

SKML deelnemersdag Parasitologie

Trippenhuis te Amsterdam
6 maart 2008

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Fecesonderzoek op parasieten

Titia Kortbeek
Theo Mank

- overzicht 2007
- 1x protozo; 1x worm
- overwegingen bij microscopisch onderzoek
- niet microscopische technieken... serologie / ELISA / PCR
- discussie / toekomst

Overzicht 2007

Helminthen

Ascaris lumbricoides
Hymenolepis nana
Opisthorchis spp
Trichuris trichiura

Protozoa

Blastocystis hominis
Chilomastix mesnili
Entamoeba coli
Entamoeba histolytica/dispar
Iodamoeba butschlii

Een protozo!!

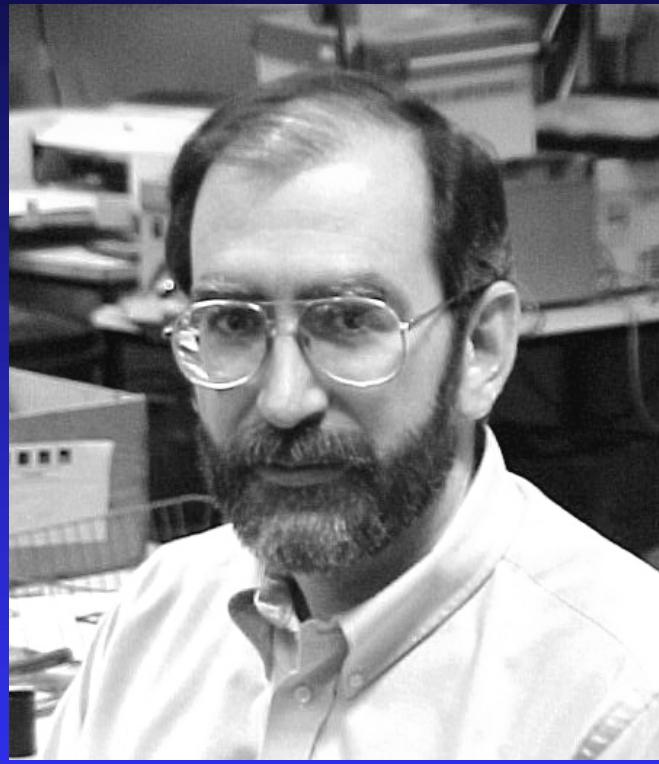
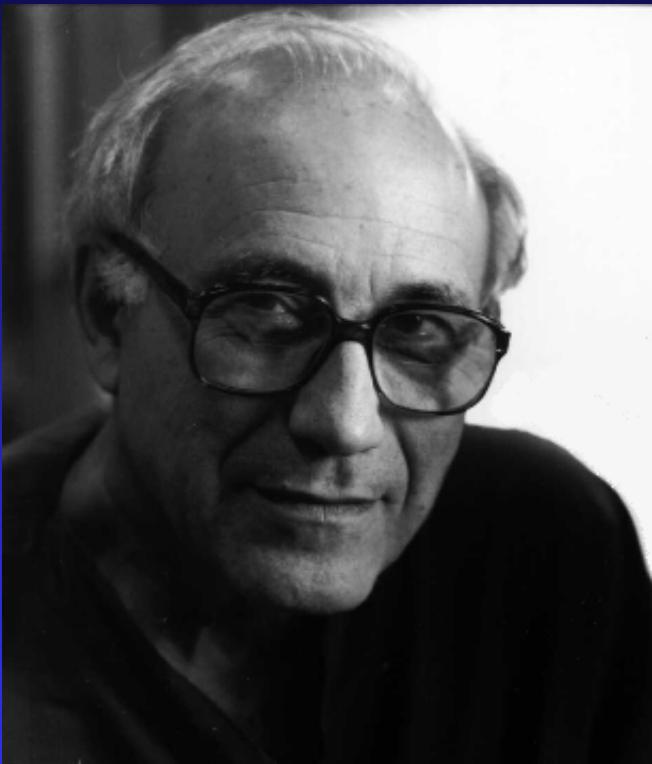


