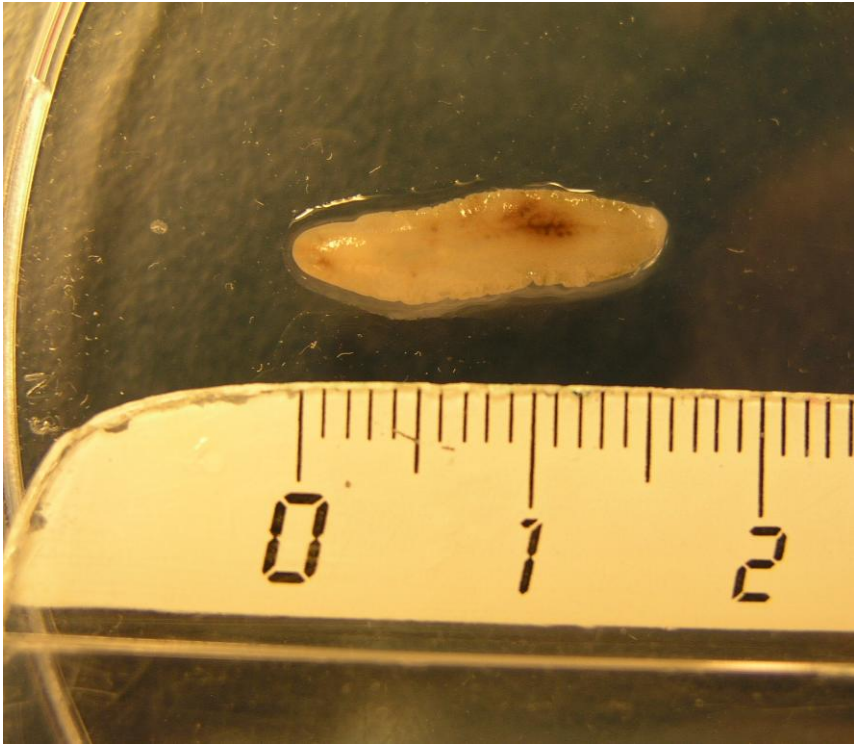


# What comes out?

STEVEN VAN DEN BROUCKE





# Woman, 73y

- Sept 2021: Referred by infectiologist. Yearly check-up Okt 2020: high eosinophilia.
- Medical history: 2014 Colon carcinoma -- > partial colectomy
- Filaria serology pos and heterogenous liver lesions: R/ DEC and doxycycline in January '21.
- 7/2021: due to persistent eosinophilia and disease activity on PET, treatment with Ivermectin and Albendazole. Also steroids.
- Since Dec 2020: now and then pain right upper abdomen: 'stitching'
- Itch on head, hear loss ++
- No rash
- Vision blurred, cataract since return
- Lived in Rwanda 1981 - 1989, then Burundi till 1996, then Ivory Coast till 1998
- Then Rwanda till July 2020
- Teacher in Belgian School. Husband architect.

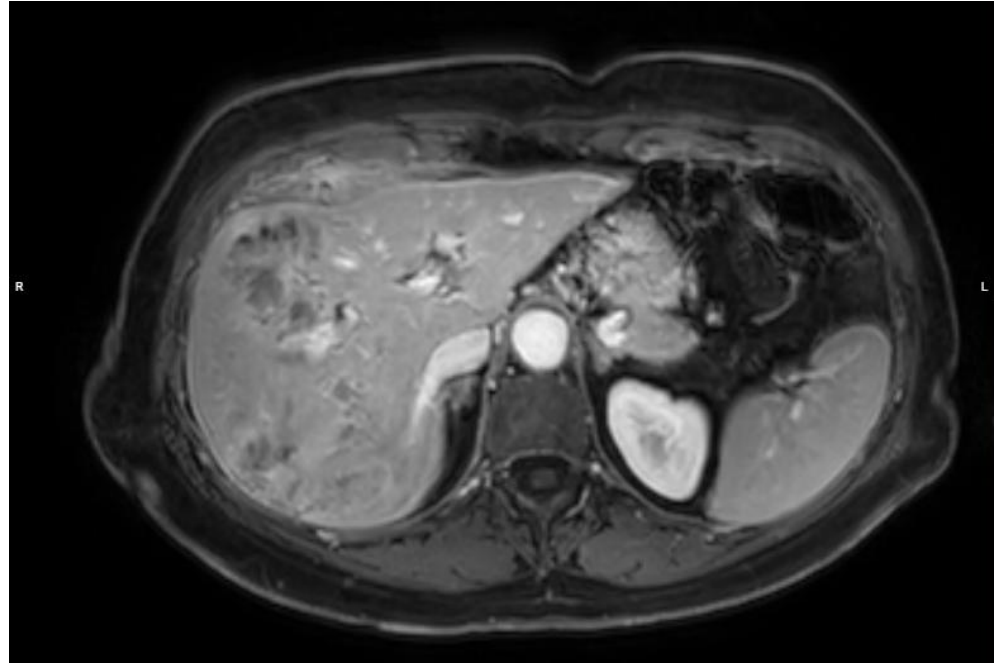
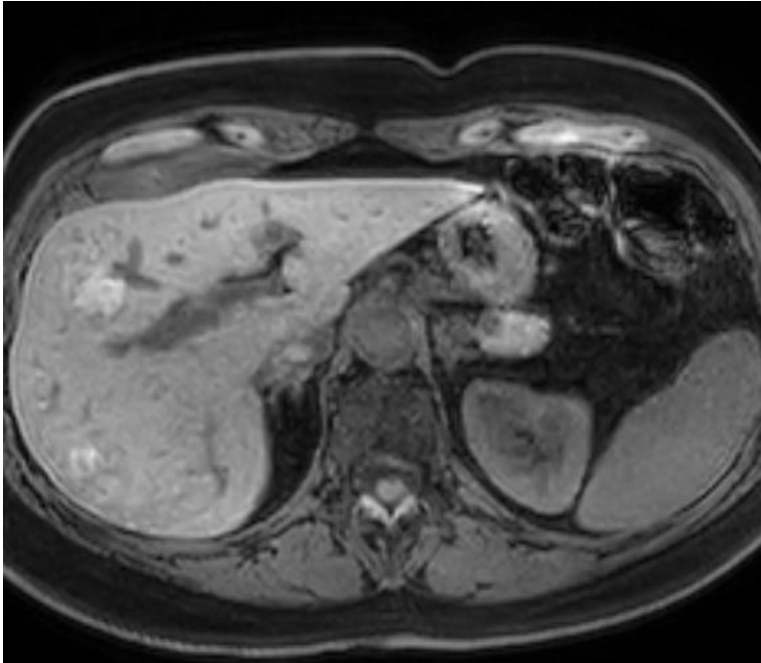
Current treatment:  
Pravastatine 40 mg 1x1  
Naproxen PRN



# Lab results

Witte bloedcellen	<b>+ 11.9</b>	10.E9/l	4.5 - 11.0
Neutrofielen	<b>- 36</b>	%	40 - 75
Eosinofielen	<b>+ 35</b>	%	0 - 6
Basofielen	1	%	0 - 1
Lymfocyten	21	%	15 - 40
Monocyten	7	%	4 - 12
Neutrofielen absoluut	4.3	10.E9/l	1.8 - 7.7
Eosinofielen absoluut	<b>+ 4.2</b>	10.E9/l	0.0 - 0.5
Basofielen absoluut	0.1	10.E9/l	0 - 0.2
Lymfocyten absoluut	2.5	10.E9/l	1 - 4.8
Monocyten absoluut	0.8	10.E9/l	0.0 - 0.8

