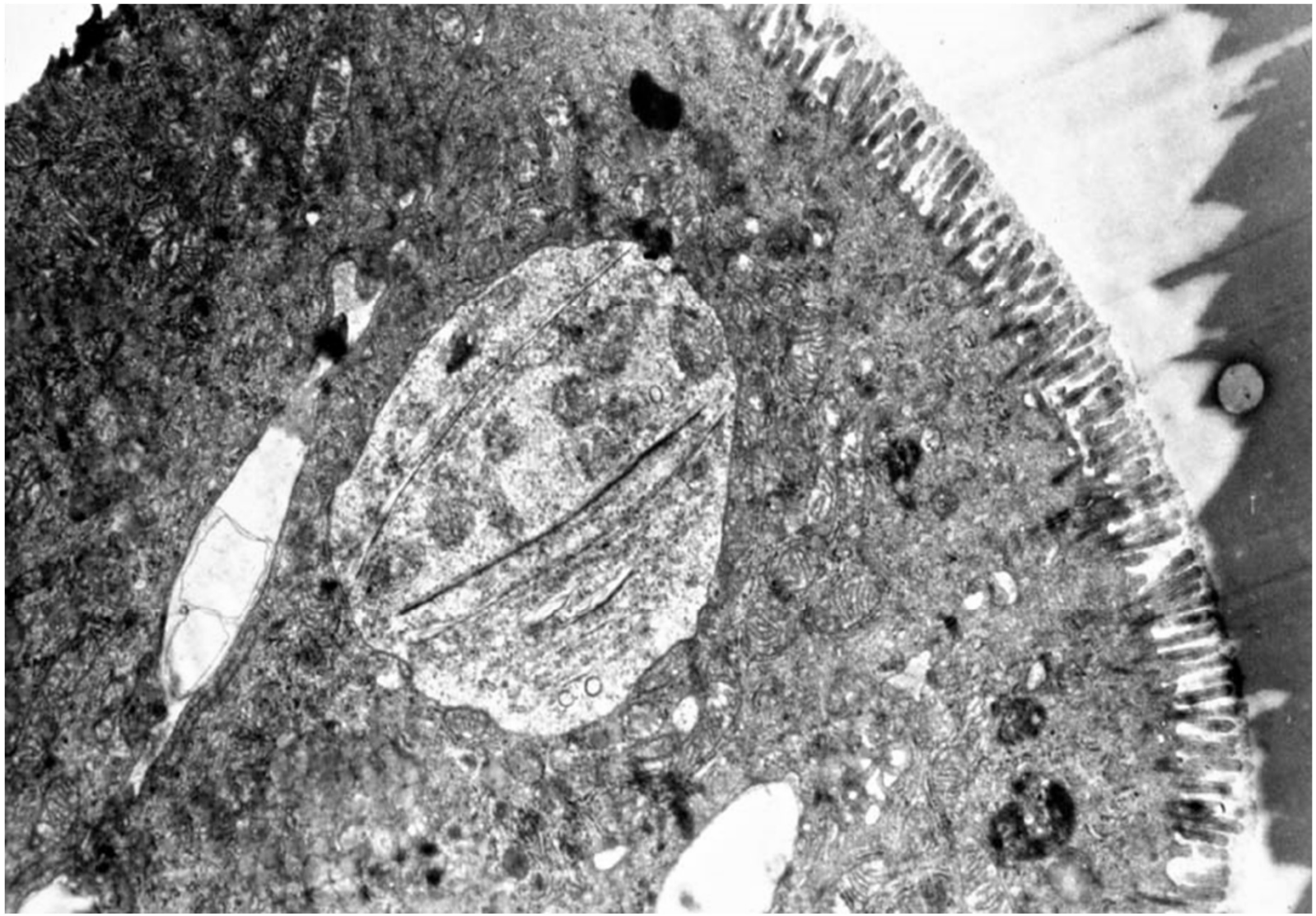
Klinische Parasitologie in Nederland:

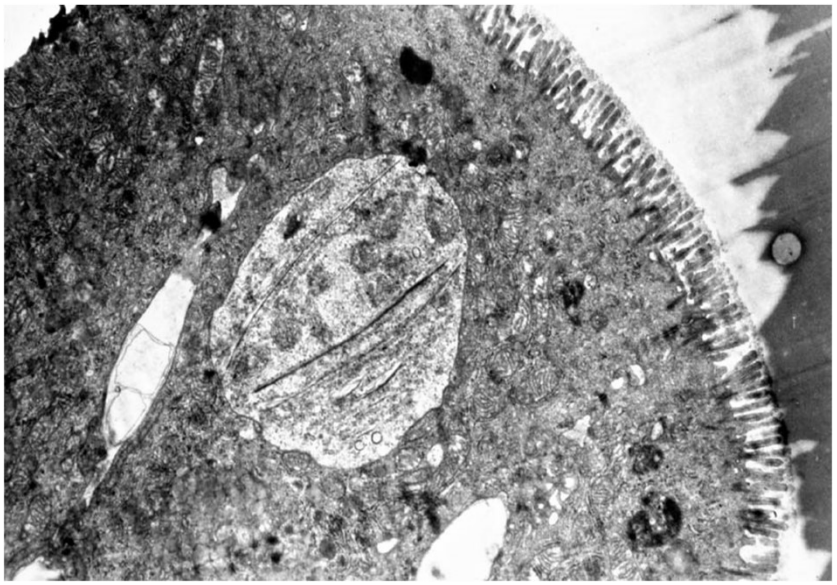
zoekt en gij zult vinden…!

Tom van Gool
Sectie Klinische Parasitologie
Amsterdam UMC

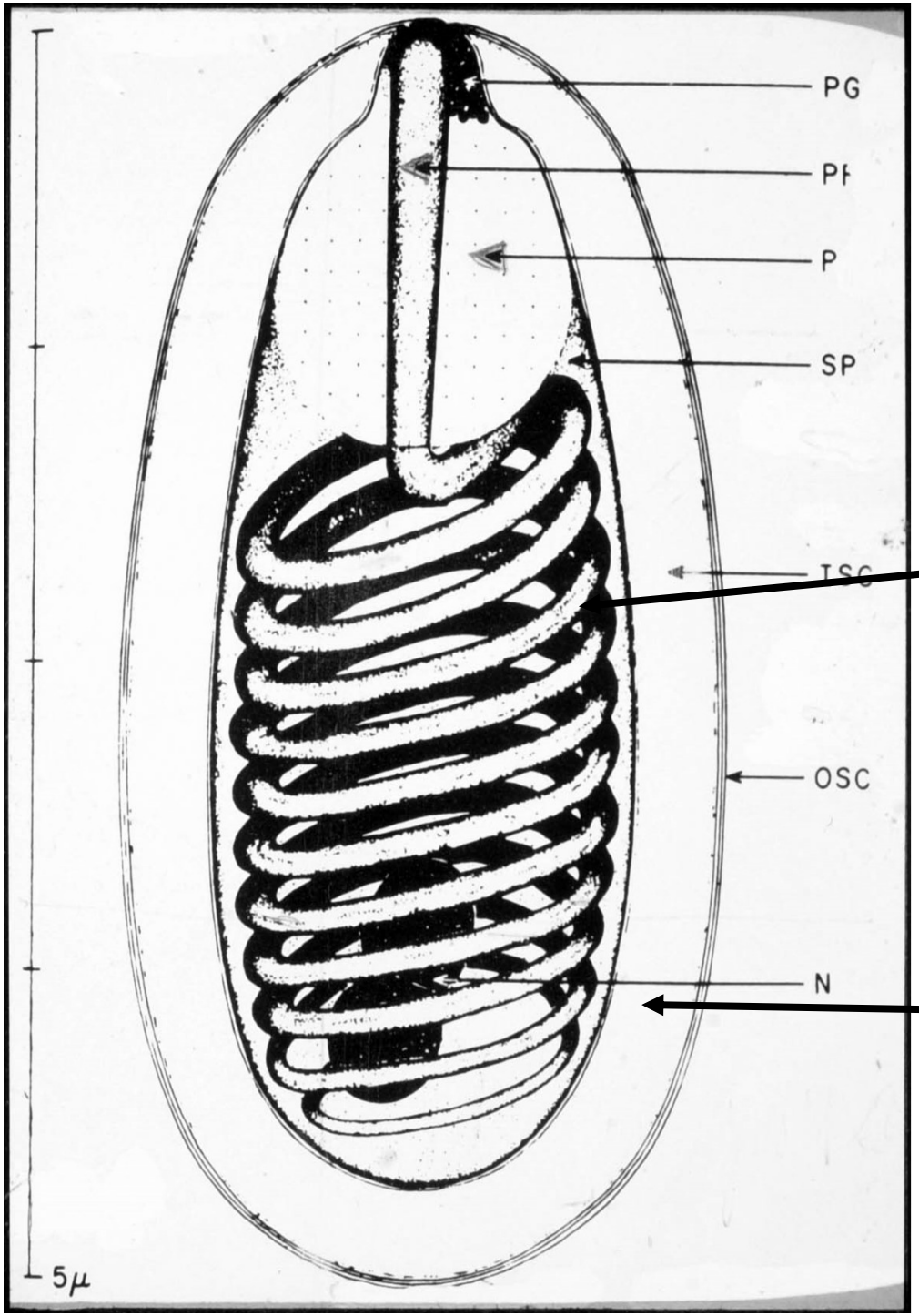


A unique finding in Paris in 1985…….

Prof. Isabelle Desportes
Hôpital Pitié-Salpêtrière (Paris)



"Small parasite belonging to the microsporidia but
Is a new, unknown species": *Enterocytozoon bieneusi*