www.sfd.gov.sa/.../food+news+3-12-2006.htm

Amoeben

- met dank aan John Ackers:
DPC dag in Dierenpark Amersfoort

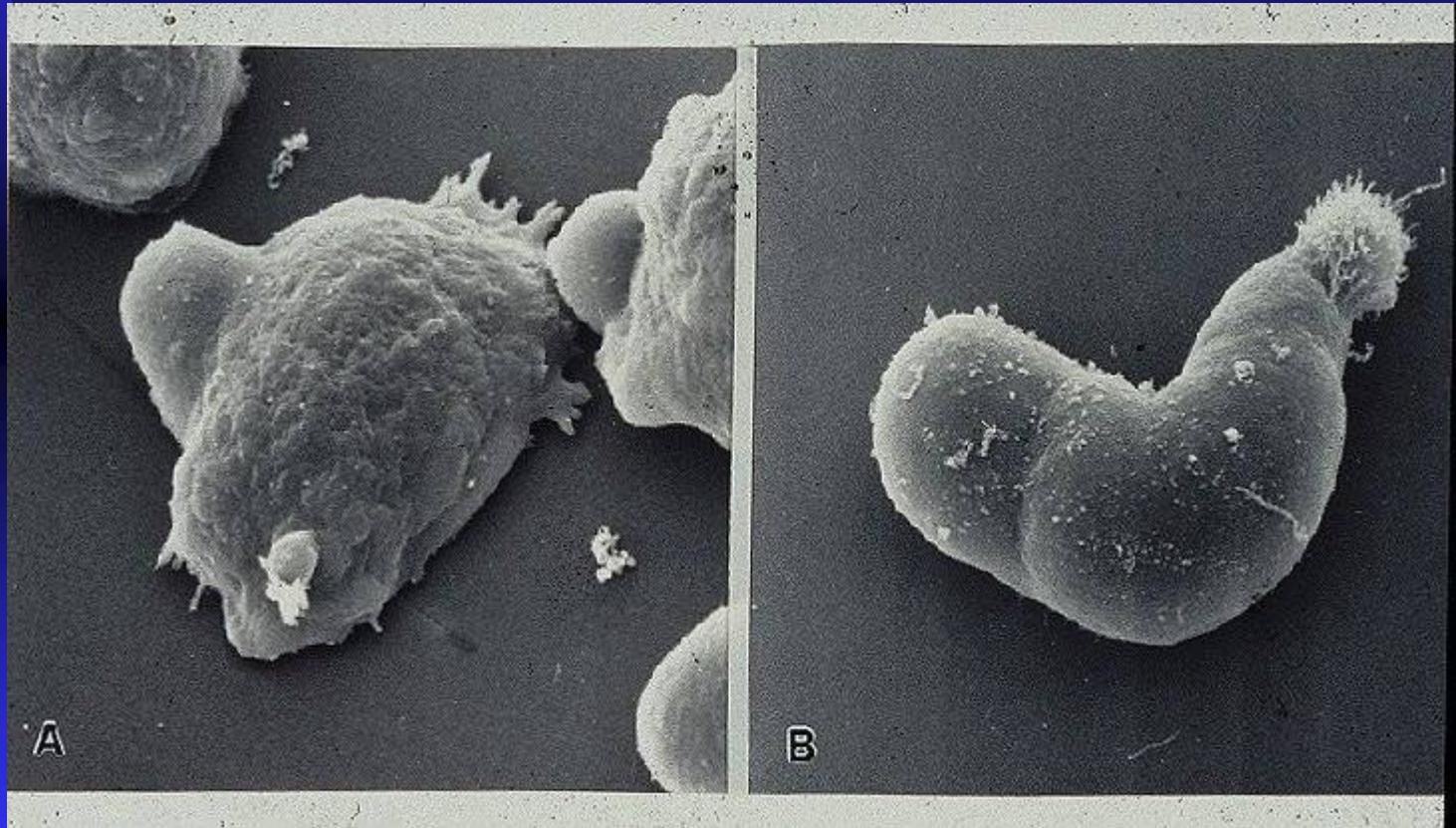
Buddy Diamond & Graham Clark



Diamond,L.S. and Clark,C.G. 1993. A redescription of *Entamoeba histolytica* Schaudinn, 1903 (Emended Walker, 1911) separating it from *Entamoeba dispar* Brumpt, 1925. *J.Euk.Microbiol* 40: 340-344