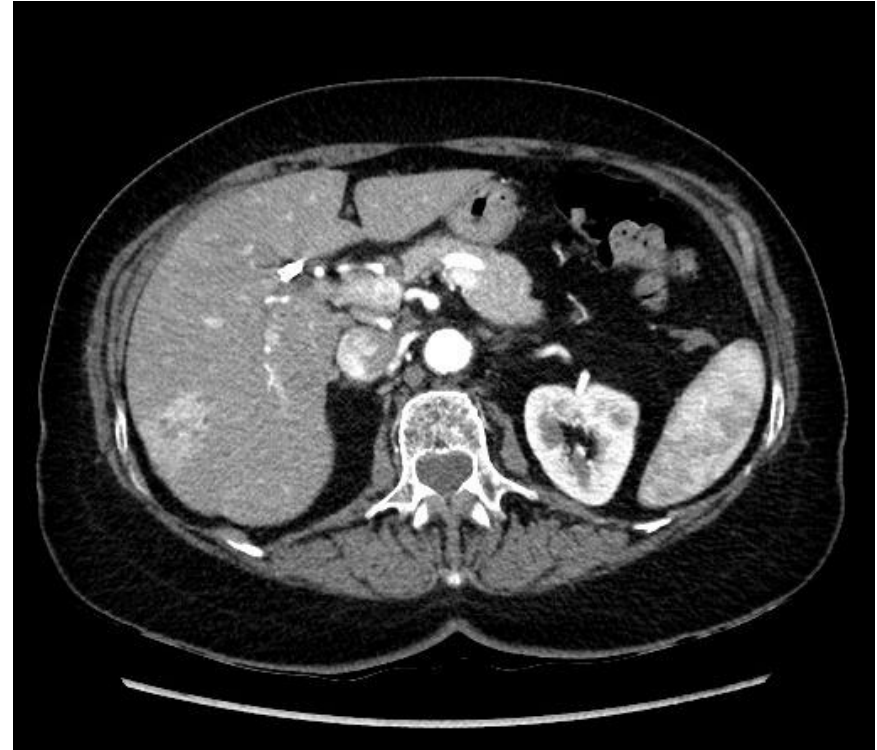
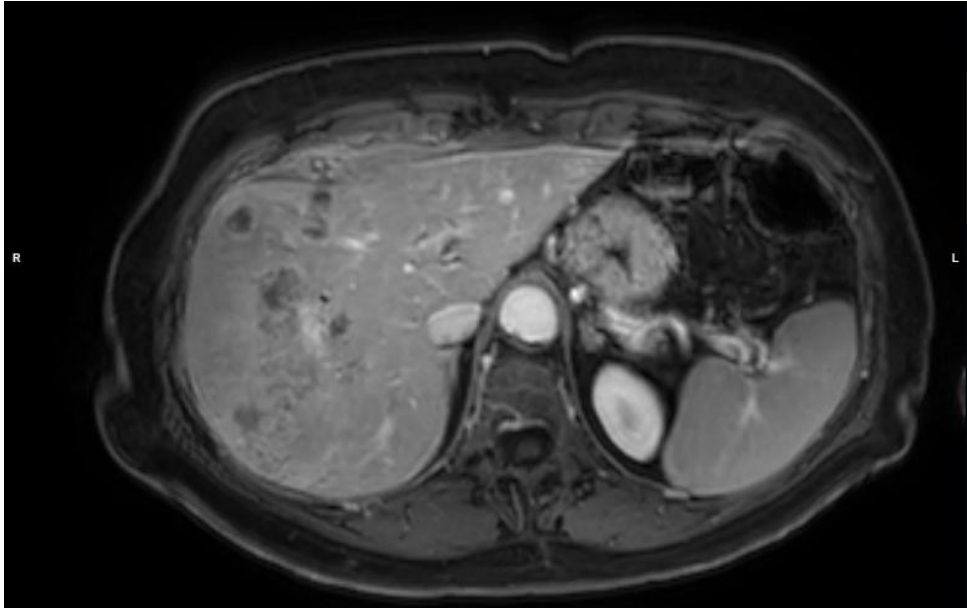
# MRI Liver



Very non-specific presentation of alterations at the level of segments 7 and 8 of the liver. There are spontaneous intensities that are suggestive of **protein-rich fluid or remnants of limited bleeding or blood pooling**. Difficult differential diagnosis of these **nonspecific** findings. The possibility of **peliosis hepatis** or **sequels of microbleeds** with a regenerative conversion of the liver parenchyma and accompanying perfusion disorders. Underlying malignancy seems unlikely.

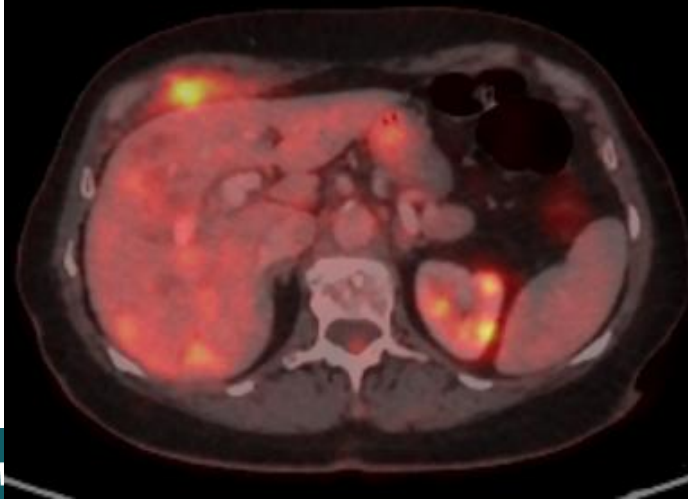
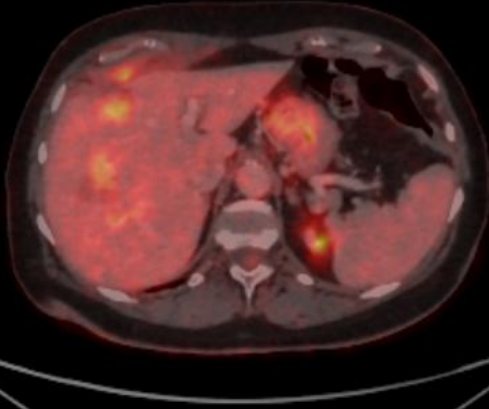
Furthermore, we notice an **alteration anterior of the liver** parenchyma that best suits a **semi-recent hematoma** formation.

## MRI and CT



## PET-CT

Hypermetabolic non-delineation, rather infiltrative image, in segments 7 and 8 of the liver. Pre-hepatically, there is a hypermetabolic structure, not clear from which structure emanates: pleura? liver capsule? added? Focal hypermetabolism projecting onto a small intestine structure against the right kidney just caudal to the pancreatic head, with no apparent correlate. Still physiological? Elsewhere no pathological hypermetabolic foci in the scanned area.