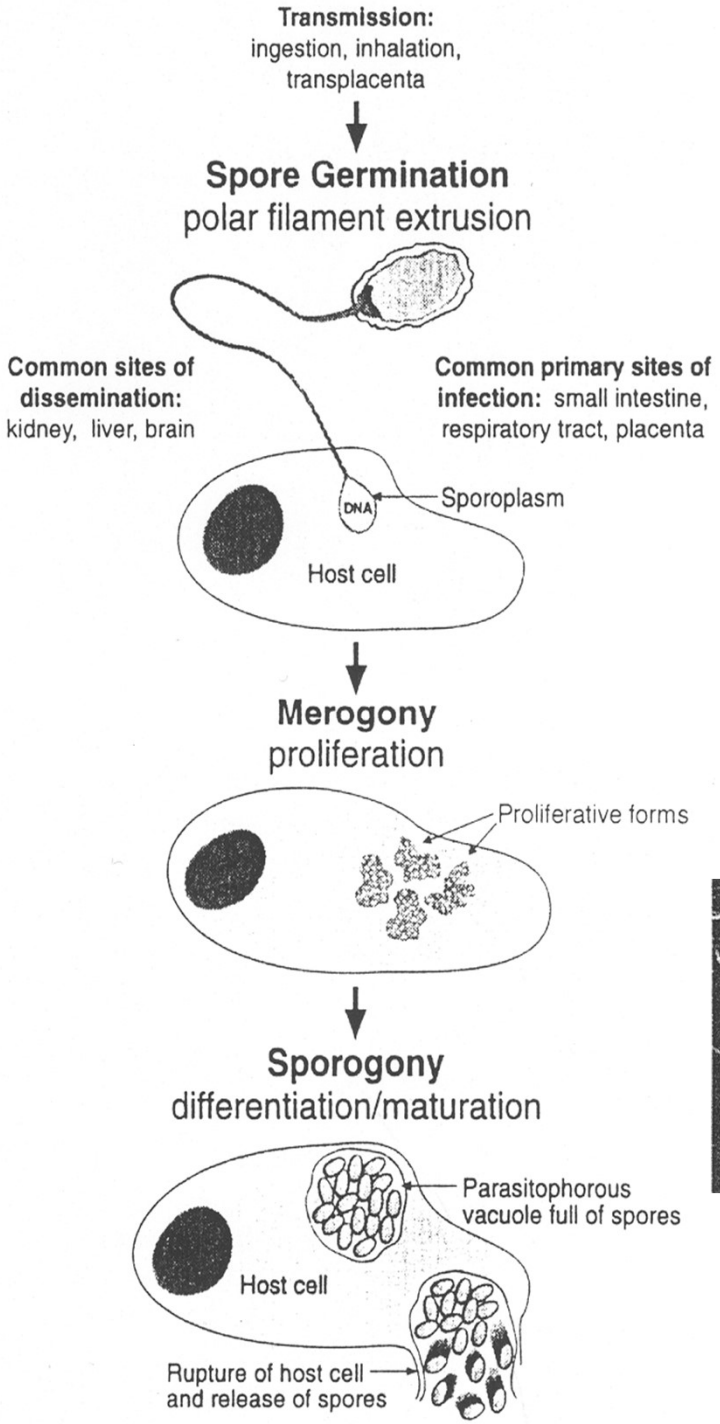


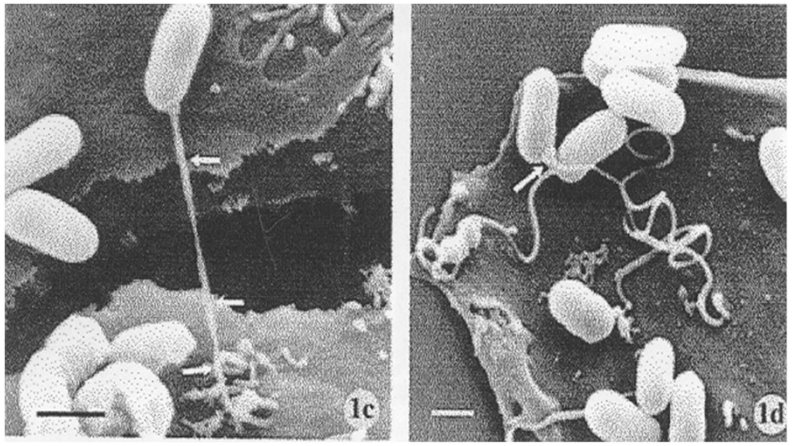
Typical spore stage of microsporidia

Coiled polar filament

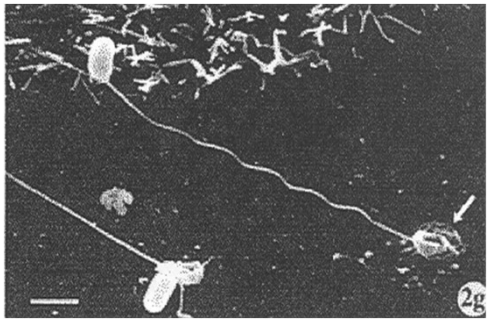
Thick exopspore with chitin

Life cycle of microsporidia (*Encephalitozoon*)

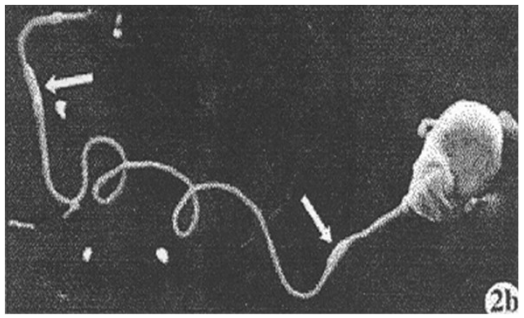




E.i.



E.h.



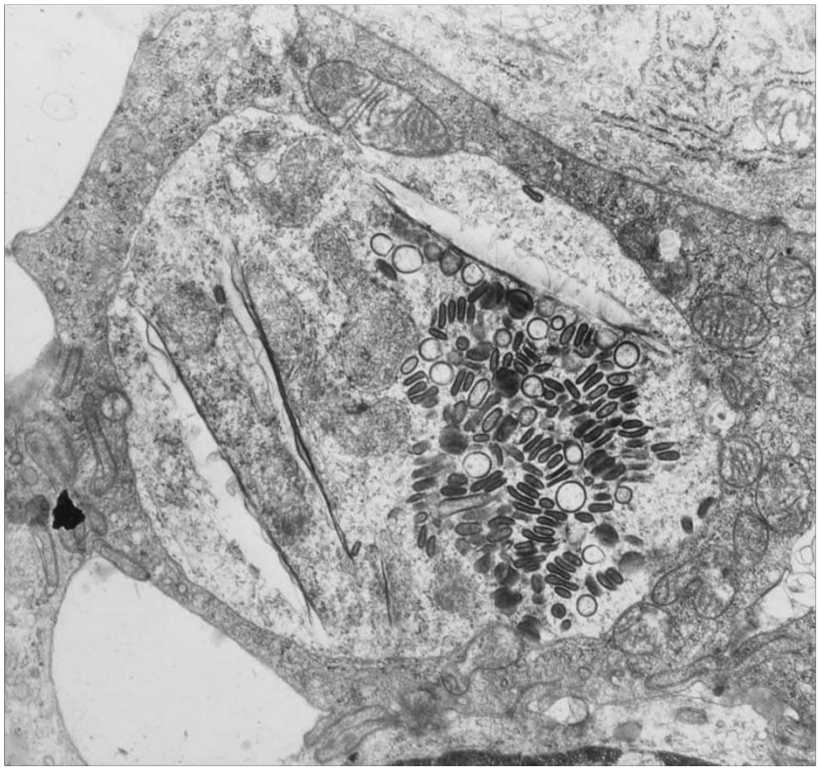
Schottelius MI 2000

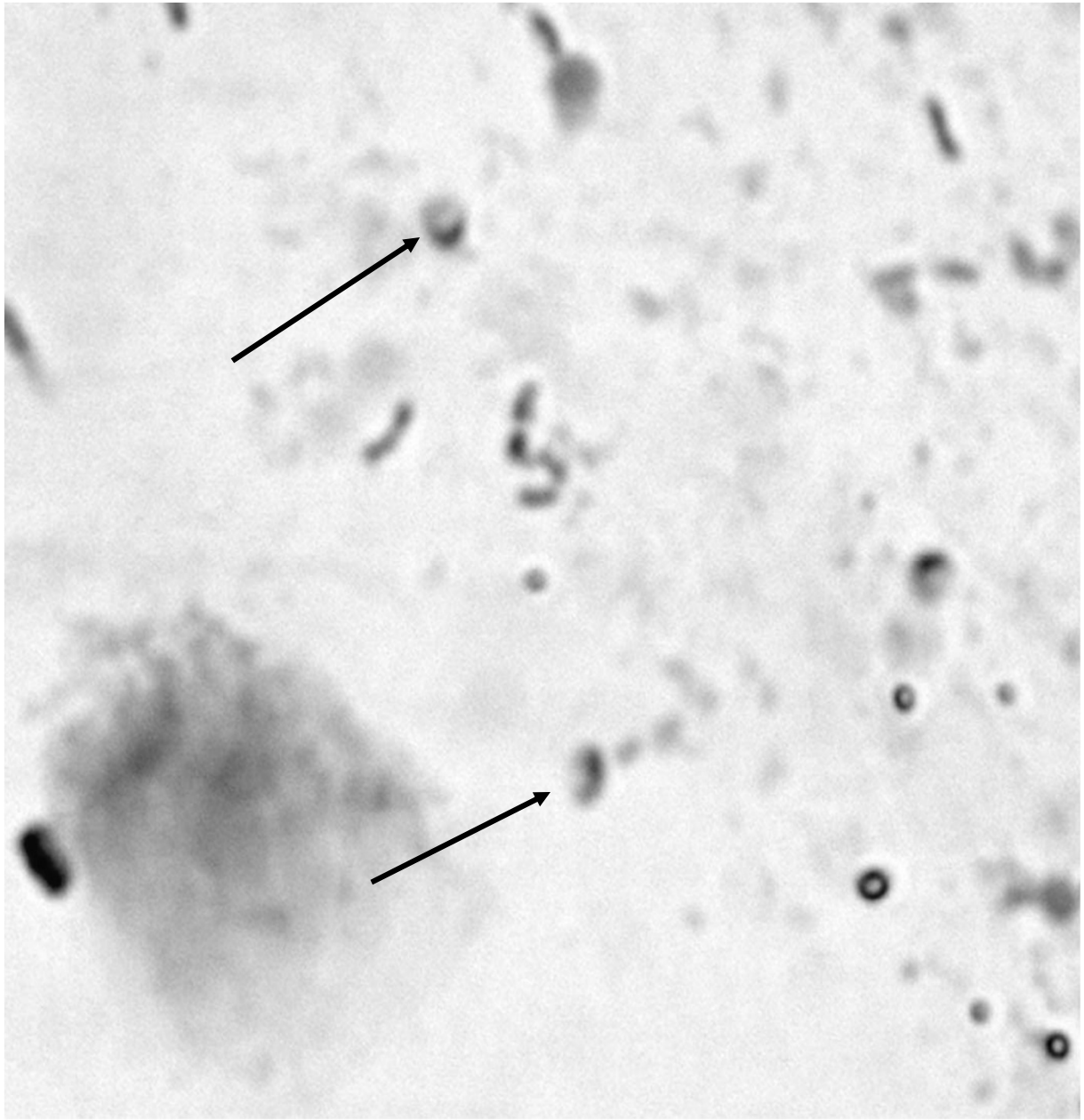
Microsporidia

- obligate intracellular protozoan parasites
- more than 144 genera and 1200 species
- important parasites in all phyla of animals

Diagnosis of human microsporidiosis 1985:

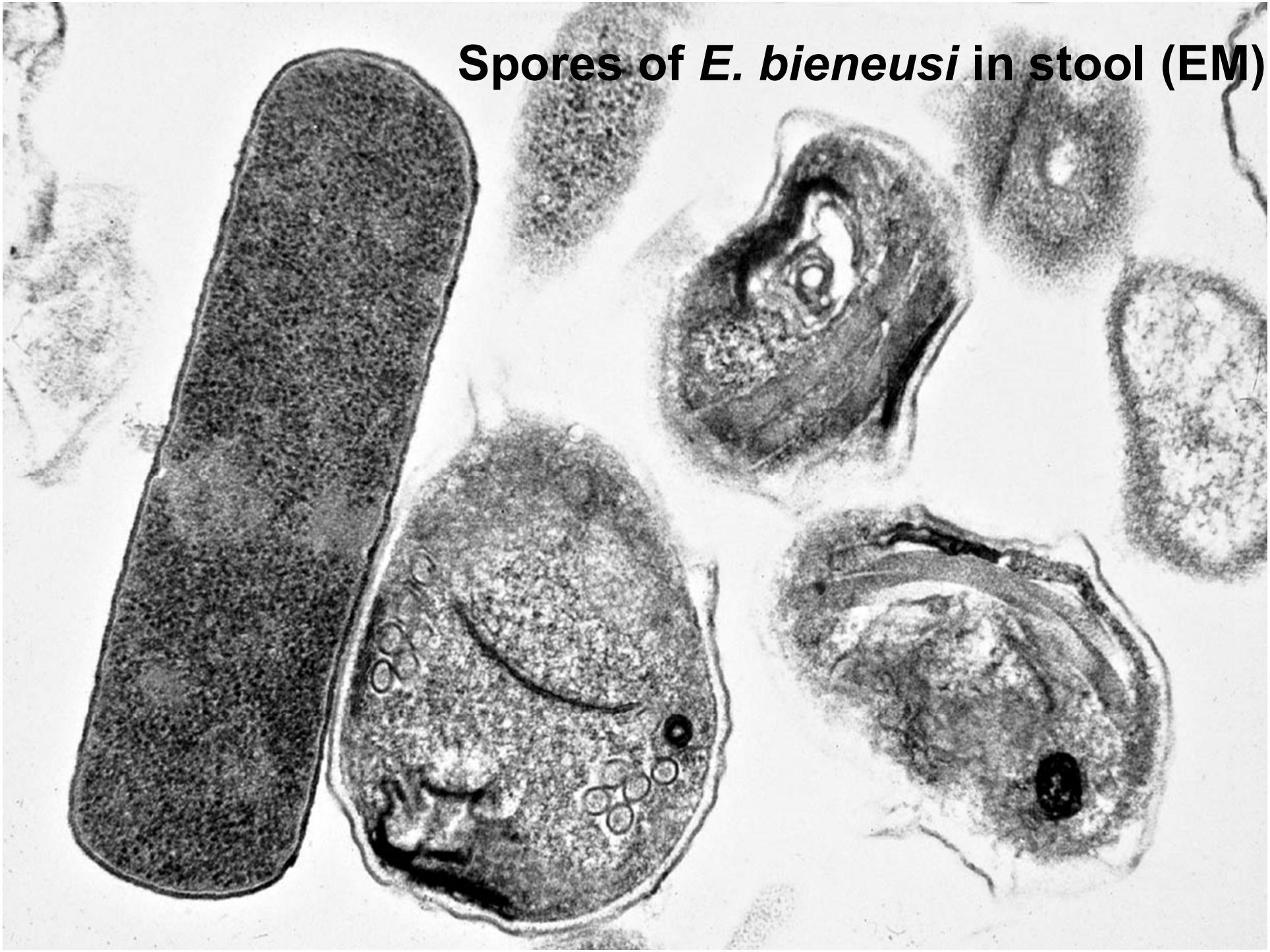
electron microscopy of duodenal biopsies