SEMs-

A: *E.histolytica*; B: *E.dispar*



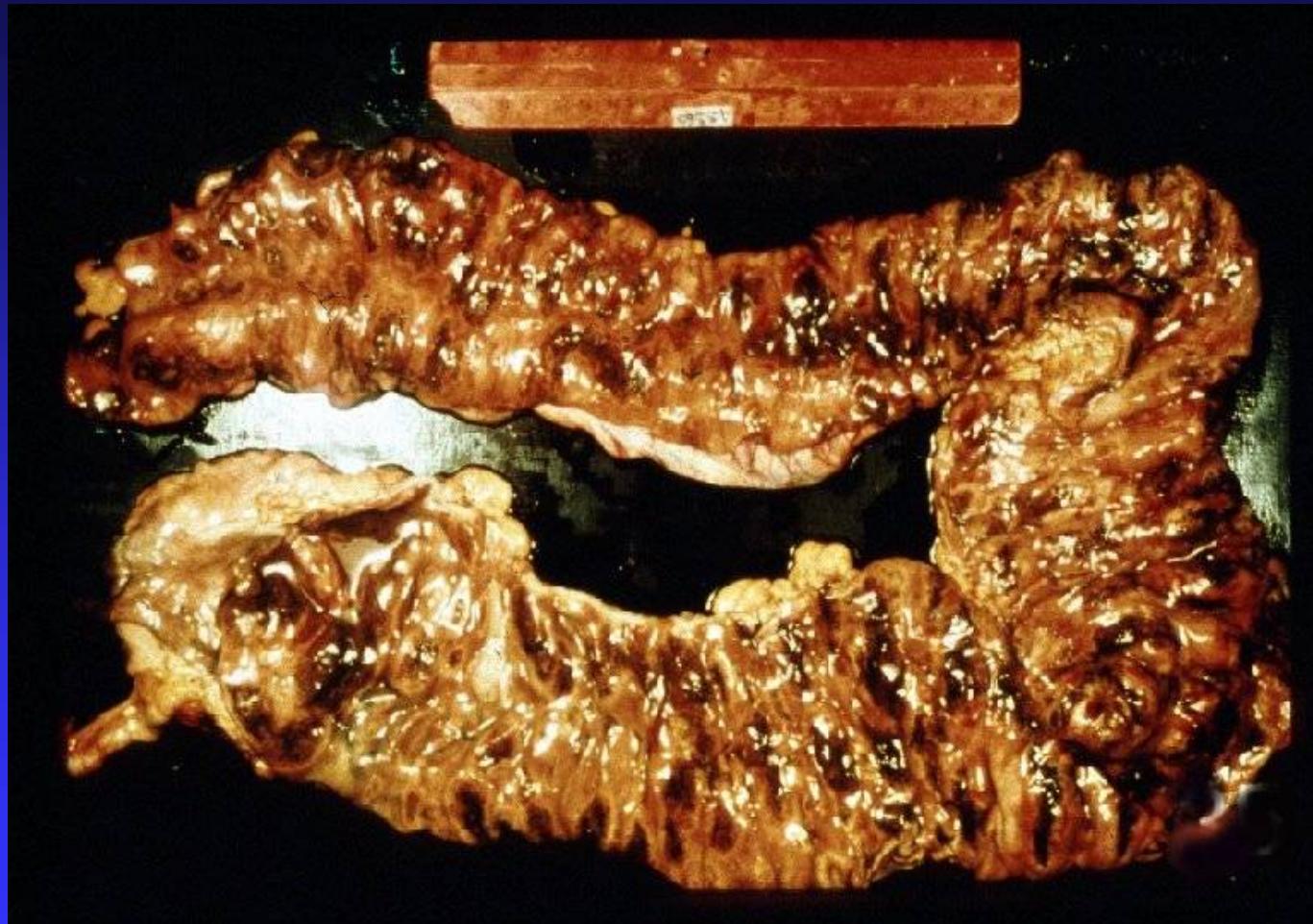
From Clark *et al.* in *Amebiasis* (Ravdin, J I Ed.) Imperial College Press, London, 2000