# Aspiration and biopsy pre-hepatic structure

## Cytology

- Neutrophils, necrotic and purulent material, few RBC, iron pigment deposition. No malignant cells.

## Biopsy

- Muscle- and connective-tissue. Several blood vessels. Discrete chronic inflammatory infiltrate. Iron pigment deposition. One giant cell with hematoidine pigment included: old bleeding. No malignancy.
- Ziehl-Nielsen, Grocott, PAS: neg

## Further anamnesis

- 2-3 Years ago, raw watercress in Belgium. Never in Africa.
- In Rwanda raw 'kapitein' from lake Victoria



- Daughter Bilharziasis 7 y ago.

## Further testing?

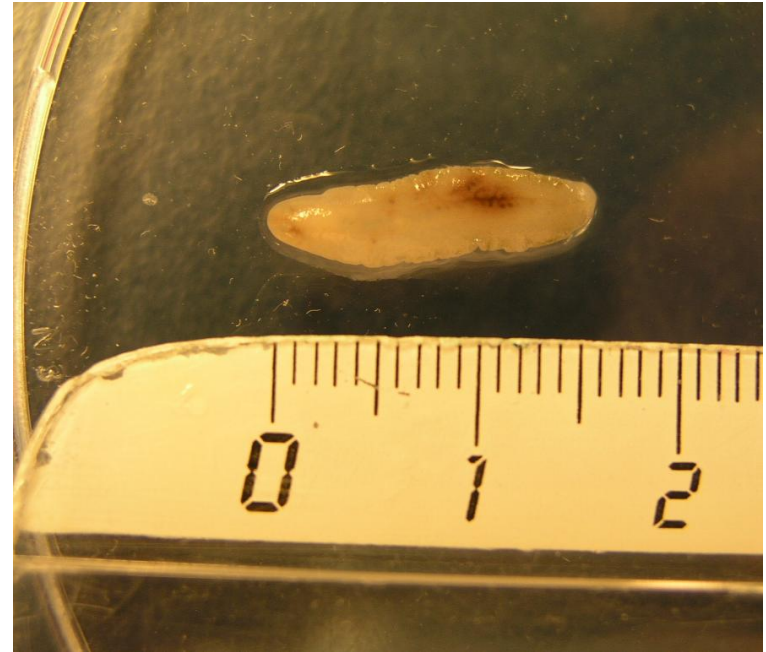
- Stools: O/P neg
- PCR *Fasciola* on stool: negative
- Fasciola serology (ELI.H.A Distoma, indirect hemagglutination):  
indetermined result
- **What would you do?**

## Further evolution

- Advised to give a trial treatment with: ...
- Triclabendazole 250 mg 1x3 tabs/d for 2 days.
  - Response to treatment = diagnostic criteria for fascioliasis
- But: pain decreased and ultrasound of the liver normalized; eosinophilia normalized (cave: had also had a steroid course)
- Nice 'thinking exercise'

## Further evolution

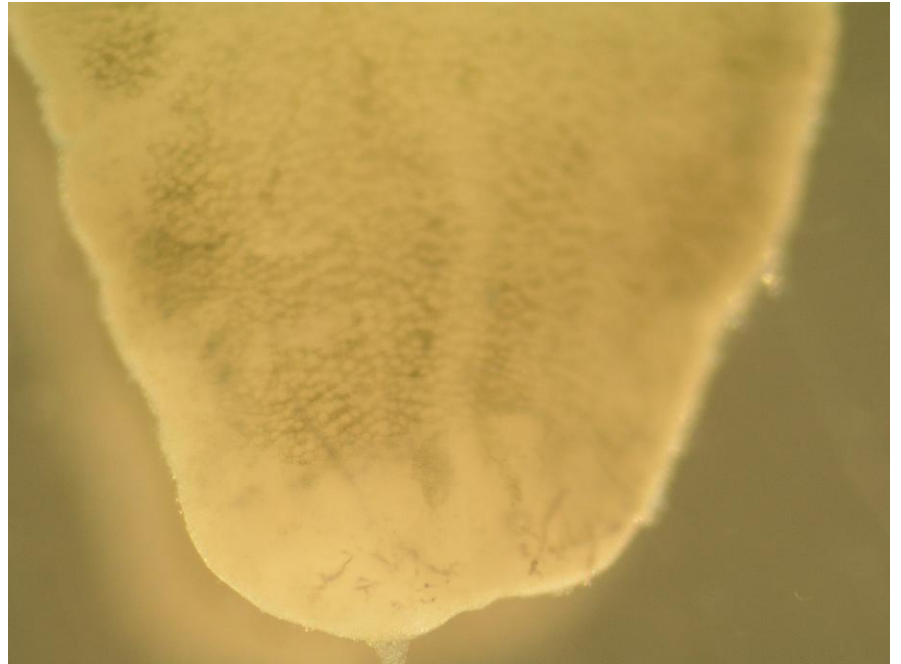
- Pain came back -- > physiotherapy
- During physio: worm comes out of the skin under the left breast



# Microscopy



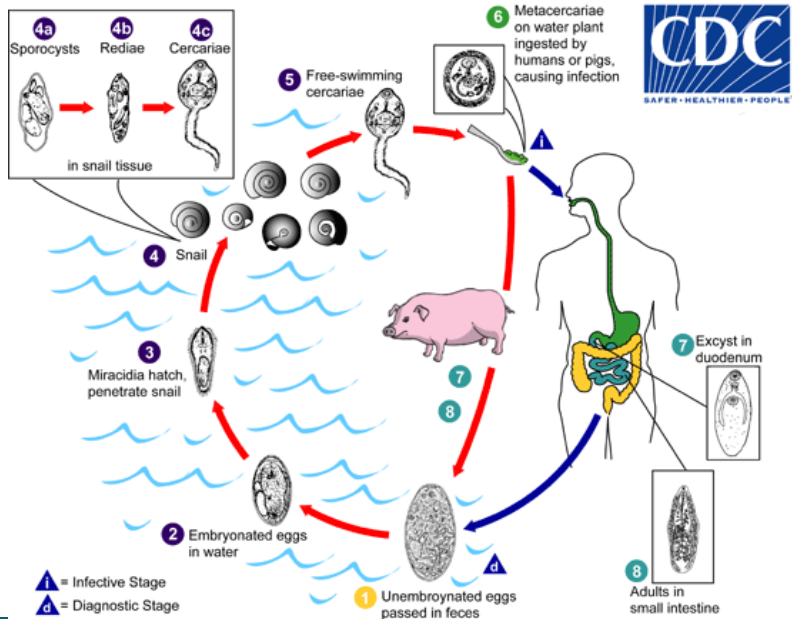
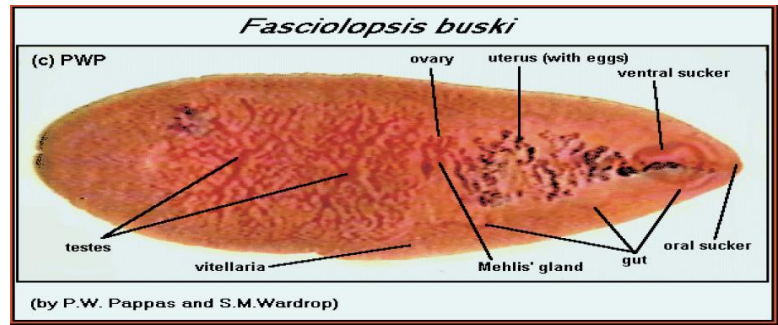
# Microscopy



Your guess?