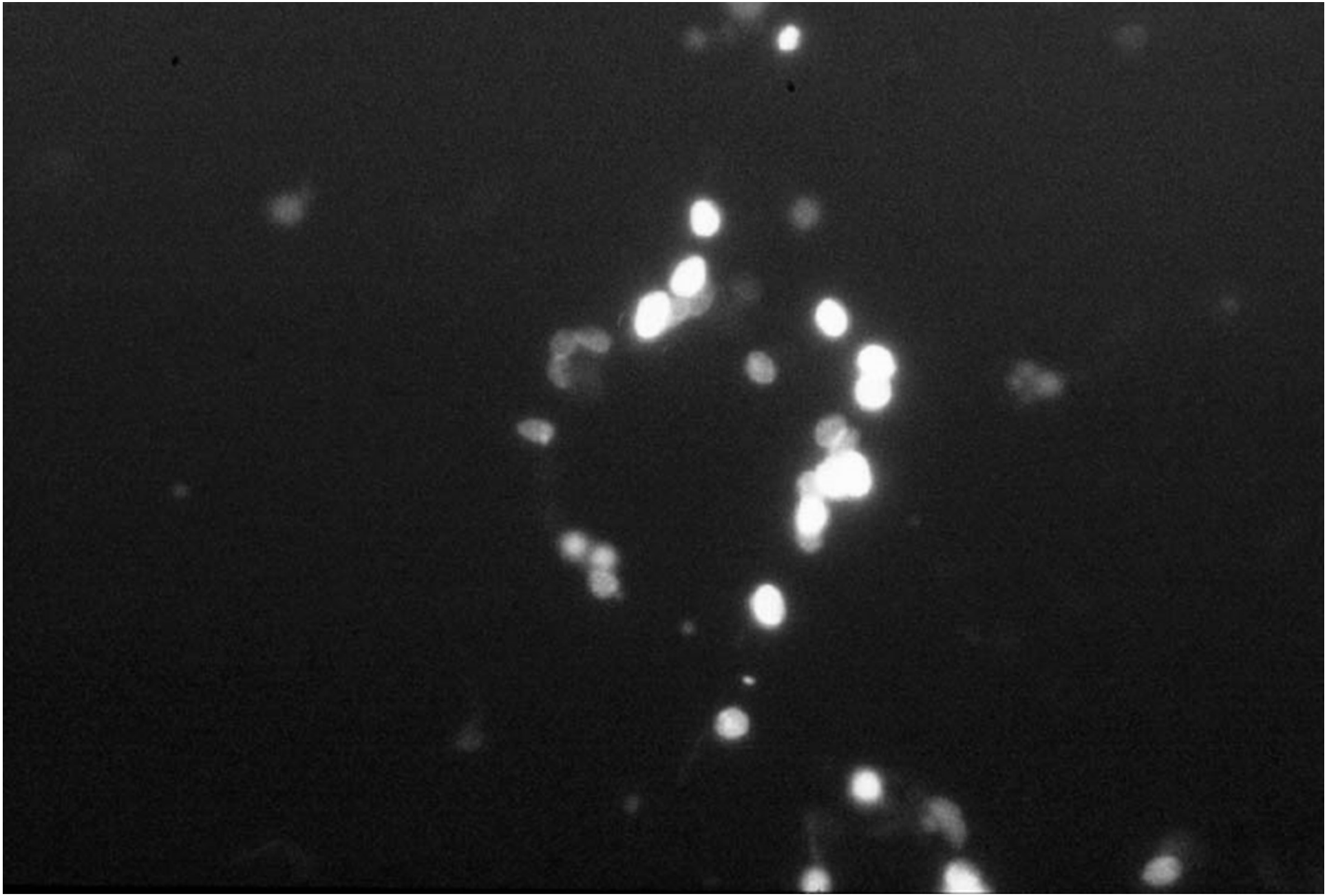




Spores of *E. bieneusi* in stool (Giemsa stain)

Spores of *E. bieneusi* in stool (EM)





E. bieneusi with Uvitex 2B stain

Clinical significance of small-intestinal microsporidiosis in HIV-1-infected individuals

J. K. M. EEFTINCK SCHATTENKERK T. VAN GOOL
R. J. VAN KETEL J. F. W. M. BARTELSMAN CARLA L. KUIKEN
W. J. TERPSTRA P. REISS

The Lancet, Clinical Practice, 1991

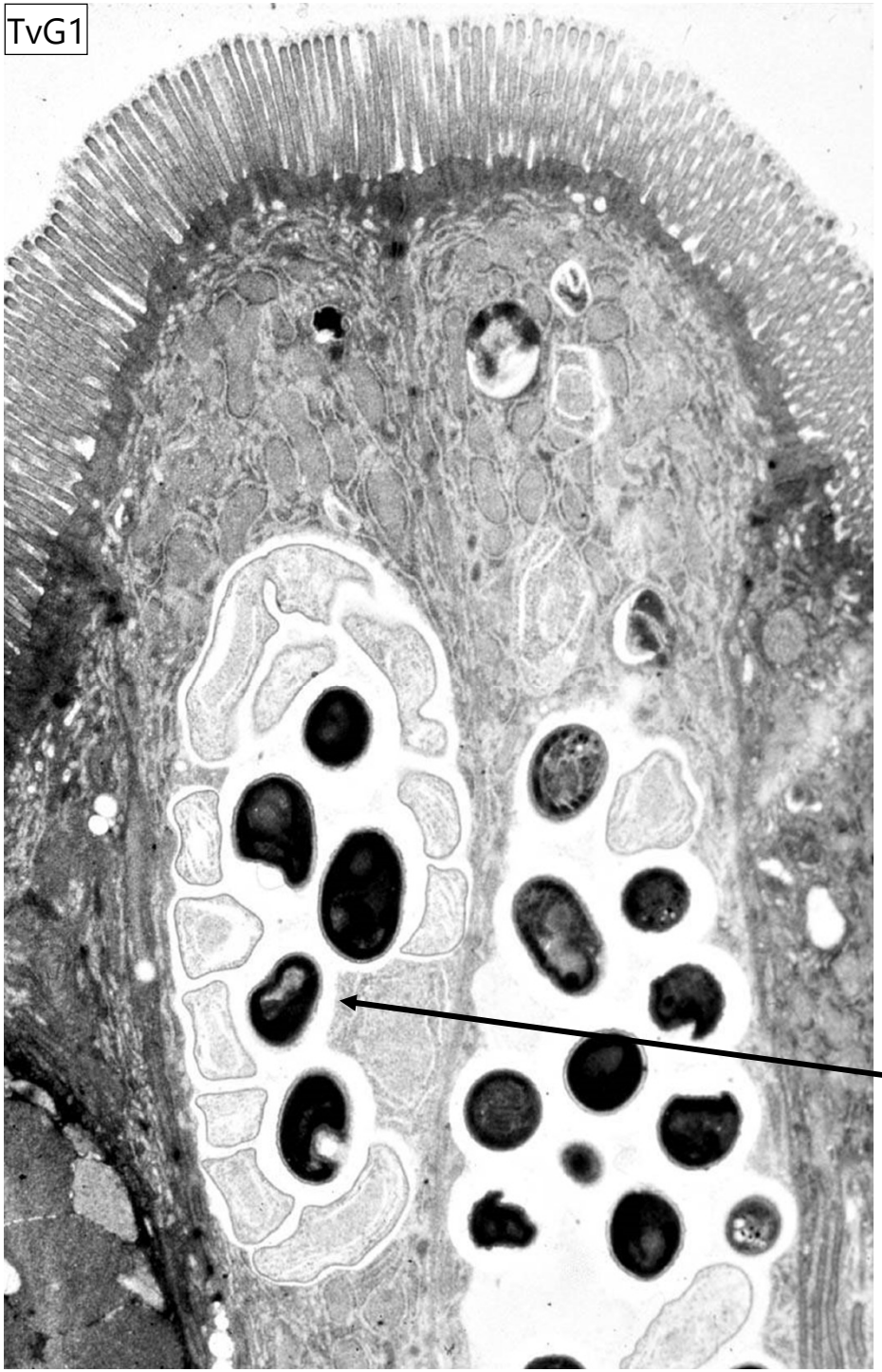
- chronic diarrhea
- cholangiopathy
- rhinosinusitis

- HIV infected patients CD 4 <100

Treatment *E. bieneusi*

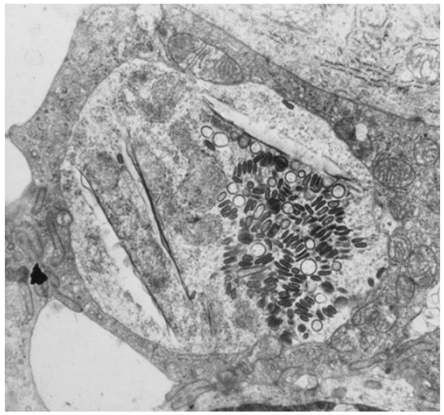
- Difficult!!
- In AIDS: fumagillin, effective but can be toxic.
- With improvement antiretroviral treatment in HIV (and improved function of immune system): disappearance of infection!
- Non-HIV immunosuppression: lowering dosage of immunosuppressive drugs can be useful

TvG1



A peculiar finding !!

Encephalitozoon spp.
in epithelial cell of small intestine



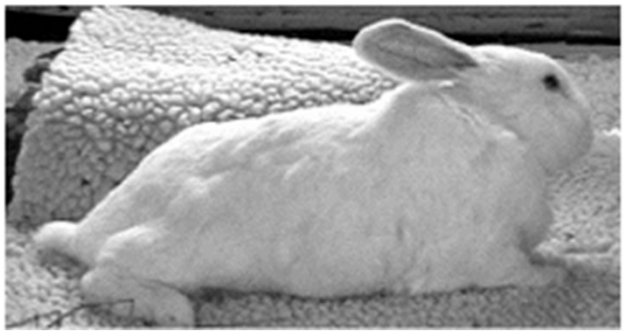
Parasitophorous vacuole with sporoblasts and spores……