Entamoeba histolytica and *E. dispar*

We now believe that *four*, not three species of *Entamoeba* live in the human large bowel:

- *Entamoeba hartmanni*
- *Entamoeba histolytica*
- *Entamoeba dispar*
- *Entamoeba coli*

Colon - amoebic dysentery



Liver abscess - CT scan



Image courtesy of Dr Rob Davidson

Liver abscess - ultrasound

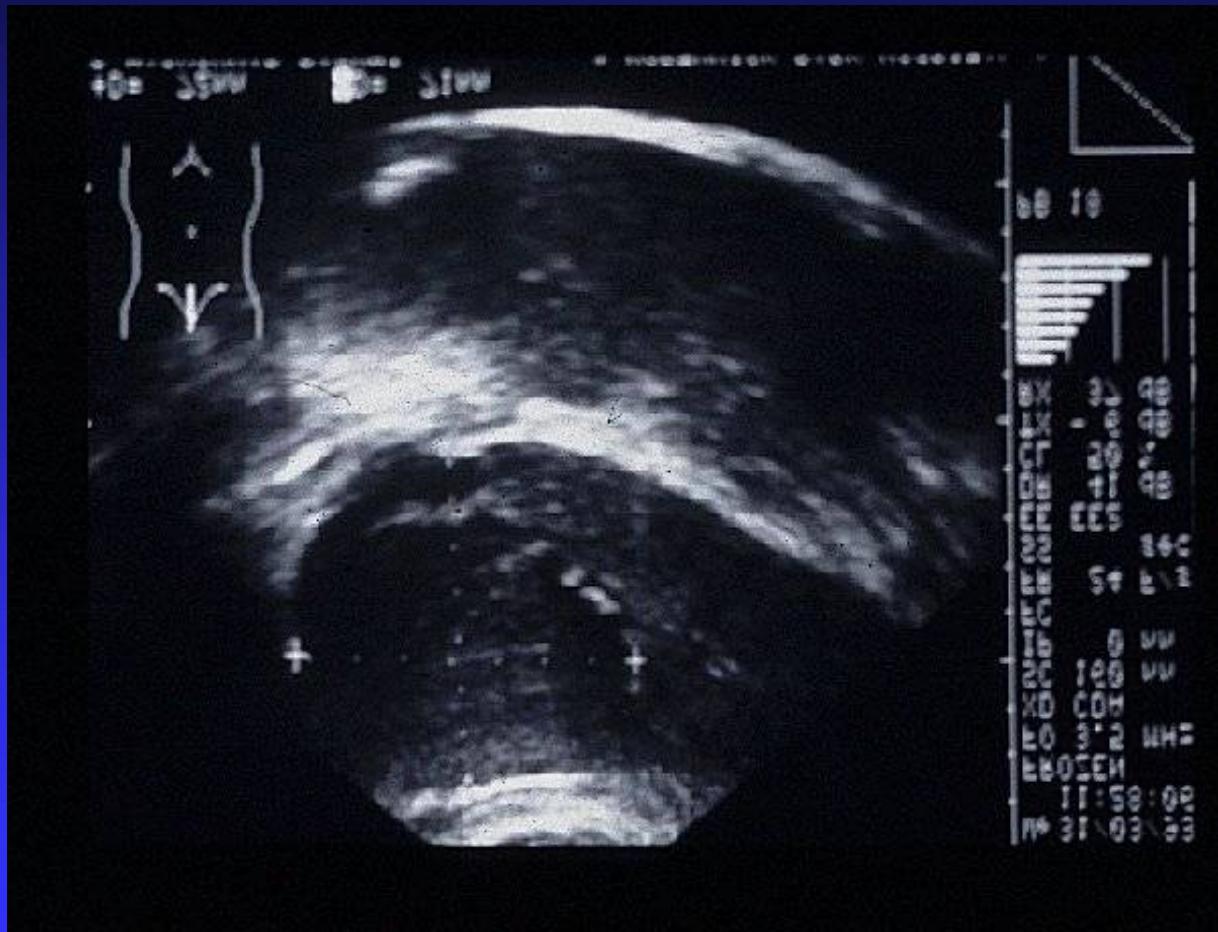


Image courtesy of Dr Rob Davidson

Diagnosis of ALA

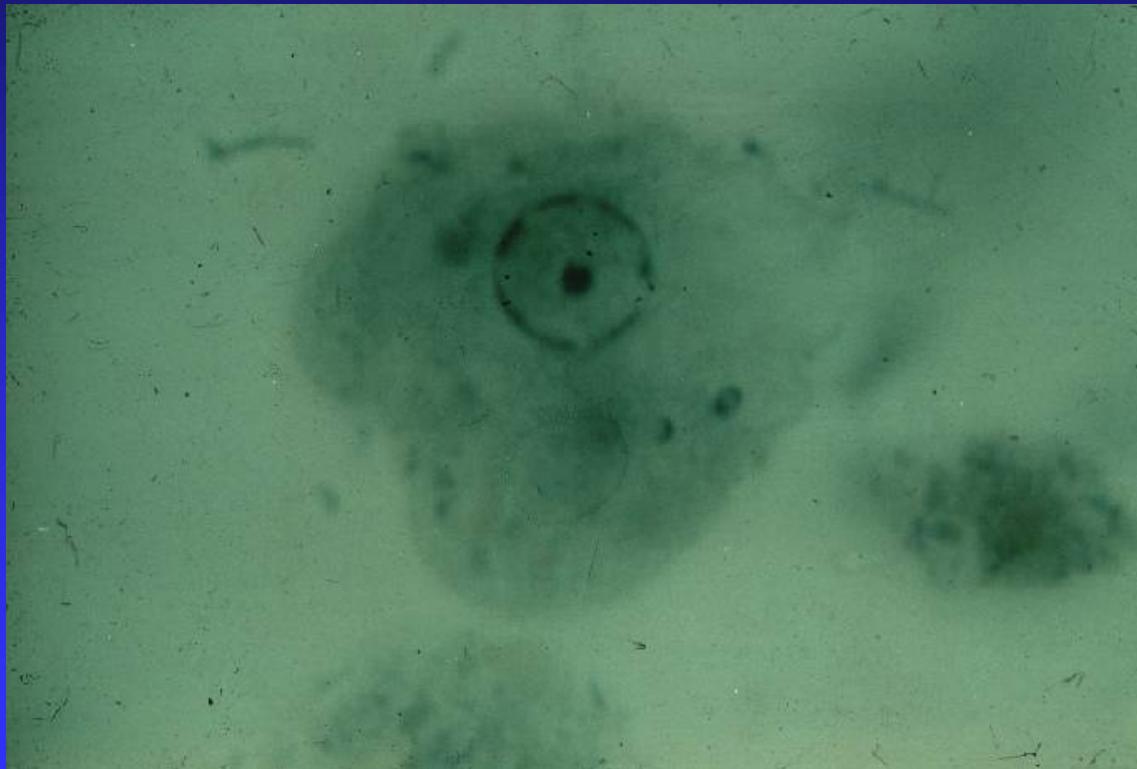
- Clinical signs and symptoms
- (Usually) strongly positive serology
- Imaging

Trophozoite with ingested RBC - unstained



© Meddia, Amsterdam

E.histolytica trophozoite - stained



© Meddia, Amsterdam

There are also a few rare species:

- "Atypical," "low temperature" or "Laredo" strains of *E.histolytica* are *E.moshkovski*
- *E.polecki*
- *E.chattoni*
- *E.gingivalis*

Wat is de klinische relevantie van de niet-histolytica soorten?

- *E.moshkovski*
- *E.hartmanni*
- *E. dispar*
- *E.coli*
- *E.polecki*
- *E.chattoni*
- *E.gingivalis*

Ellen J. Pritham¹, Cédric Feschotte¹ and Susan R. Wessler

Unexpected Diversity and Differential Success of DNA Transposons in Four Species of Entamoeba
Protozoans Mol Biol Evol. 2005 Sep;22(9):1751-63. Epub 2005 May 18

Fotedar R, Stark D, Beebe N, Marriott D, Ellis J, Harkness J.