# Fasciolopsis (*Fasciolopsis buski*)

■ Our worm more prominent cephalic cone



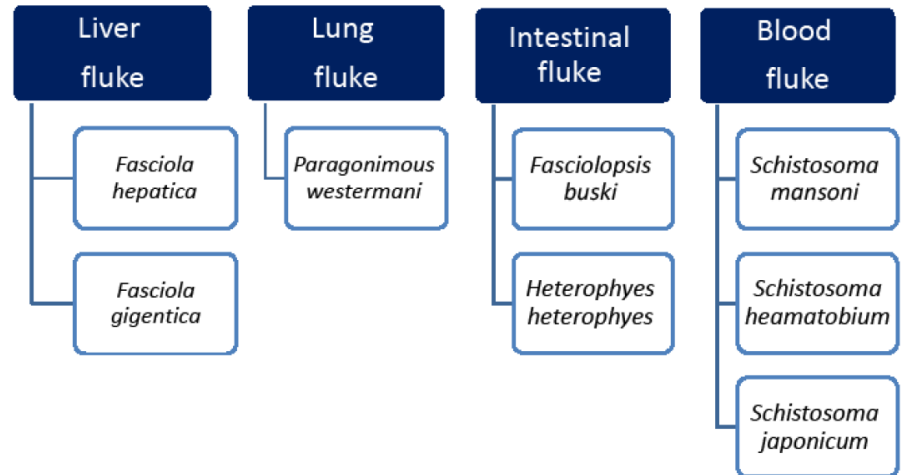
- Abdominal pain and diarrhea possible 1 or 2 months after infection
- Heavy infections: **obstruction, abdominal pain, nausea, vomiting, and fever.**

# Paragonimus

- Most likely juvenile stage: too big for *Dicrocoelium* or *Paragonimus*



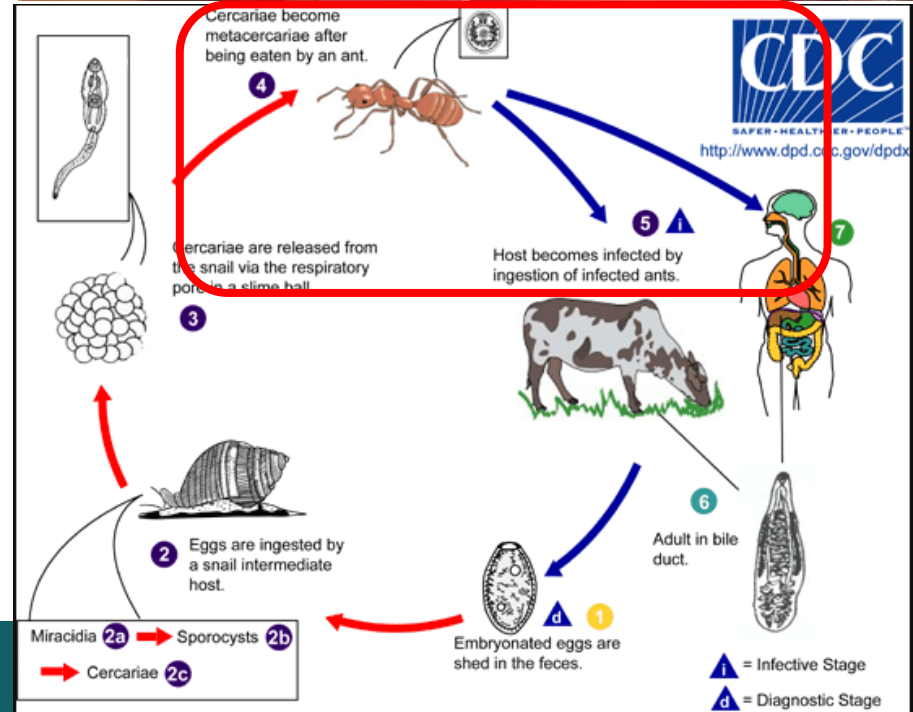
## Medically Important flukes



# Dicrocoelium dendriticum



- In more intense infections, symptoms may include cholecystitis, liver abscesses, and generalized gastrointestinal/abdominal distress. Occasional cases involving flukes in subcutaneous masses have been reported



# Gastrodiscoides hominis

- Too elongated for Gastrodiscoides
- Mostly no symptoms
- Diarrhea, fever, abdominal pain, colics



*Fasciolopsis buski*



*Artyfechinostomum sufrartyfex*



*Gastrodiscoides hominis*



*Fasciola gigantica*



*Opisthorchis noverca*



*Eurytrema pancreaticum*



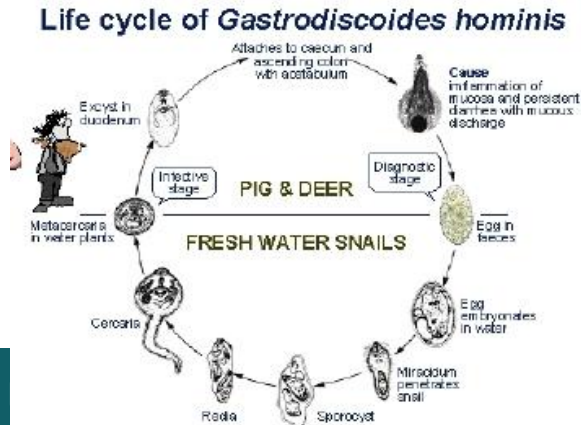
*Clinostomum complanatum*  
(excysted metacercaria)



*Paragonimus westermani*  
(Adult\* & excysted metacercaria)

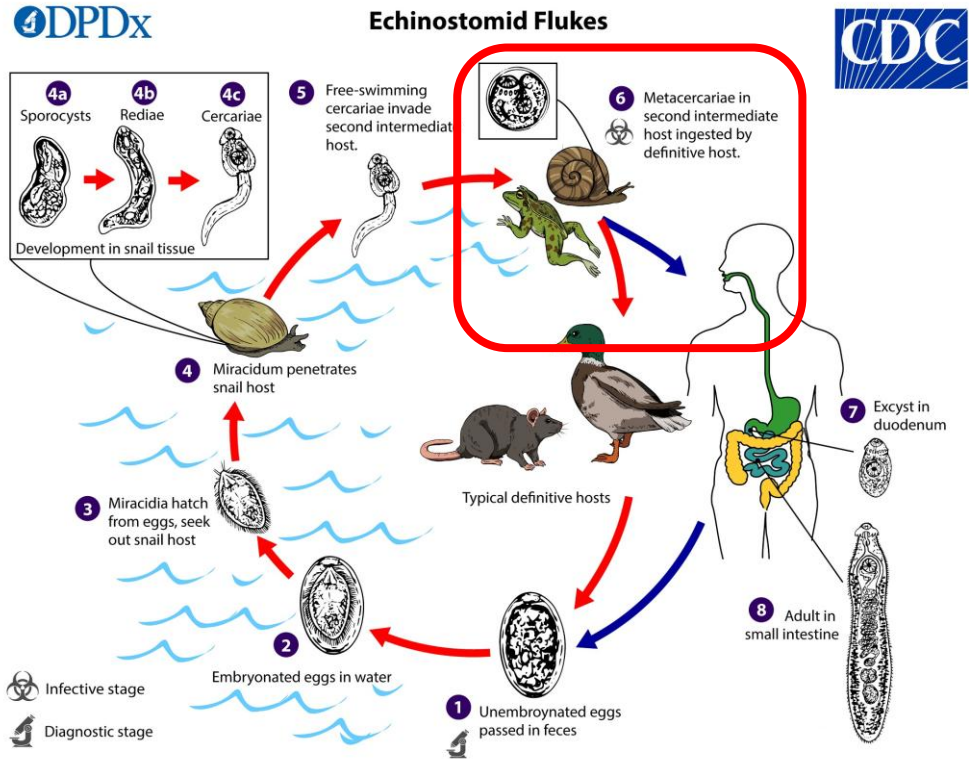


*Procerovum*  
sp.