TvG1 Tom van Gool; 12-02-2020

Parasite in EM much resemblance with *Encephalitozoon cuniculi*

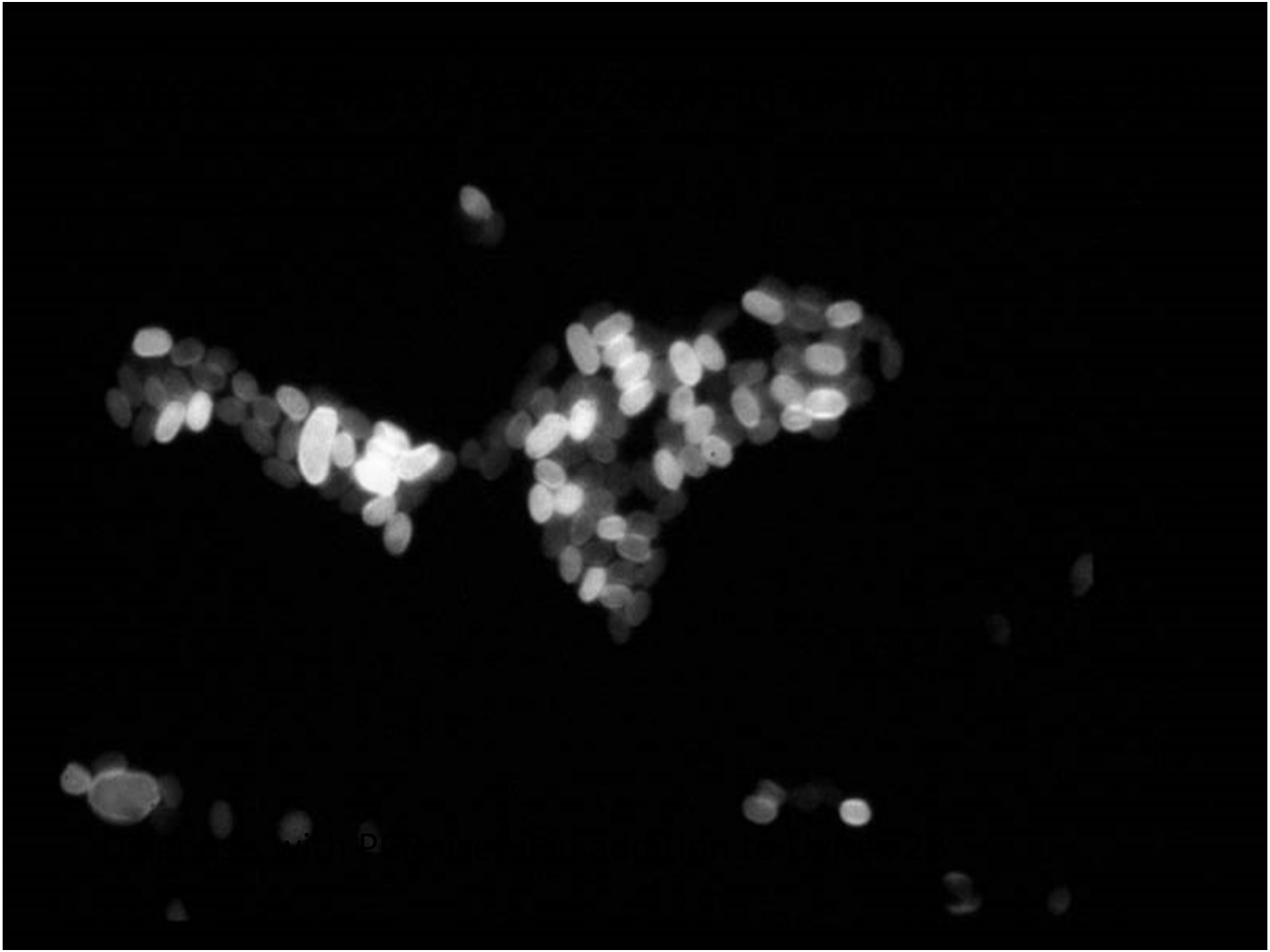
→ Severe pathology in animals i.e. CNS and kidneys

HOUSE RABBIT SOCIETY



"Phoebe"

Torticollis



Pathology due to *Encephalitozoon* infections in AIDS: involvement of multiple organs

Diarrhea and disseminated infection:

cholangitis, hepatitis, nephritis, rhinosinusitis,

keratoconjunctivitis, bronchopneumonia,

urethritis, encephalitis

Treatment of *Encephalitozoon* species

- Albendazole 400 mg twice a day for 4 weeks
  - Rapid disappearance of spores from body fluids
  - Prolonged treatment necessary to prevent relapses

Microsporidiosis in immunosuppression
other than AIDS: increased no. of positives !!

- Solid organ tranplantation (kidney, heart- long and liver)
- Bone marrow transplant recipiënts.

E. bienusi: prolonged diarrhea

Encephalitozoon spp.: multipe organ involvement

Microsporidosis most common in persons with AIDS:

up to 10-20% positives in cases

with chronic diarrhea!

Infection also in immunocompetent individuals?

Microsporidia in immunocompetent persons

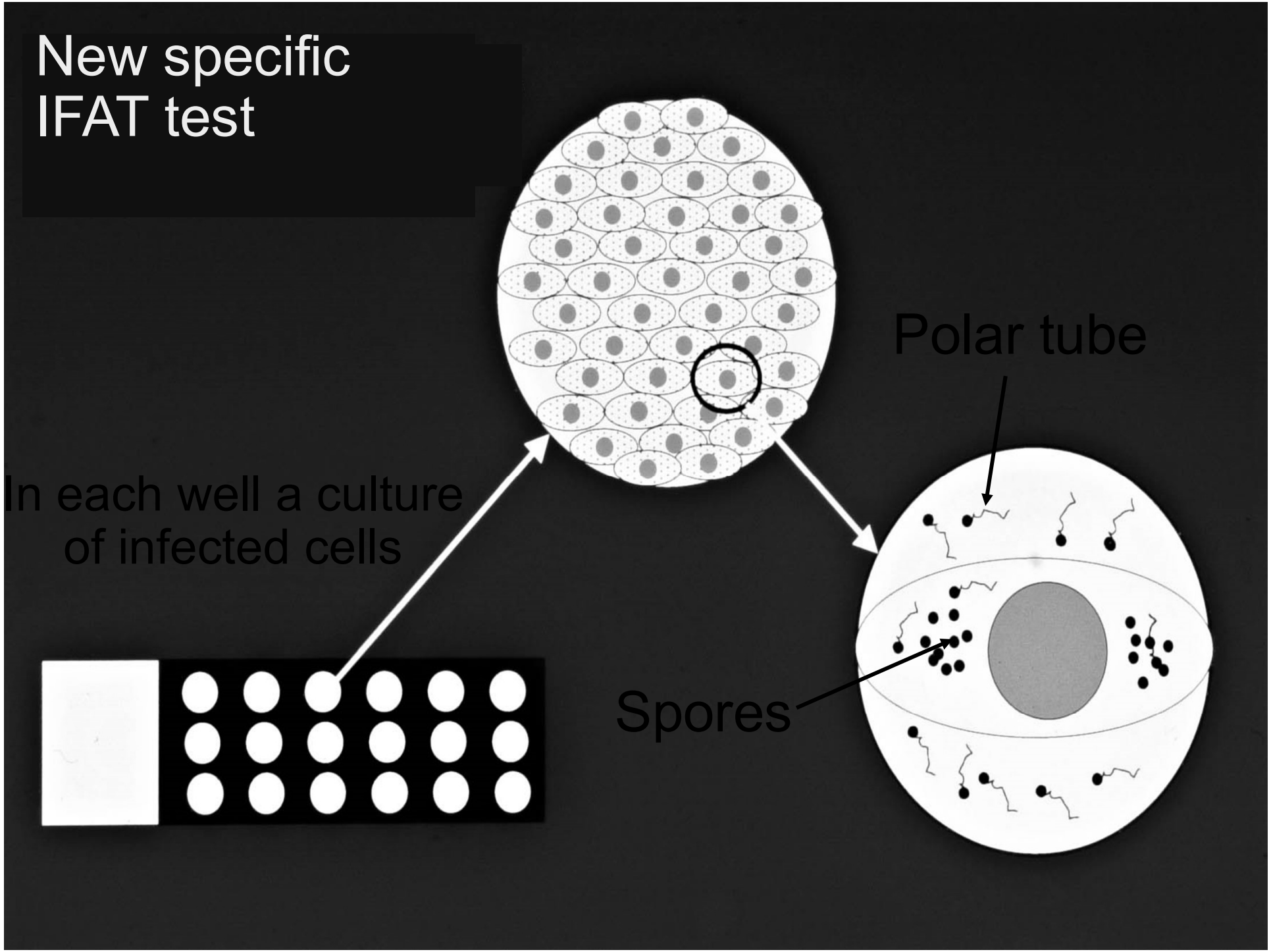
- often non-severe, self limiting diarrhea (travellers)
- associated with eye infection
- frequent in rural areas, developing coutnries
- microsporidia reported: i.e, *E. bieneusi*, *E. intestinalis*

But in *immunocompetent cases* other diagnostic methods could be useful:

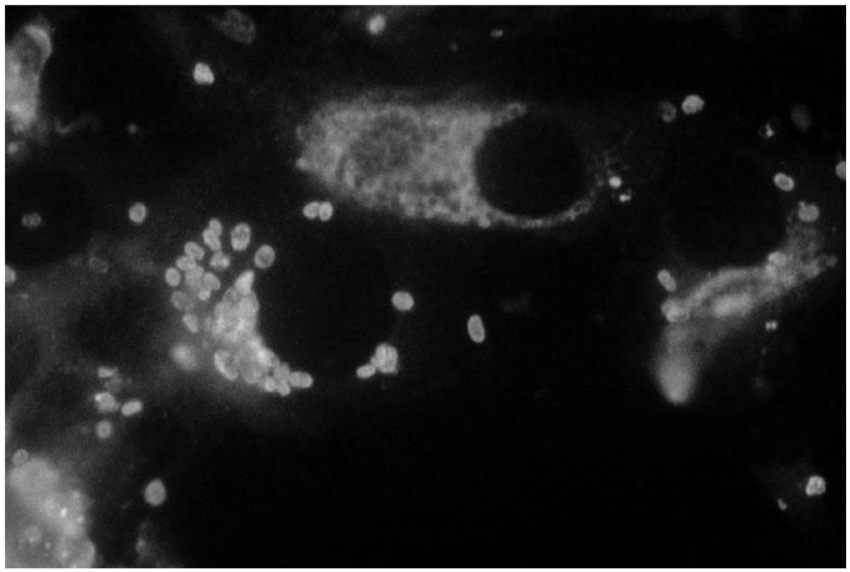
⇨ Because of *short duration of shedding* detection of spores less useful

⇨ Serodiagnosis (antibodies are long persisting) potential useful!

New specific IFAT test

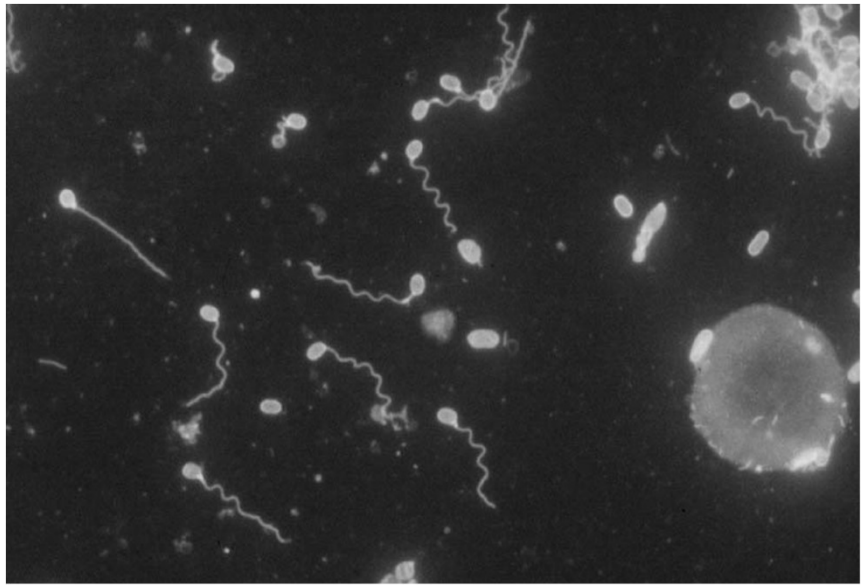


Different antigens recognized with different sera!



sporewall only

polar tube only



sporewall and polartube

Three groups studied (total : 1053 persons)