Laboratory diagnostic techniques for Entamoeba species. Clin Microbiol Rev. 2007 Jul;20(3):511-32,

Wat is de klinische relevantie van de niet-histolytica soorten?

Fotedar R, Stark D, Beebe N, Marriott D, Ellis J, Harkness J.

Laboratory diagnostic techniques for *Entamoeba* species. Clin Microbiol Rev. 2007 Jul;20(3):511-32

Entamoeba dispar :

- Apathogeen
- Bij dieren wel focale lesies
- Er zijn rapportages van (milde) intestinale symptomen

Wat is de klinische relevantie van de niet-histolytica soorten?

WHO richtlijnen:

World Health Organization (WHO). Amoebiasis. WklyEpidemiol Rec 1997;/72:/97-9.

Recommendations

- Optimally, *E. histolytica* should be specifically identified and, if present, treated.
- If only *E. dispar* is identified, treatment is unnecessary. If the infected person has gastrointestinal symptoms, other causes should be sought.
- Species identification based on culture can never exclude the presence of *E. histolytica*.
- In asymptomatic individuals, treatment is not appropriate when *E. histolytica*/*E. dispar* has been detected but *E. histolytica* has not been specifically identified, unless there are reasons to suspect infection with *E. histolytica*, including high specific antibody titres, a history of close contact with a case of invasive amoebiasis, or an outbreak of amoebiasis.

WEEKLY EPIDEMIOLOGICAL RECORD, No. 14, 4 APRIL 1997

- If *E. histolytica*/*E. dispar* has been detected in symptomatic patients, it should not be assumed that *E. histolytica* is the cause of the symptoms and other explanations should also be considered.
- These recommendations are appropriate for managing all individuals, including male homosexuals, travellers returning from endemic areas, pregnant women, and individuals infected with HIV.
- Antiamoebic drugs are of 2 classes: tissue amoebicides (such as 5-nitroimidazoles) and luminal amoebicides (such as diloxanide furoate and paromomycin). Invasive disease should be treated with a tissue amoebicide followed by a luminal amoebicide. Tissue amoebicides are not appropriate for treatment of asymptomatic individuals, unless other evidence for invasive amoebiasis exists.
- Chemoprophylaxis is never appropriate.

Wat is de klinische relevantie van de niet-histolytica soorten?



Available online at www.sciencedirect.com



International Journal of Medical Microbiology 296 (2006) 397–403



www.elsevier.de/ijmm

Diagnostic methods for differentiation of *Entamoeba histolytica* and *Entamoeba dispar* in carriers: Performance and clinical implications in a non-endemic setting

Leo G. Visser^{a,*}, Jaco J. Verweij^b, Marjan Van Esbroeck^c, Willeke M. Edeling^a, Jan Clerinx^c, Anton M. Polderman^b

^aDepartment of Infectious Diseases, Leiden University Medical Center, Room C5-P-41, PO Box 9600, 2300 RC Leiden, The Netherlands

^bDepartment of Parasitology, Leiden University Medical Center, The Netherlands

^cDepartment of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

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Table 1. Patient characteristics of different patient groups

Characteristics	<i>E. histolytica</i> ^a	<i>E. dispar</i> ^a	Controls ^b
	Percentage (number)		
Male	74% (18)	66% (171)	43% (57)
Female	26% (7)	34% (87)	57% (76)
Median age [range]	41 [13–74] yr	34 [1–75] yr	30.5 [0–76] yr
Clinical symptoms			
Asymptomatic	16 (4)	28 (72)	15 (20)
Abdominal complaints	16 (4)	12 (31)	7 (9)
Diarrhoea	40 (10)	48 (125)	66 (88)
Colitis	24 (6)	<1 (2)	10 (14)
Missing	4 (1)	11 (28)	1 (2)
Total	100 (25)	100 (258)	100 (133)

^aDetection of DNA of *E. histolytica* or *E. dispar* in stool by real-time PCR.

^bNo detection of DNA of *E. histolytica* or *E. dispar*, and no cysts of *E. histolytica*/*E. dispar*.