# Echinostoma

- Too long, too wide
- No armed "collar" around the oral suction cup
- Intestinal ulceration and inflammation



## *Fasciola hepatica*

- “Wider shoulders”: anterior part wide and becomes thinner towards the back



# *Fasciola gigantica*

## *Fasciola sp.* cont.

### *Fasciola hepatica*

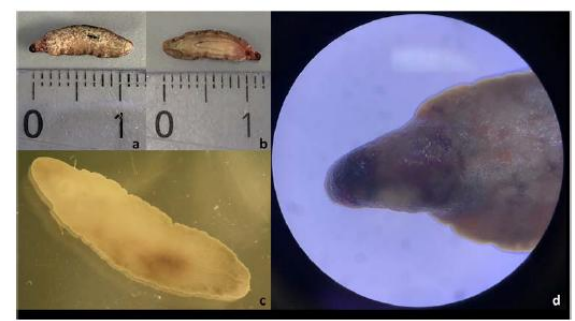


cephalic cone, 2 shoulders,  
converging margins, smaller  
in size

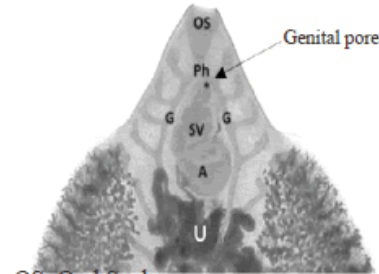
### *Fasciola gigantica*



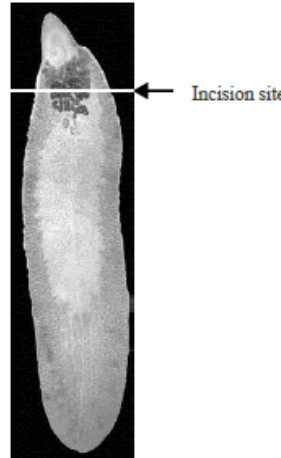
Less prominent  
shoulders,  
parallel  
margins, larger  
in size



**Figure 2.** Macroscopy and microscopy of the emerged worm. Macroscopic dorsal view (a) and ventral view (b) showing a worm measuring 12 mm in length. Microscopic view (c) demonstrating parallel margins lacking prominent shoulders with a pronounced cephalic cone (d), suggestive of juvenile stage of *Fasciola gigantica* (adult *F. gigantica* measures on average 25–75 mm in length).



OS: Oral Sucker  
Ph: Pharynx  
G: Main Ceca Of The Gut  
Sv: Seminal Vesicle  
A: Acetabulum Or Ventral Sucker  
U: Uterus Filled With Eggs



**Figure 1:** Location where eggs of the adult *Fasciola gigantica* were extracted for positive control.



# Quid skin penetration?

JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2007, p. 648–650  
0095-1137/07/\$08.00+0 doi:10.1128/JCM.01151-06  
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Vol. 45, No. 2

## Molecular Confirmation that *Fasciola gigantica* Can Undertake Aberrant Migrations in Human Hosts<sup>∇</sup>

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Pierre Dorny,<sup>5</sup> Thanh Giang Thi Nguyen,<sup>1</sup> and Donald P. McManus<sup>6</sup>

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Received 5 June 2006/Returned for modification 6 July 2006/Accepted 9 October 2006

**Two cases of aberrant migration by the liver fluke *Fasciola gigantica* in humans are reported. In both cases, subadult worms emerged through the skin. The identity of the worms was confirmed from their DNA sequences. This uncommon human pathogen might be more likely than *F. hepatica* to undertake aberrant migrations in humans.**

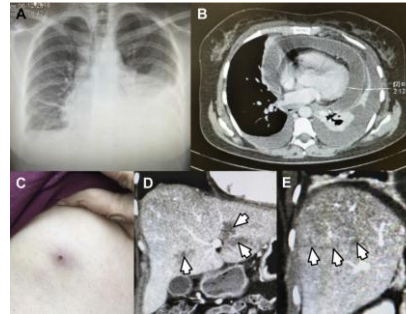


## Other extra-hepatic sites



# Other worms that might emerge through the skin?

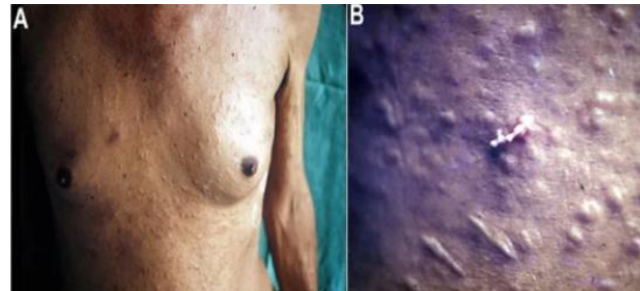
■ *Paragonimus*



■ *Gnathostoma*



■ Sparganosis



■ *Dracuncululus*, Guinea worm

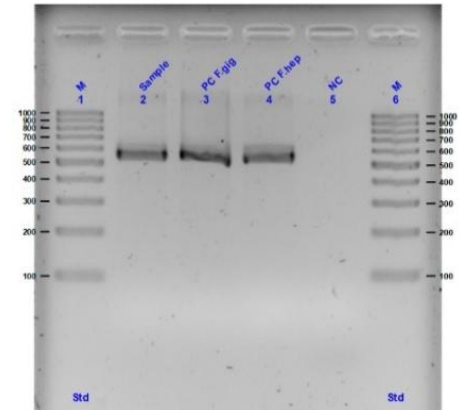


# Molecular testing

1. DNA extraction on the worm
2. ITS-2 (Internally transcribed spacer 2) = ribosomal RNA gene amplified with PCR
  1. 550 bp amplicon identified as *Fasciola* spp

**Fig. 1S:** 3 gels showing the PCR results. Outer lanes: 100-1000 bp DNA ladder, lanes 2: worm sample

**a. PCR for ITS-2:** the 550 bp band of the sample indicates *Fasciola* spp. positivity<sup>1</sup>, the positive controls are *F. gigantica* (*Fg*) (lane 3) and *F. hepatica* (*Fh*) (lane 4)