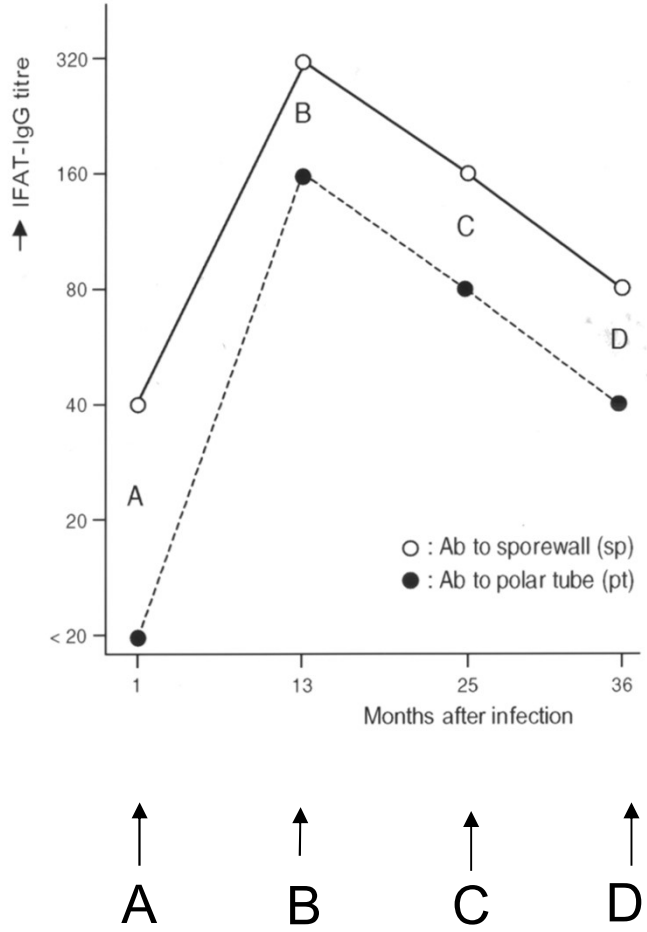
France (Paris): 490 pregnant women

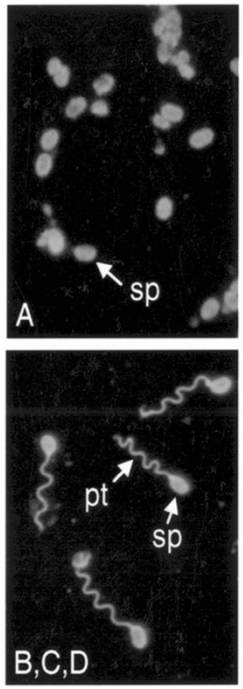
Netherlands : 210 pregnant women

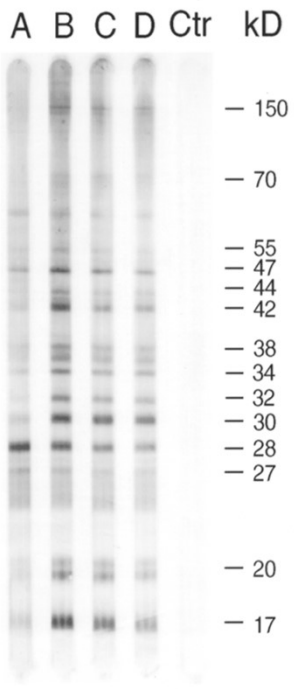
Netherlands: 350 (HIV- negative) homosexual men

Prevalence of specific antibodies up to 25%!!

Serological responses in immunocompetent person (1-36 months after infection)







Microsporidia from humans in other hosts and environment

How do people come in contact with microsporidia?

Humans: *E. bieneusi, E. intestinalis, E. hellem, E. cuniculi, Vittaforma corneae,, Nosema ocularum, Brachiola spp., Trachipleisthophora spp*., *Pleistophora spp and Microsporidum spp.*

Animals: +++
Enterocytozoon bieneusi i.e.: cats, chickens, dogs, goats, pigs, cattle, rats
Encephalitozoon intestinalis i.e.: donkeys, dogs, pigs, cows, goats, gorillas
Encephalitozoon cuniculi: i.e.: rabbits, rodents, foxes, goats, horses, birds
Encephalitozoon hellem i.e.: birds

Water: + *E. bieneusi, E. intestinalis,, Nosema, Pleistophora spp, Vittaforma cornea*

Food: +/- *Pleistophora* spp.

Insects: +/- *Brachiola algera, Nosema cornea*

Microsporidia are (indeed) close by……

studies among Dutch pigeons

Study of 331 pigeon feces collected in several townships of Amsterdam

Examined for psittacosis: 26 positive (7.9 %)

Frequent Occurrence of Human-Associated Microsporidia in Fecal Droppings of Urban Pigeons in Amsterdam, The Netherlands[▽]

Aldert Bart,[1]* Ellen M. Wentink-Bonnema,[1] Edou R. Heddema,[1,3] Jan Buijs,[2] and Tom van Gool[1,4]

Sequence confirmed *Microsporidia* positive PCR: 41/331 (12%)
pigeon feces positive for microsporidia

36/331 (11%) contain human pathogens:

18 (5.4%) *Enterocytozoon bieneusi*
11 (3.3%) *Encephalitozoon hellem*
6 (1.8%) *Encephalitozoon cuniculi*
1 (0.3%) *Encephalitozoon intestinalis*

5/331 other Microsporidia

Examined for psittacosis (331): 26 positive (7.9 %)* !

Contact of humans with microsporidia easy:

Sweeping surfaces which are contaminated with excreta (guano) from pigeons……*

- Roof building in Baltimore:
- stay for 10 - 550 pigeons/ per day
- effect of sweeping 30 min for humans:

Air, personal sampler: ingestion 3500 viable spores *E. bieneusi* in 30 min.!!!

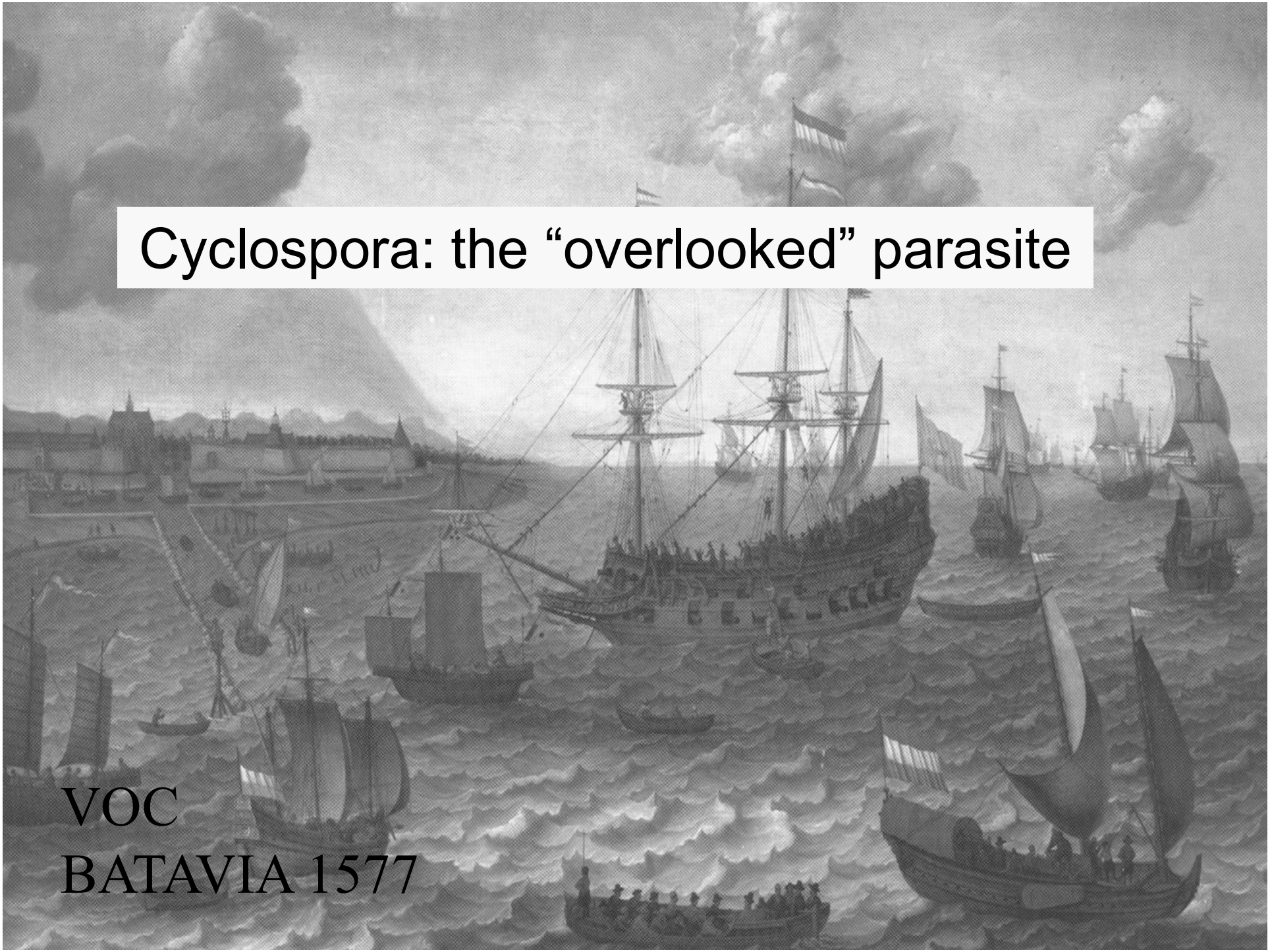
• Graczyk et al, 2007 Applied Environdmental Microbiology

Microsporidia and humans

- Frequent contact microsporidia with humans
- With proper immune system, in healthy persons, infection most often early aborted
- Can be cause of pathology not yet properly recognised (i.e.diarrhoea, encephalitis)

Pigeons excreta in the car:

On the sponge after washing : a nice microsporidia coctail with Enterocytozoon sp, Encephalitozoon sp, Vittaforma sp. and others……..


Cyclospora: the “overlooked” parasite
VOC
BATAVIA 1577

History

1979 Ashford. Papua New Guinea.
Undescribed coccidian parasite. Oocyts with 4 sporozoites

1986 Soave. Patients in Haiti and Mexico with diarrhea. Structures 8 -10 μm, defined wall, granular material inside: coccidian body/fungal spore.

JOURNAL OF CLINICAL MICROBIOLOGY, June 1990, p. 1101–1104
0095-1137/90/061101-04$02.00/0

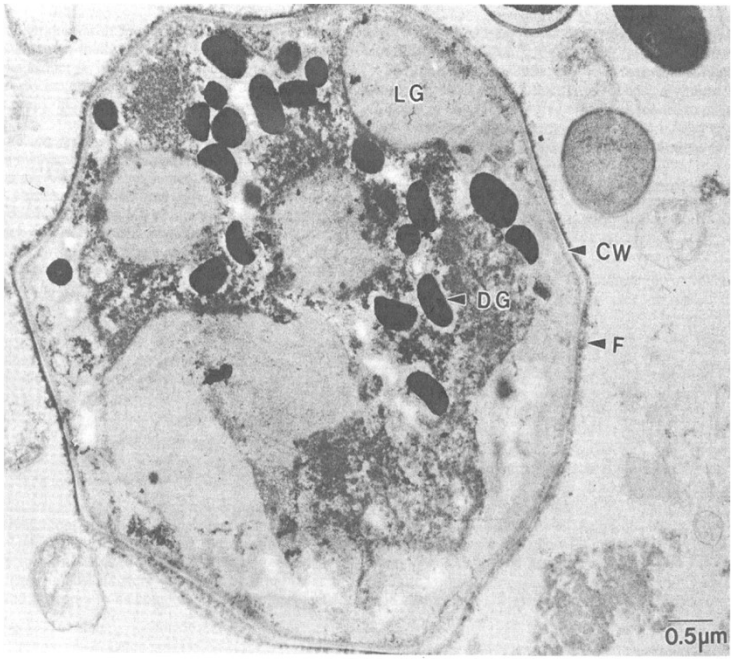
Vol. 28, No. 6

Alga Associated with Diarrhea in Patients with Acquired Immunodeficiency Syndrome and in Travelers

EARL G. LONG,[1]* ADELEH EBRAHIMZADEH,[2] ELIZABETH H. WHITE,[1] BILLIE SWISHER,[1] AND CAREY S. CALLAWAY[1]

Experimental Pathology Branch, Division of Immunologic, Oncologic, and Hematologic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333,[1] and Parasitology Laboratory, New York City Department of Health Bureau of Laboratories, New York, New York 10016[2]