Conclusie:

We found that infection with *E. histolytica* is a rare event in a non-endemic setting. In as many as 24% of patients with carriers of *E. dispar* another possible cause of diarrhoea was found. In comparison to real-time PCR the performances of *Entamoeba* testTM and *E. histolytica* IITM lacked sensitivity for a reliable diagnosis of *E. histolytica/E. dispar* infection. In carriers of *E. histolytica/E. dispar* from non-endemic countries the high specificity of serology can be used to establish the diagnosis of *E. histolytica* infection if antibodies are present.

Wat is de klinische relevantie van de niet-histolytica soorten?

Lebbad M, Svärd SG. PCR differentiation of *Entamoeba histolytica* and *Entamoeba dispar* from patients with amoeba infection initially diagnosed by microscopy. Scand J Infect Dis. 2005;37(9):680-5.

207 patienten *E. histolytica/E. dispar*,

PCR: 165 patients *E. dispar*, 10 patients *E. histolytica*.

R. ten Hove, T. Schuurman, M. Kooistra, L. Möller, L. van Lieshout, J. J. Verweij (2007)
Detection of diarrhoea-causing protozoa in general practice patients in The Netherlands by
multiplex real-time PCR Clinical Microbiology and Infection 13 (10) , 1001-1007

Studie in huisartsenpatienten Groningen: 950 pat.

Geen *Entamoeba* gevonden

Wat is de klinische relevantie van de niet-histolytica soorten?

Fotedar R, Stark D, Marriott D, Ellis J, Harkness J.

Entamoeba moshkovskii infections in Sydney, Australia. Eur J Clin Microbiol Infect Dis. 2008 Feb;27(2):133-137. Epub 2007 Oct 24.

Entamoeba moshkovskii: freeliving amoeba;
morphologically similar
biochemically and genetically different

E. moshkovskii may be a "potential" pathogen,
diarrhea and other intestinal disorders.

110 patients microscopically positive for the Entamoeba species,
55/110 (50%) positive *E. moshkovskii* in the test group of patients
presenting with diarrhea.

healthy controls : No DNA of *E. moshkovskii*

?? *E. moshkovskii* may not simply be a commensal of the human
gastrointestinal tract but a "potential" pathogen in the case of
diarrhea and other gastrointestinal disorders ??

Wat is de klinische relevantie van de niet-histolytica soorten?

- *E.hartmanni*
- *E. dispar*
- *E.coli*
- *E.polecki*
- *E.chattoni*

Apathogene darmbewoners ;
Behandeling niet nodig

Moet je ze dan wel benoemen ?

Een ei!!



Het ei

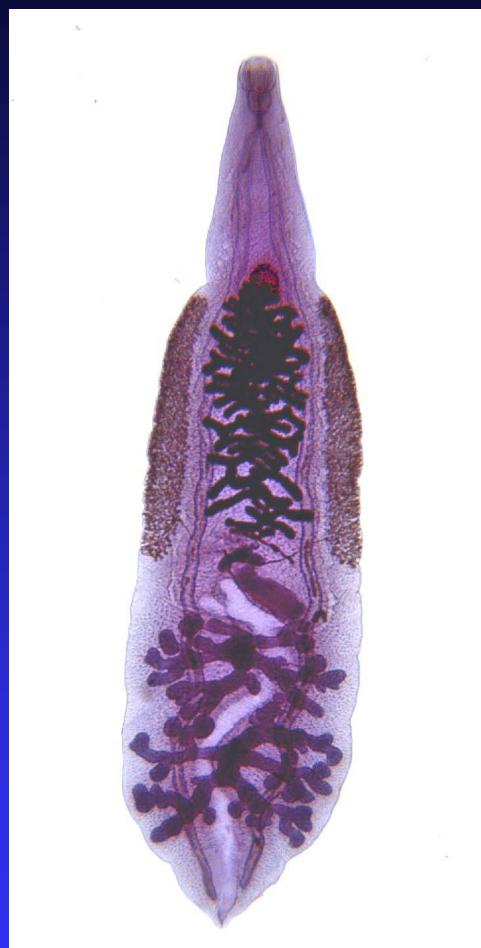
- Grootte: 28×17 mu
- Operculum
- Geen "schouders"
- Miracidium

Het ei

- *Clonorchis sinensis*
- *Metagonimus yokogawai*
- *Heterophyes heterophyes*
- *Opisthorchis spp*

Geographic distribution of *Clonorchis sinensis* and *Opisthorchis viverrini*



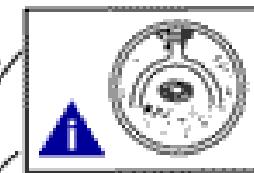






<http://www.dpd.cdc.gov/dpdx>

Metacercariae in flesh or skin of fresh water fish are ingested by human host.