3. PCR product sent to BaseClear ([www.baseclear.com](http://www.baseclear.com)) for sequencing

## Sequence results

Africa: *F. gigantea* dominant

More temperate regions North and South and at higher altitude: *F. hepatica* and hybrids

```
TGCAAAGTGCATACTGCTTTGAACATCGACATCTTGAACGCATATTGCGGCCATGGGTTAGCCTGTGGCCACG
CCTGTCCGAGGGTCGGCTTATAAACTATCACGACGCCCAAAAAGTCGTGGCTTGGGTTTTGCCAGCTGGCGTG
ATCTCCTCTATGAGTAATCATGTGAGGTGCCAGATCTATGGCGTTTCCCTAATGTATCCGGATGCACCCTTGTC
TTGGCAGAAAGCCGTGGTGAGGTGCAGTGGCGGAATCGTGGTTTAATAATCGGGTTGGTACTCAGTTGTCAG
TGTGTTGGCGATCCCCTAGTCGGCACACTCATGATTTCTGGGATAATTCCATAACCAGGCACGTTCCGTTACTG
TACTTTGTCATTGGTTTGATGCTGAACTTGGTCATGTGTCTGATGCTATTTCATATAACGACGGTACCCTTCG
```

**Fig. 2S:** DNA amplicon sequencing result of the worm sample. T, C, T, T, -, A on positions 207, 231, 270, 276, 324 and 334 of the ITS-2-region

- Corresponded best with *F. gigantea* (C, T, T, -, A on positions 231, 270, 276, 324 and 334 of the ITS-2-region). But the T nucleotide on position **207** compatible with *F. hepatica*, hinting to the possibility of a *F. gigantea* × *F. hepatica* hybrid. However, it has been shown there is a marked geographical variation at this location for *F. gigantea* specimens, and thus the ITS-2 PCR may not allow for the differentiation of pure specimens from hybrids.

## *F. hepatica* vs *gigantica* : ITS-2

Table 2

Comparison of nucleotides at six variable positions in the ITS-2 sequences of *Fasciola* spp. from different geographical locations

Species	Geographical origin	Variable positions in ITS-2 sequence						Reference	-
		207	231	270	276	324	334		
<i>F. hepatica</i>	France	T	T	C	C	T	G	Present study	
	Sichuan	T	T	C	C	T	G	Present study	
	Australia	T	T	C	C	T	G	Adlard et al. (1993)	
	Japan	T	T	C	C	T	G	Itagaki and Tsutsumi (1998)	
	Uruguay	T	T	C	C	T	G	Itagaki and Tsutsumi (1998)	
	Korea	T	T	C	C	T	G	Agatsuma et al. (2000)	
	Hungary	T	T	C	C	T	G	Adlard et al. (1993)	
	New Zealand	T	T	C	C	T	G	Adlard et al. (1993)	
	Mexico	T	T	C	C	T	G	Adlard et al. (1993)	
<i>F. gigantica</i>	Guangxi	C	C	T	T	-	A	Present study	
	Japan	C	C	T	T	-	A	Itagaki and Tsutsumi (1998)	
	Malaysia	C	C	T	T	-	A	Adlard et al. (1993)	
	Indonesia	C	C	T	T	-	A	Itagaki and Tsutsumi (1998)	
	Korea	C	C	T	T	-	A	Agatsuma et al. (2000)	
	<i>Fasciola</i> sp.	Heilongjiang	T	T/C	C/T	C/T	T/-	G/A	Present study
	Korea	T/C	T/C	C/T	C/T	T/-	G/A	Agatsuma et al. (2000)	

# Thymine SNP at pos 791

specimens and sequences retrieved from GenBank (Table I). It revealed that 1 of the species determining variable sites, position 791, proved problematic for species determination in *F. gigantica*. While this site seems to be “T” consistently in *F. hepatica* specimens, it appears to vary geographically in *F. gigantica* flukes.

- Africa: mostly Thymine
- Asia: Cytosine

*Journal of Parasitology* 2020 106(2) 316–322  
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## Journal of Parasitology

journal homepage: [www.journalofparasitology.org](http://www.journalofparasitology.org)



**Table 1.** Comparison of nucleotides at 11 variable positions of the *ITS1/2* marker of *Fasciola* samples collected from Chad, Côte d'Ivoire, and Switzerland and sequences deposited in NCBI GenBank. Abbreviations: n/a, no sequence for this part. Y and W indicate heterozygous positions with nucleotides C/T and A/T, respectively.

Species	Origin*	n†	Variable positions in <i>ITS1/2</i> sequences											Accession no.
			18	108	202	280	300	791	815	854	860	911‡	918	
<i>Fasciola hepatica</i>	Switzerland§	1	C	A	C	T	C	T	T	C	C	T	G	MK321597-602
	France	1	C	A	C	T	C	T	T	C	C	T	G	JF708034
	Spain	1	C	A	C	T	C	T	T	C	C	T	G	JF708036
	Niger	5	C	A	C	T	C	T	T	C	C	T	G	AM850107 AM900370
	Tunisia	1	C	A	C	T	C	T	T	C	C	T	G	GQ231546
	Tunisia	1	C	A	C	T	C	T	T	C	C	T	G	GQ231547
	Iran	1	C	A	C	T	C	T	T	C	C	T	G	JF432072
	Egypt	17	n/a	n/a	n/a	n/a	n/a	T	T	C	C	T	G	AB553720-36
	Egypt	67	C	A	C	T	C	n/a	n/a	n/a	n/a	n/a	n/a	LC076128-48 LC076150-52 LC076154-96
	Egypt	1	C	A	C	T	C	n/a	n/a	n/a	n/a	n/a	n/a	HE972273
<i>Fasciola gigantica</i>	Saudi Arabia	1	C	A	C	T	C	n/a	n/a	n/a	n/a	n/a	n/a	JF708026
	China	1	C	A	C	T	C	T	T	C	C	T	G	AB207145
	Japan	1	C	A	C	T	C	n/a	n/a	n/a	n/a	n/a	n/a	AM900371
	Niger	13	T	T	T	A	T	C	C	T	T	—	A	AM850108
	Chad§	26	T	T	T	A	T	T	C	T	T	—	A	MK321603-643
	Ivory Coast§	15	T	T	T	A	T	T	C	T	T	—	A	MK321603-643
	Burkina Faso†	1	T	T	T	A	T	T	C	T	T	—	A	AJ853848
	Kenya	1	T	T	T	A	T	n/a	n/a	n/a	n/a	n/a	n/a	EF612472
	Kenya	1	n/a	n/a	n/a	n/a	n/a	T	C	T	T	—	A	EF612484
	Mauritania	2	T	T	T	A	T	T	C	T	T	—	A	HQ197358, HQ197359
	Zambia	6	T	T	T	A	T	n/a	n/a	n/a	n/a	n/a	n/a	AB207142
	Zambia	6	n/a	n/a	n/a	n/a	n/a	T	C	T	T	—	A	AB010976
	Iran	1	T	T	T	A	T	T	C	T	T	—	A	JN828953
	Egypt	1	T	T	T	A	T	T	C	T	T	—	n/a	KF425321
	Egypt	9	n/a	n/a	n/a	n/a	n/a	T	C	T	T	—	A	AB553694-702
	Saudi Arabia	1	T	T	T	A	T	n/a	n/a	n/a	n/a	n/a	n/a	HE972274
	Indonesia	1	T	T	T	A	T	n/a	n/a	n/a	n/a	n/a	n/a	AB207143
	Thailand	1	T	T	T	A	T	n/a	n/a	n/a	n/a	n/a	n/a	AB207144
	Indonesia: Thailand	1	n/a	n/a	n/a	n/a	n/a	C	C	T	T	—	A	AB207149
	China	3	T	T	T	A	T	C	C	T	T	—	A	JF496709, JF496714 KF543340
Japan	1	T	T	T	A	T	n/a	n/a	n/a	n/a	n/a	n/a	AB207146	
Japan	2	n/a	n/a	n/a	n/a	n/a	C	C	T	T	—	A	AB207151, AB207152	
Vietnam	1	n/a	n/a	n/a	n/a	n/a	C	C	T	T	—	A	EU260063	
South Korea	1	n/a	n/a	n/a	n/a	n/a	C	C	T	T	—	A	HQ821455	
India	1	n/a	n/a	n/a	n/a	n/a	C	C	T	T	G	A	KJ720004	
China	1	n/a	n/a	n/a	n/a	n/a	T	T	C	C	T	G	AJ557570 clone I	
China	1	n/a	n/a	n/a	n/a	n/a	T	C	T	T	—	A	AJ557571 clone II	
China	1	Y	W	Y	W	Y	n/a	n/a	n/a	n/a	n/a	n/a	AJ628428	
Egypt	2	C	A	C	T	C	n/a	n/a	n/a	n/a	n/a	n/a	LC076149, LC076153	
Egypt	2	Y	W	Y	W	Y	n/a	n/a	n/a	n/a	n/a	n/a	AB553691, AB553692	
Egypt	1	n/a	n/a	n/a	n/a	n/a	T	Y	Y	Y	T	R	AB553737	
Egypt	1	n/a	n/a	n/a	n/a	n/a	T	Y	Y	Y	—	R	AB553738	
Saudi Arabia	1	C	A	T	A	A	n/a	n/a	n/a	n/a	n/a	n/a	HE972275	
Japan	1	Y	W	Y	W	Y	n/a	n/a	n/a	n/a	n/a	n/a	AB207147	
Japan	1	n/a	n/a	n/a	n/a	n/a	Y	Y	Y	Y	T	R	AB207153	
South Korea	7	Y	W	Y	W	Y	n/a	n/a	n/a	n/a	n/a	n/a	AB211237	
Chad§	1	Y	W	Y	W	Y	T	Y	Y	Y	—/T	R	MK321644-645	

