate (4 mg/kg/d, daily for 10 days)
- Nitazoxanide (500 mg bd for 7 days)
- Bithionol (not registered for human use)
- several combinations of triclabendazole and other anti-helminthics
- less effective for acute stage infection
- no good diagnostic tools to monitor in humans

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## Microscopy “gold” standard



*Clonorchis sinensis*  
(Chinese or oriental liver fluke)  
≈ 27-35 µm x 11-20 µm



*Opisthorchis viverrini*  
(Southeast Asian liver fluke)  
*O. felineus* (cat liver fluke)  
≈ 19-30 µm x 10-20 µm



*Fasciola hepatica*  
(sheep liver fluke)  
≈ 130-150 µm x 60-90 µm



## Serology for trematodes

Early diagnosis

Higher sensitivity/specificity

Exclude passage of eggs

Follow-up after treatment

Published\* *Fasciola*:

- Commercial ELISA (E/S), Sens/Spec
- Recombinant Ag

New developments (veterinarian):

- CAg detecting tests, intensity depending
- POC-test for Ab, milk screening



\*) Reviews: Keiser et al., (2010) *Curr Opin Infect Dis* 23:513-520

Cabada and White (2012) *Curr Opin Infect Dis* 25:518-522

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## Serology for trematodes

Early diagnosis

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Exclude passage of eggs

Follow-up after treatment

LUMC: *Fasciola* in-house test

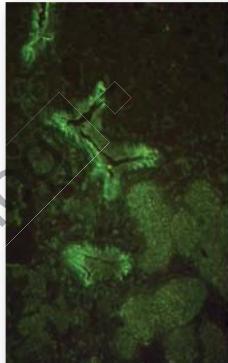
ELISA (E/S) IgG <1:32

IFA (aw-RF-gut) IgG <1:40

↓ cross-reactivity *Schistosoma*

Exchange Basel (n=20); 85% concordance  
(2 inconclusive/negative; 1 positive/negative)

LUMC: Source of antigen?



## Source of antigen – LUMC serology



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## Serology for trematodes

<2008: Assumed cross-reactivity for other trematodes (?)

2008: 29 sera of proven *Clonorchis* infections Korea (egg positive and/or specific *Clonorchis* serology positive)

Most of these sera negative or +/- when tested at LUMC *Fasciola* serology



## LUMC *Fasciola* serology

### 2001-2016 – 21 serology positive cases

N=4 proven cases: egg positive & eosinophilia

Serology IFA and EIA positive

N=7 highly suspected cases (including 2 presented here)

Serology IFA and EIA positive

N=4 *Opisthorchis/Clonorchis* cases (n=3 PCR proven)

Serology IFA or EIA positive; except *Clonorchis* case => serology negative

N=6 clinically indecisive cases or no information

Serology IFA and/or EIA positive, several just above cut-off

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## Conclusions

Limitation of small numbers of proven *Fasciola* cases

Serology, in particular for acute phase

- Follow-up after treatment (more cases need)
- Not clear-cut what serology does for non-*Fasciola* Trematodes

PCR – human diagnosis? How sensitive? Acute stage? Stool?

(European) collaboration on diagnosis of human food-born trematodes?

Need for alternative treatment

- treatment failure of Triclabendazole
- combination therapy?

## Thank you!



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