Received 15 November 1989/Accepted 8 February 1990

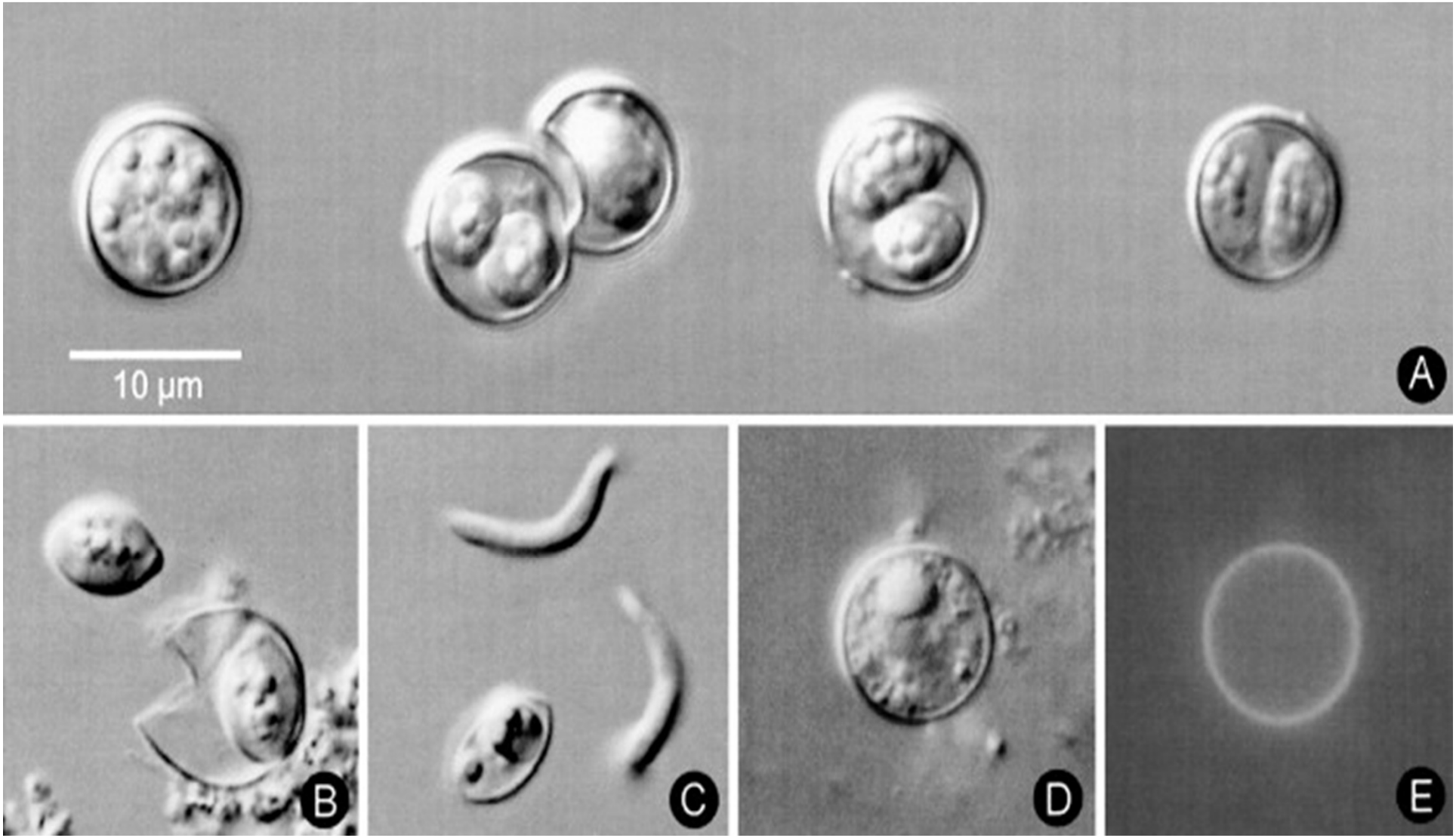


1992 Ortega. Peru.

Sporulation and excystation of oocysts in 2 sporocysts each with 2 sporozoites.

EM of sporozoites characteristic for coccidian protoza ! No alga at all…..

Developmental stages of oocysts of Cyclospora

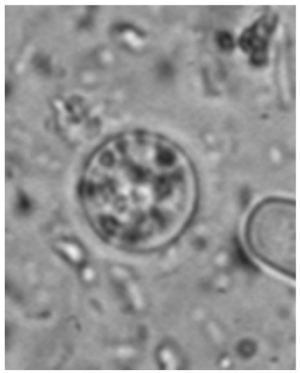


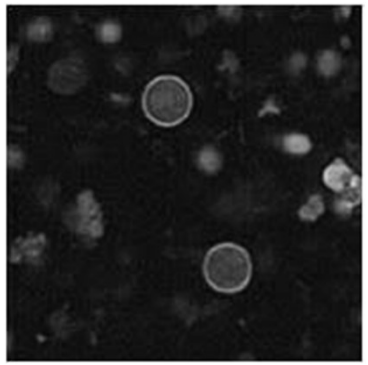
Coccidian parasite belongs to genus *Cyclospora*

- Oocyst with 2 sporocysts, each with two sporozoites
- Human infection due to other *Cyclospora* species not described
- *Cyclospora* spp. well known in insects, reptiles and rodents, especially moles. Animal cyclosporons have larger oocysts.
- Life cycle of human *Cyclospora* unknown: intermediate host can be involved

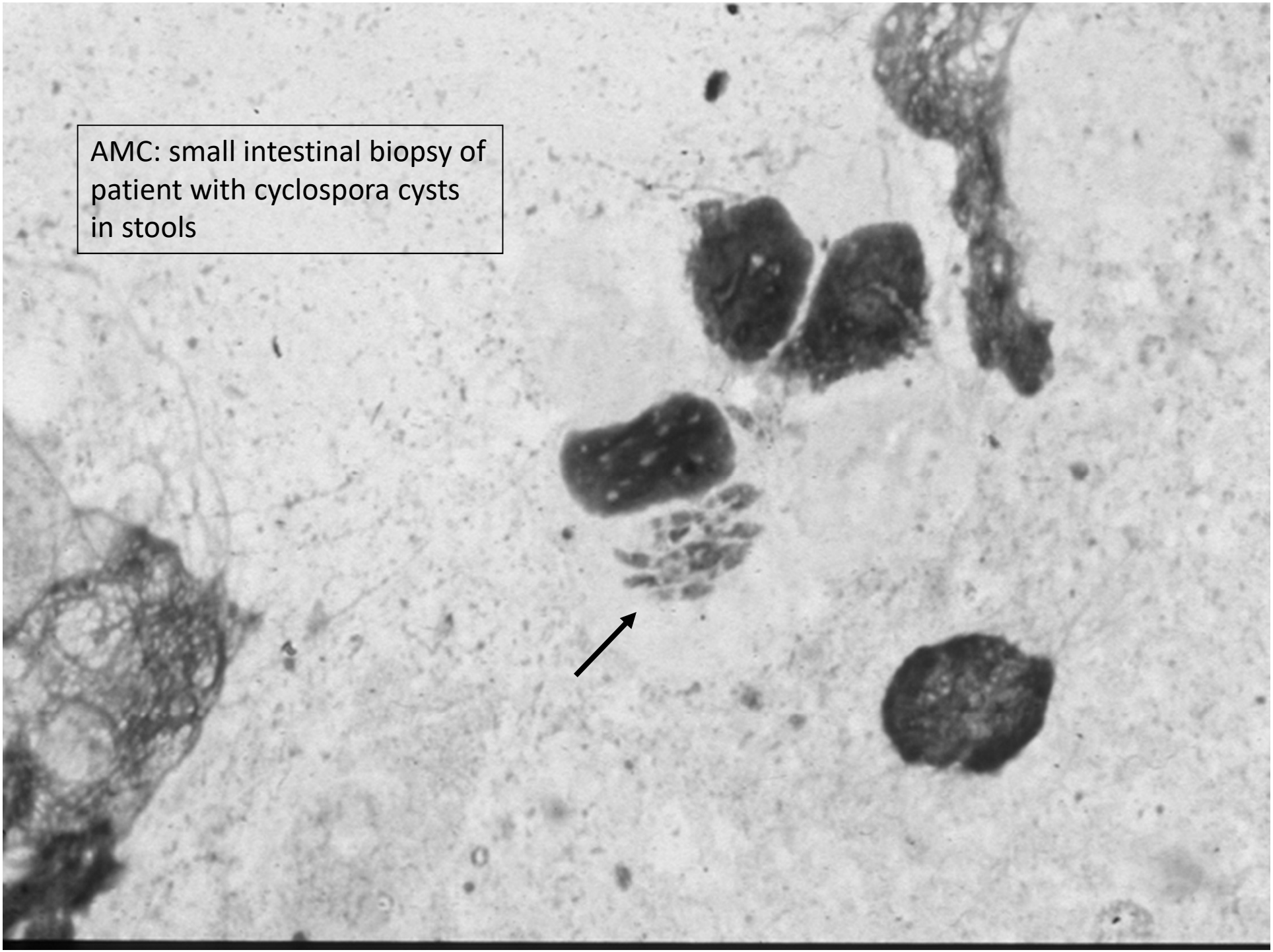
Diagnosis: relative easy with microscopy!

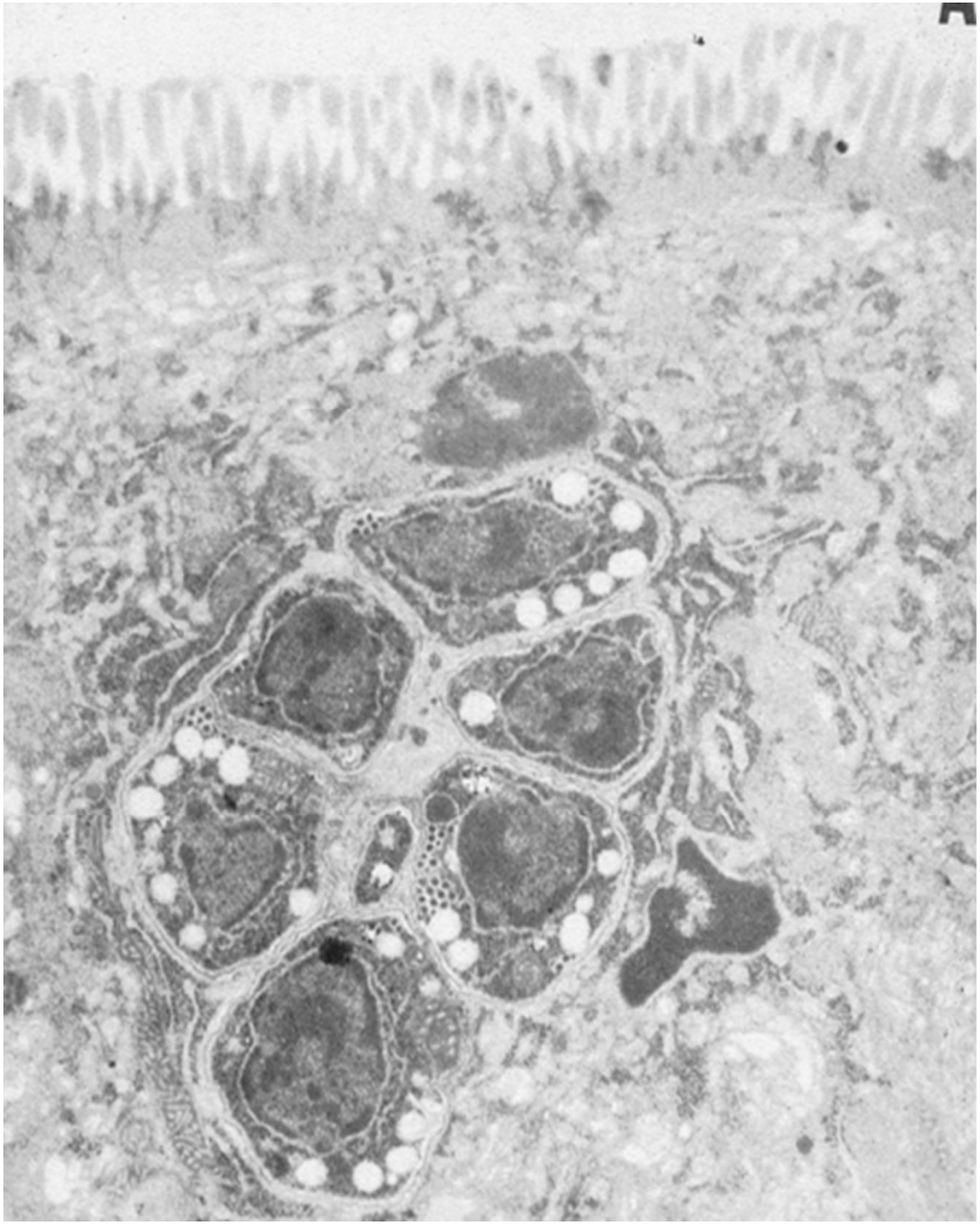
- 8-10 um with several globlets inside.
- when properly trained technicians “cannot miss it”!!