\* Origin as reported in GenBank.

† Number of samples investigated.

‡ Dashes (—) = Deletion.

§ Specimens from this study.

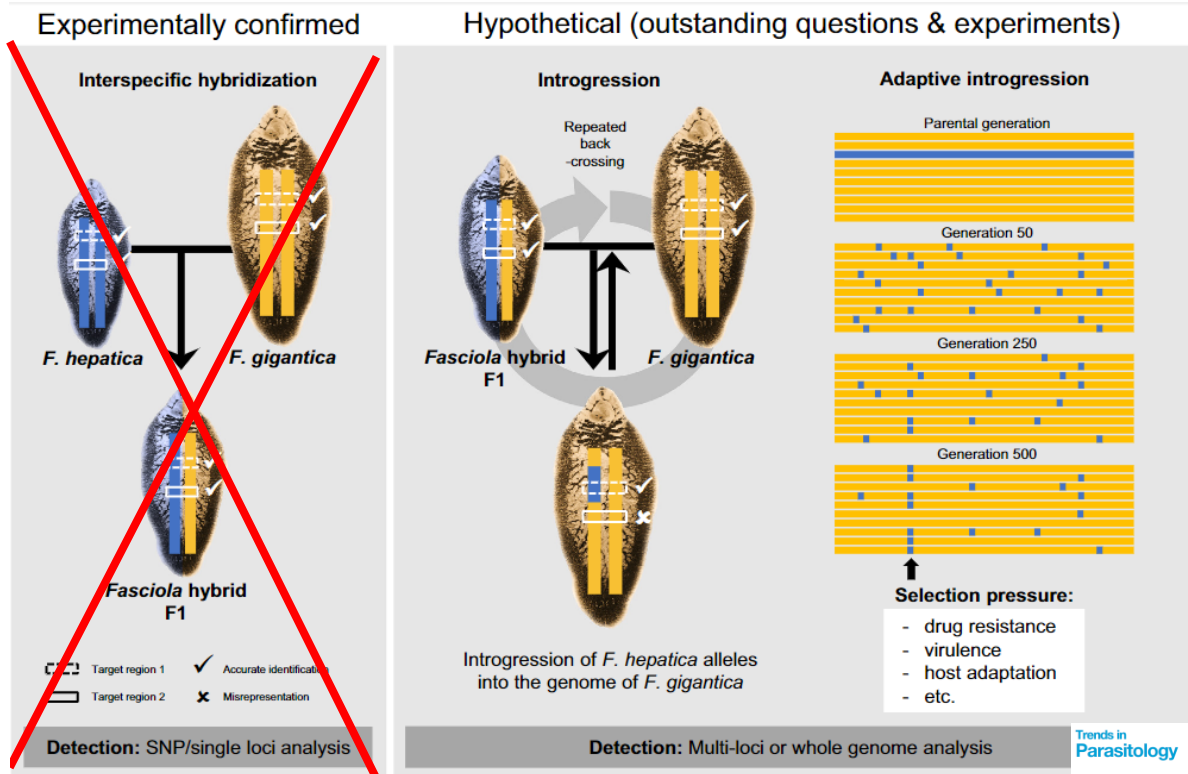
|| One entry in GenBank, but appears to be consensus sequence from numerous specimens from the publication.

# Site 791 for this specimen is reported as “C” in Liu et al. (2014), but as “T” in Amor et al. (2011a,b), it is reported as “T” in GenBank.

# Quid hybrid?

- Africa: *F. gigantica* dominant
- More temperate regions North and South and at higher altitude: *F. hepatica* and hybrids

- Hybride 1st generation: heterozygote profile for all SNP's expected
- But was clearly homozygote