4

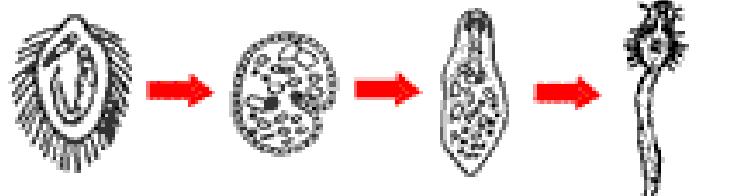
Free-swimming cercariae encyst in the skin or flesh of fresh water fish. 3



3

Eggs are ingested by the snail. 2

2



Miracidia Sporocysts Rediae Cercariae
2a 2b 2c 2d

1 Embryonated eggs passed in feces.

i = Infective Stage
d = Diagnostic Stage

5 Excyst in duodenum



6 Adults in biliary duct



- Trematode (zuigworm/bot)
 - Schistosomen: m/v definitieve gastheer: zoogdier + 1 tussengastheer = slak
 - Hermafrodiete boten: definitieve gastheer: zoogdier + 2 tussengastheren: slak + crustaciën/vis/plant
- Eten van rauwe vis
 - Nematode - vis?
 - Cestode - vis?
- Voor NL importziekte / Geografie!
- Galgangen / lever
- Worm leeft +/- 30 jaar 2400 eieren /dag



Figure 2. *Opisthorchis* (solid lines) and *Clonorchis* (broken lines) endemic areas in the former USSR. Original map was obtained from the United Nations Development Programme Web site (www.undp.org).

Emerg Infect Dis. 2004 Dec;10(12):2122-6. Opisthorchiasis from imported raw fish. Yossepowitch O, Gotesman T, Assous M, Marva E, Zimlichman R, Dan M.

Archive Number 20070529.1733

Published Date 29-MAY-2007

Subject PRO/AH/EDR> Opisthorchiasis - Russia (Altay region)

OPISTHORCHIASIS - RUSSIA (ALTAY REGION)

Date: Mon 28 May 2007

Source: IA: Amic.ru [translated by Assoc. Mod.NP, edited]

<http://www.amic.ru/news/?news_id=70108>

High level of opisthorchiasis morbidity in the Altay region

A total of 700 cases of opisthorchiasis have been registered in the territory of the Altay region for the first 3 months of 2007, including 61 cases among children. Meanwhile 674 cases of opisthorchiasis, including 48 cases in children under 14 years old were observed for the same period of 2006. Staff of the RosPotrebNadzor [Federal Supervision Agency for Customer Protection and Human Welfare - Mod.NP] in the Altay region warn against self-treatment, as only a doctor can determine the necessity of choice of drug and dosage.

- Kliniek: afhankelijk van wormlast
- Laag geïnfecteerden: asymptomatisch
- Bij hoge wormlast: klachten
 - Hyperplasie epitheel galwegen
 - Fibrose van galwegen / cholangitis / pancreatitis
 - Obstructie en stuwing
 - Eosinofilie / diarree (slijmerig) / buikpijn
 - Angiocarcinoma
- Diagnostiek: aantonen eieren in feces (gal) / serologie / beeldvormende technieken
- Therapie: Praziquantel

- overzicht 2007
- 1x protozo; 1x worm
- overwegingen bij microscopisch onderzoek
- niet microscopische technieken... serologie / ELISA / PCR
- toekomst

Overwegingen bij microscopisch onderzoek

- expertise microscopist
 - Training!
 - Controle?
- Aard materiaal (fixatie, meervoudig)
 - Rapportage naar aanvrager?
- Sensitiviteit
 - Aard materiaal / soort onderzoek / expertise
- Parasitaire species