Parasites in stool but what is the site of infection in the intestine?


AMC: small intestinal biopsy of patient with cyclospora cysts in stools



Electron microscopy of
Cyclospora in small intestine

Symptomatology associated with Cyclospora infection

- patients often feel very ill!

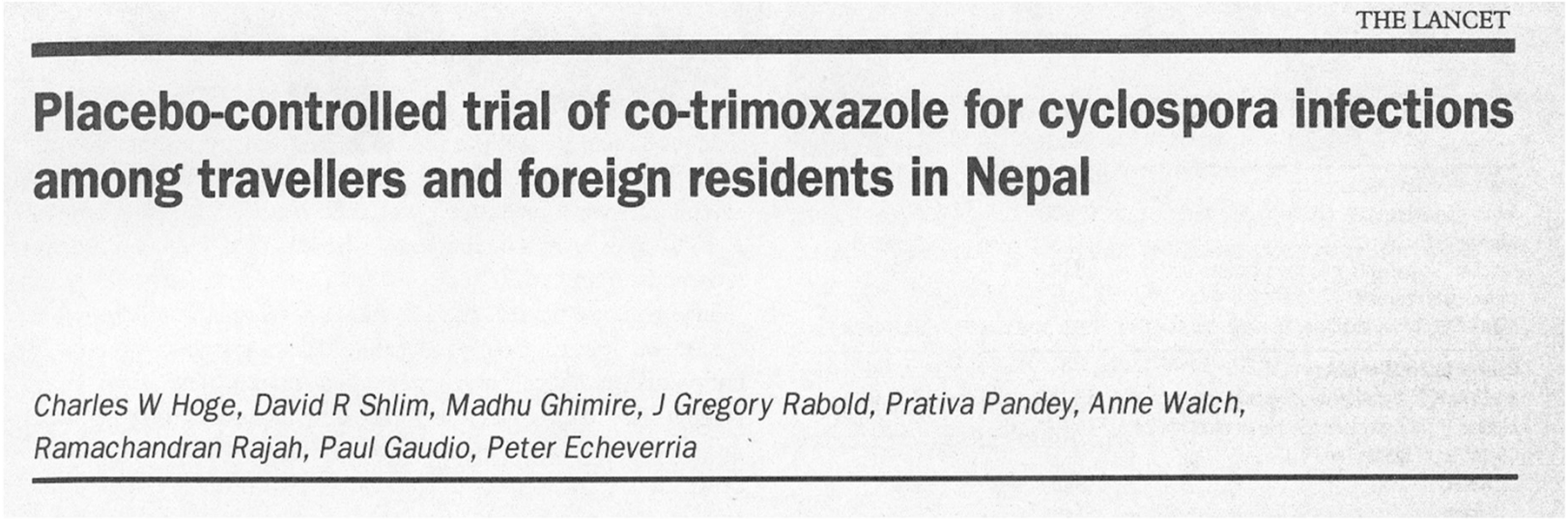
- predominant symptom: watery diarrhea often in relapsing, cyclical pattern

- important **associated** symptoms:
  - heartburn-like symptoms, abdominal cramps
  - fatigue
  - anorexia, weight loss (up to 10 kg!) and vomiting

- infection may last for weeks (but is self-limiting)

Treatment of Cyclospora infection ?

AMC, Amsterdam: two patients treated with co-trimoxazole strong relief of complaints, but not sufficient evidence for proof of efficacy.



THE LANCET

Placebo-controlled trial of co-trimoxazole for cyclospora infections among travellers and foreign residents in Nepal

Charles W Hoge, David R Shlim, Madhu Ghimire, J Gregory Rabold, Prativa Pandey, Anne Walch, Ramachandran Rajah, Paul Gaudio, Peter Echeverria

Lancet: March 1995

In case of severe complaints:

Co-trimoxazole (trimethoprim - sulfamethoxazole (160/800 mg) 2x dd for 7-10 days

Fast effect after start of treatment!

But….

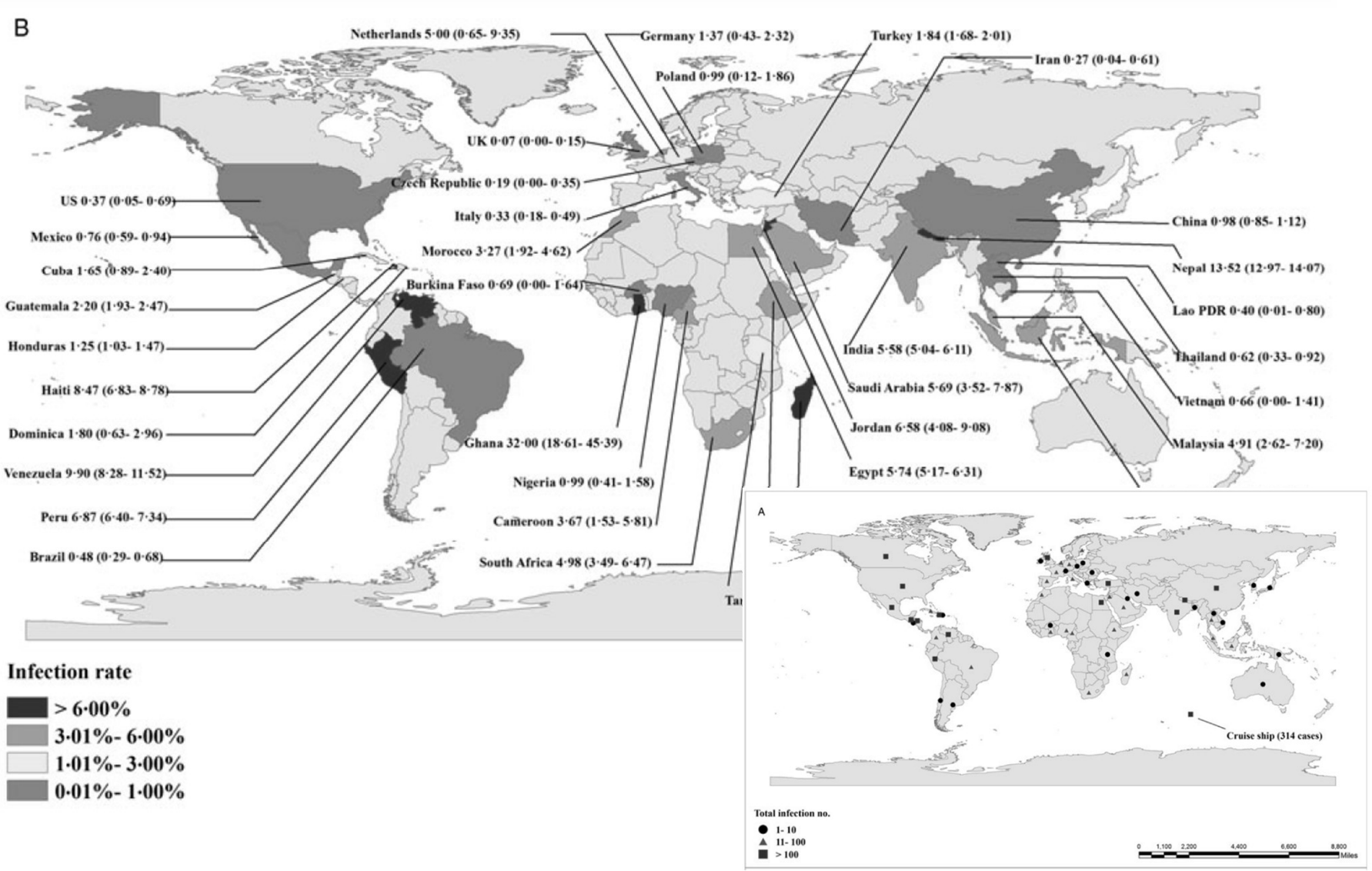
Cyclospora infection is self-limiting:

symptoms mild or absent: no treatment !

Epidemiology

- High prevalence in tropical countries
- Low prevalence in western countries
- Occasionally observed in travellers from tropics

Prevalence Cyclospora infection worldwide



Junqiang Parasitology 2019

Transmission: contaminated food (i.e.produce), water..

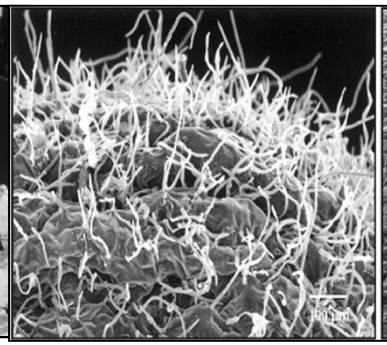
Prevalence:
many countries

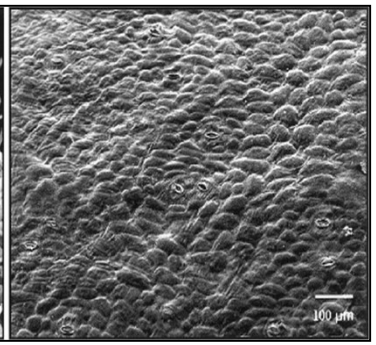
- Latin America
●Guatamala
● Peru
● Mexico
- India
- SE Asia
- Also USA….

USA 2018: multiple outbreaks > 2000 cases (no int. travel)

A foodborne outbreak of
Cyclospora cayetanensis at a wedding

Fleming C.A. et al. Arch Intern Med
Vol 158, May 25, 1998



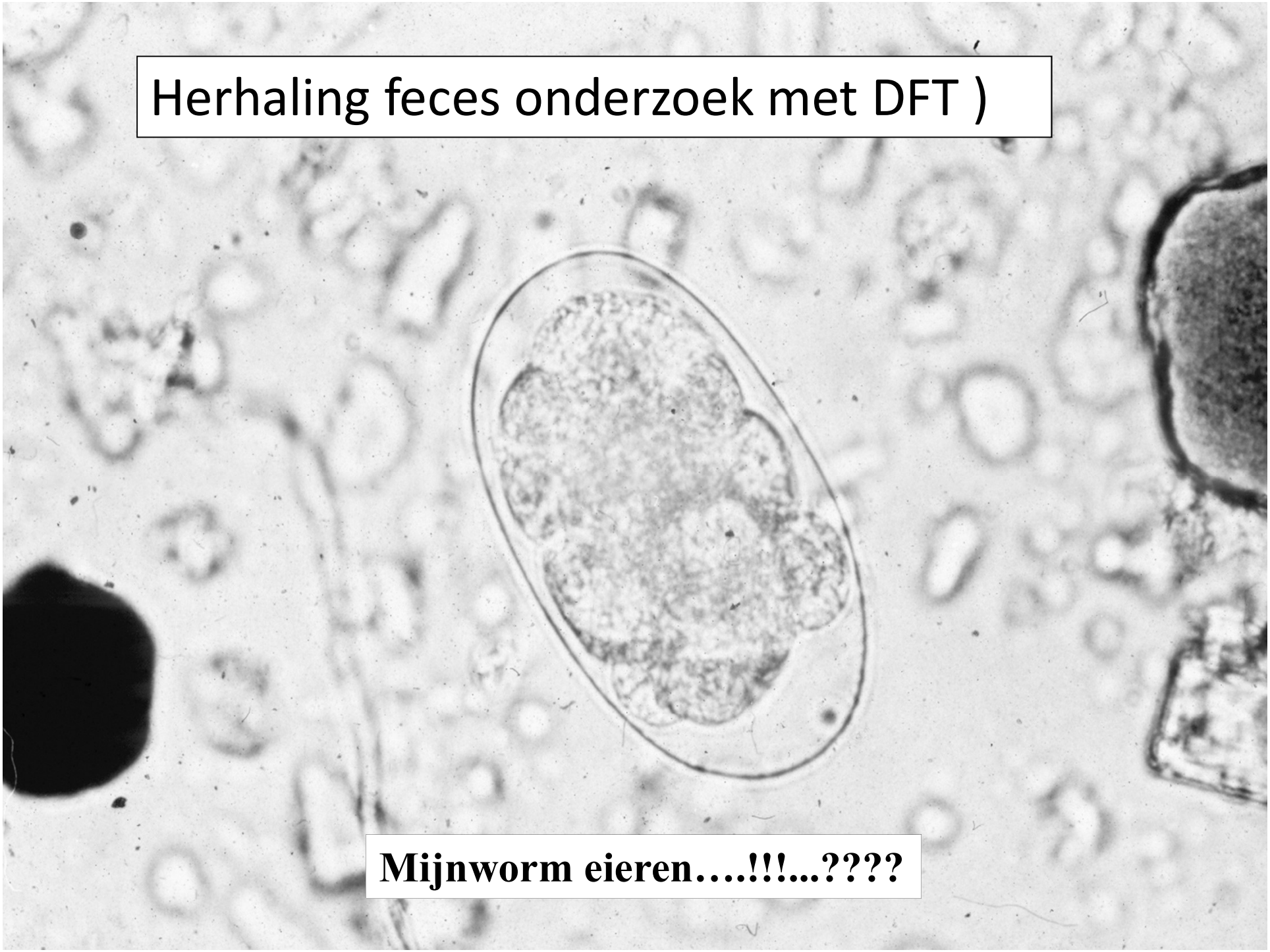


Contaminated raspberries from Guatamala

Heftige jeuk én productie van wormeieren in de darm bij reizigers…..