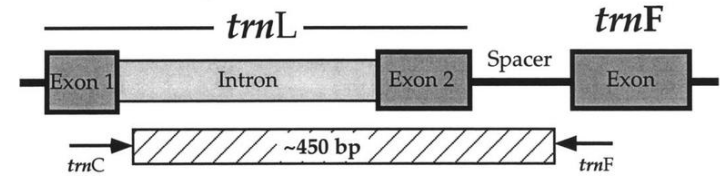
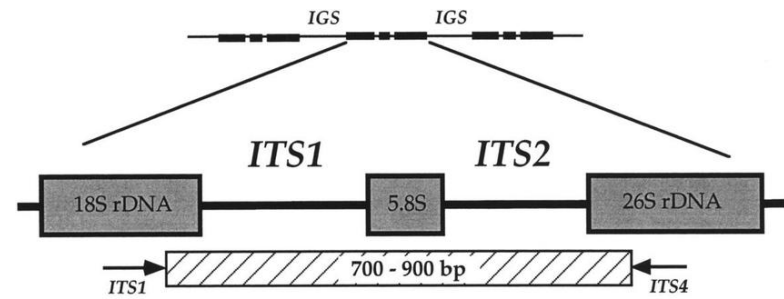
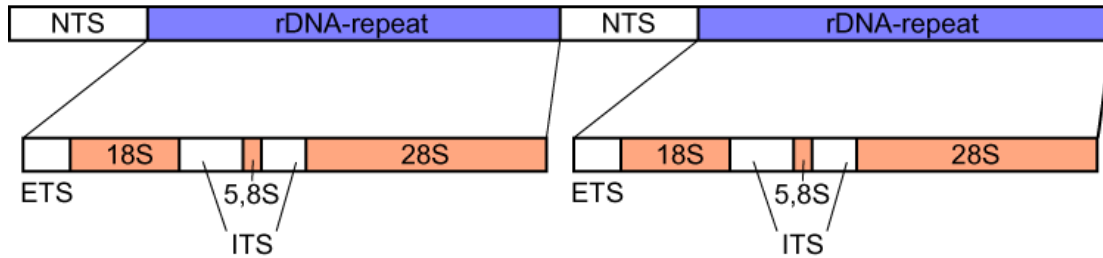


Hybride of further generation? By crossing back (of the hybrid) with *F. gigantica*, certain SNPs of the hepatic genome could have been preserved by introgression

Table 2  
Comparison of nucleotides at six variable positions in the ITS-2 sequences of *Fasciola* spp. from different geographical locations

Species	Geographical origin	Variable positions in ITS-2 sequence						Reference
		207	251	270	276	324	334	
<i>F. hepatica</i>	France	T	T	C	C	T	G	Present study
	Sichuan	T	T	C	C	T	G	Present study
	Australia	T	T	C	C	T	G	Adlard et al. (1993)
	Japan	T	T	C	C	T	G	Itugaki and Tsunoda (1998)
	Ungway	T	T	C	C	T	G	Itugaki and Tsunoda (1998)
	Korea	T	T	C	C	T	G	Aganuma et al. (2000)
	Hungary	T	T	C	C	T	G	Adlard et al. (1993)
	New Zealand	T	T	C	C	T	G	Adlard et al. (1993)
	Mexico	T	T	C	C	T	G	Adlard et al. (1993)
<i>F. gigantica</i>	Guangxi	C	C	T	T	-	A	Present study
	Japan	C	C	T	T	-	A	Itugaki and Tsunoda (1998)
	Malaysia	C	C	T	T	-	A	Adlard et al. (1993)
	Indonesia	C	C	T	T	-	A	Itugaki and Tsunoda (1998)
	Korea	C	C	T	T	-	A	Aganuma et al. (2000)
<i>Fasciola</i> sp.	Heilongjiang	T	T	C	C	T	G	Present study
	Sri Lanka	T	T	C	C	T	G	Aganuma et al. (2000)

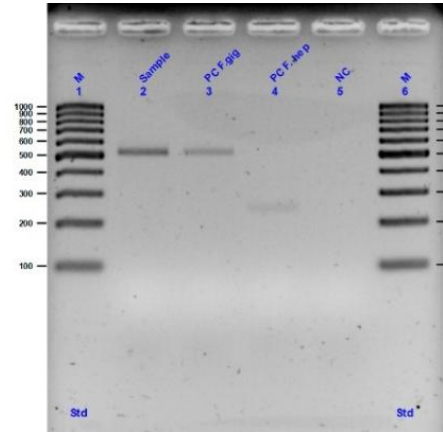
# ITS (internal transcribed spacer)



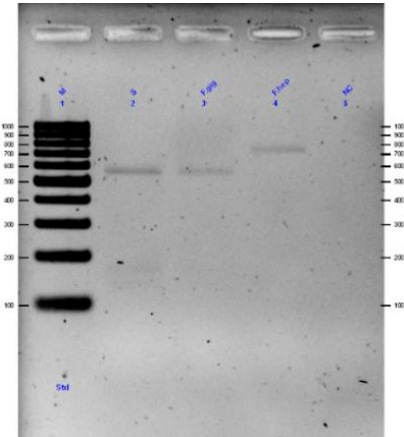
ITS1 is a spacer DNA located between 18S and 5.8S rRNA genes in eukaryotes, while ITS2 is a spacer DNA located between 5.8S and 28S rRNA genes in eukaryotes

# Definite species determination

- Multiplex PCR for phosphoenolpyruvate carboxykinase genes (*pepck*) and a PCR-Restriction Fragment Length Polymorphism (RFLP) for DNA polymerase delta (*pold*), both nuclear genes
- Band patterns for both *pepck* and *pold* were **compatible with *F. gigantea***, **definitively excluding the possibility of hybridization with *F. hepatica***



**Multiplex PCR for *pepck*:** the 509 bp band of the sample is specific for *Fg*, the *Fg* and *Fh* controls show a band of 509 and 241 bp respectively (lanes 3 and 4)



**PCR-RFLP for *pold*,** the 544 and 164 bp bands of the sample are specific for *Fg*, the positive control is *Fg* (the 164 bp band not visible); a *Fh* control shows a *Fh* specific 708 bp band



Short communication

Novel methods for the molecular discrimination of *Fasciola* spp. on the basis of nuclear protein-coding genes



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- **Sympatry** is the occurrence of different types of organisms or populations in the same area.
- **Introgression** is the gene flow of genes from the gene pool of one species to that of another species by repeated recrossing of an interspecific mongrel with one of its parent species.
- Introgression is a form of hybridization, but hybridization is usually used when two whole haploid genomes come together in an offspring (which then has 50% of its genetic material from one parent and 50% from the other parent). Introgression is when it comes to certain genes or phenotypes that end up in another genome through natural or intended crossbreeding.



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Clinical Pearls

## Juvenile *Fasciola gigantica* emerging through the skin in a returning traveller

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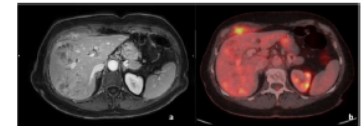
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**Key words:** Fasciola, *Fasciola gigantica*, ectopic migration, liver lesions, skin emergence

### Case presentation

An asymptomatic 73-year-old Belgian woman, with a history of cholecystectomy and colon cancer in remission 6 years earlier, was found with a high eosinophil count (3890/ $\mu$ l; 40% of the total leucocytes) during a check-up, 3 months after resettlement in Belgium. The patient lived in Rwanda, Burundi, Ivory Coast and Rwanda again for 9, 7, 2 and 22 years, successively. During the subsequent workup, liver imaging (Figure 1) showed multiple hypermetabolic nodular liver lesions predominantly in the right liver lobe and a hypermetabolic structure between the liver and abdominal wall. A biopsy of the pre-hepatic structure demonstrated a chronic inflammatory infiltrate without eosinophils



**Figure 1.** MRI and PET-CT of the liver. (a) MRI of the liver with intravenous gadolinium showed T1 intensities with heterogeneous contrast uptake in segments 7 and 8 and an elongated heterogeneous zone anterior of the liver with T1 hyperintensities. (b) PET-CT described a hypermetabolic diffuse infiltration in segments 7 and 8 and a pre-hepatic hypermetabolic structure.

# Gourmet liver sandwich... would you like flukes with that?!





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