Michèle van Vugt,
Aldert Bart,
Tom van Gool

Herhaling feces onderzoek met DFT)

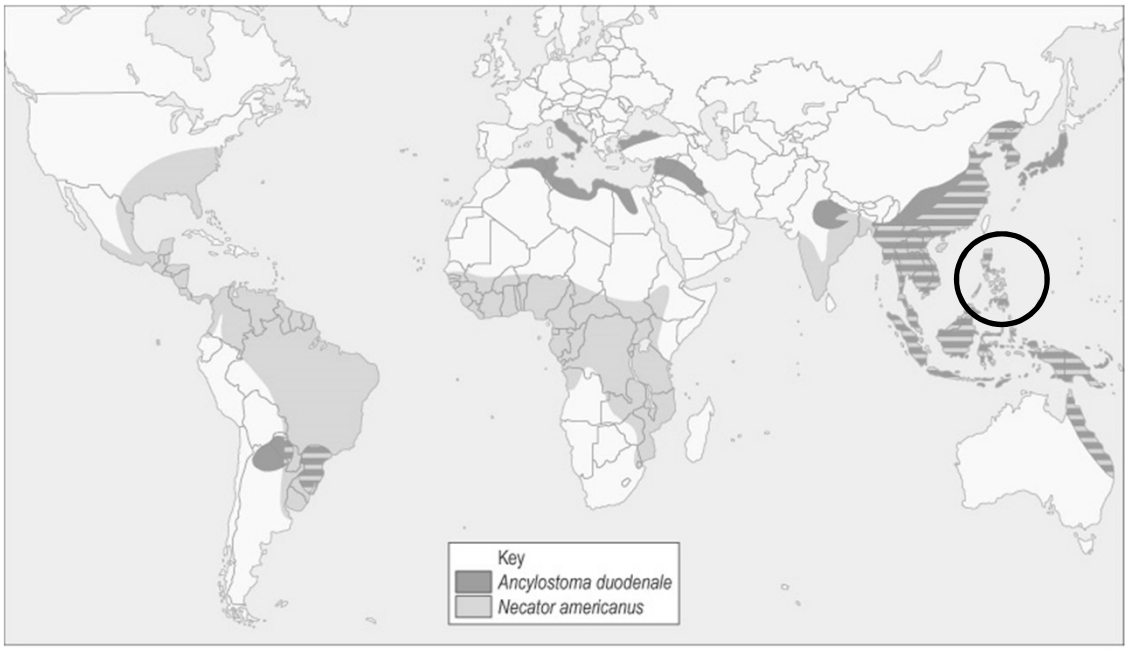


Mijnworm eieren….!!!...????

Mijnwormen bij de mens

- Infectie met mijnwormen komt frequent voor: naar schatting bij 1 miljard mensen waarvan de helft in de Azië/ Pacific

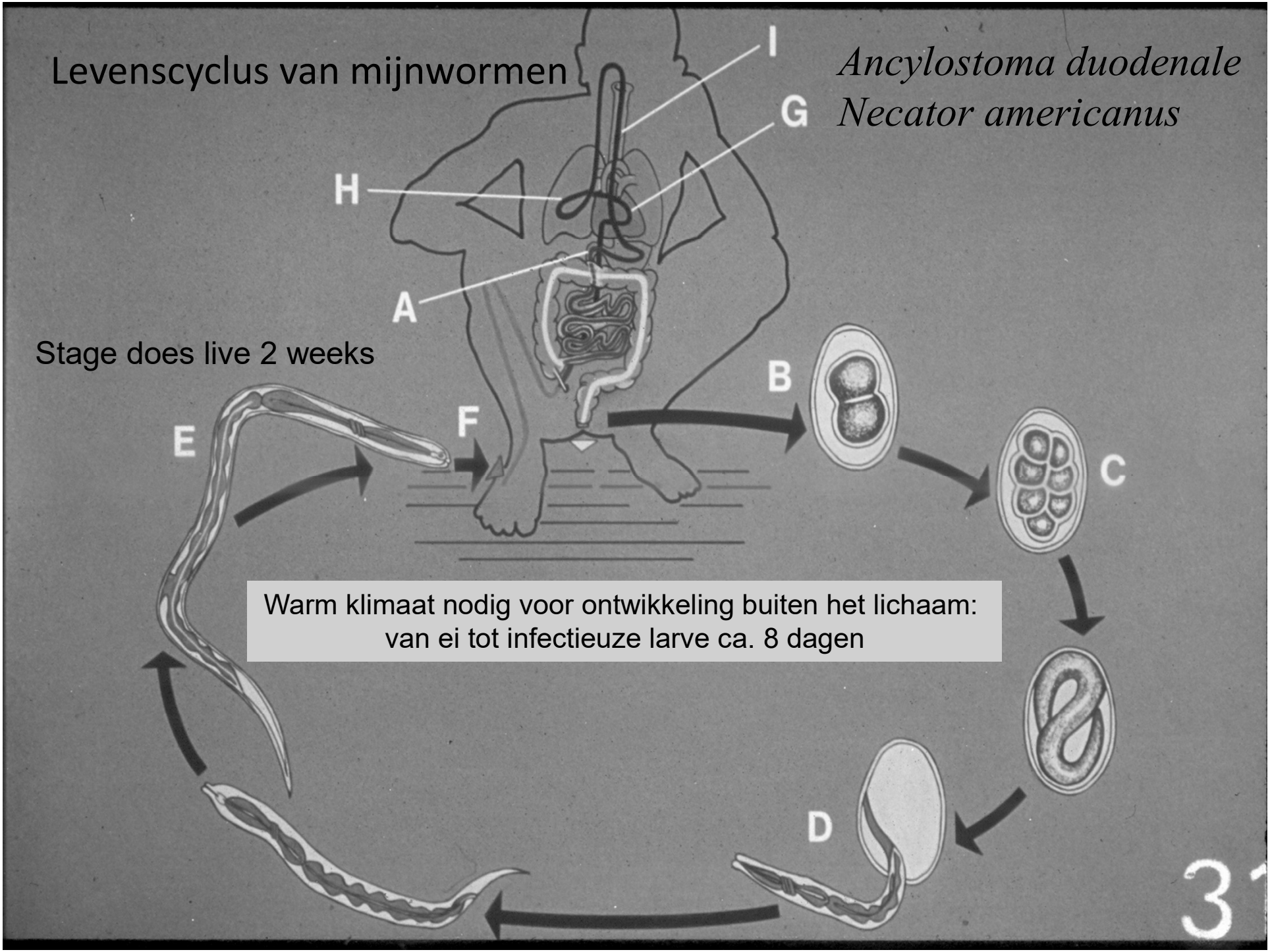
- Bij de mens de meest voorkomende soorten *Ancylostoma duodenale en Necator americanus*



Peters and Pasvol: Atlas of Tropical Medicine and Parasitology 6th edition © 2006 Elsevier Ltd

Levenscyclus van mijnwormen

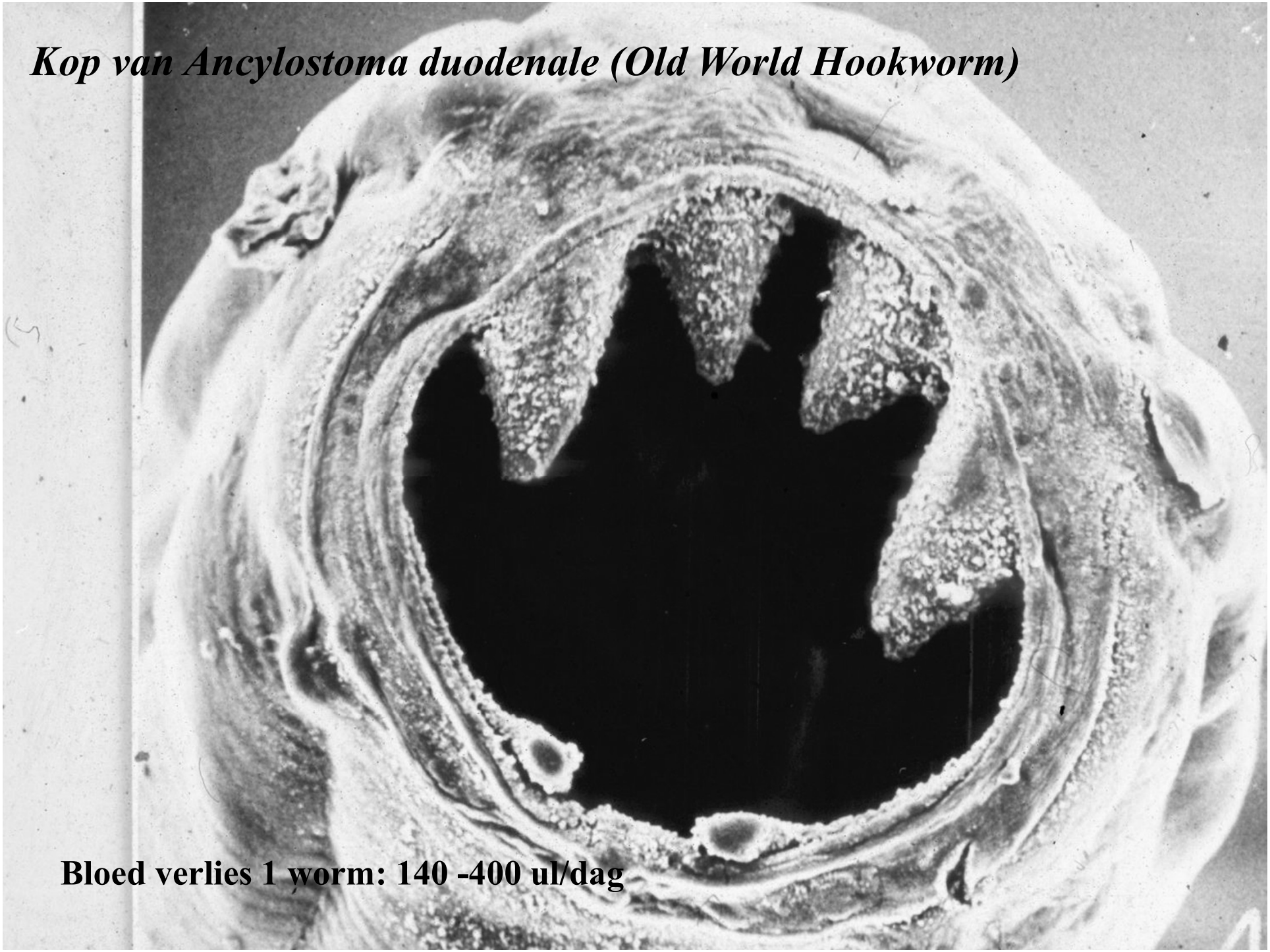
Ancylostoma duodenale
Necator americanus



Filariforme larvae van mijnwormen penetreren de huid…..

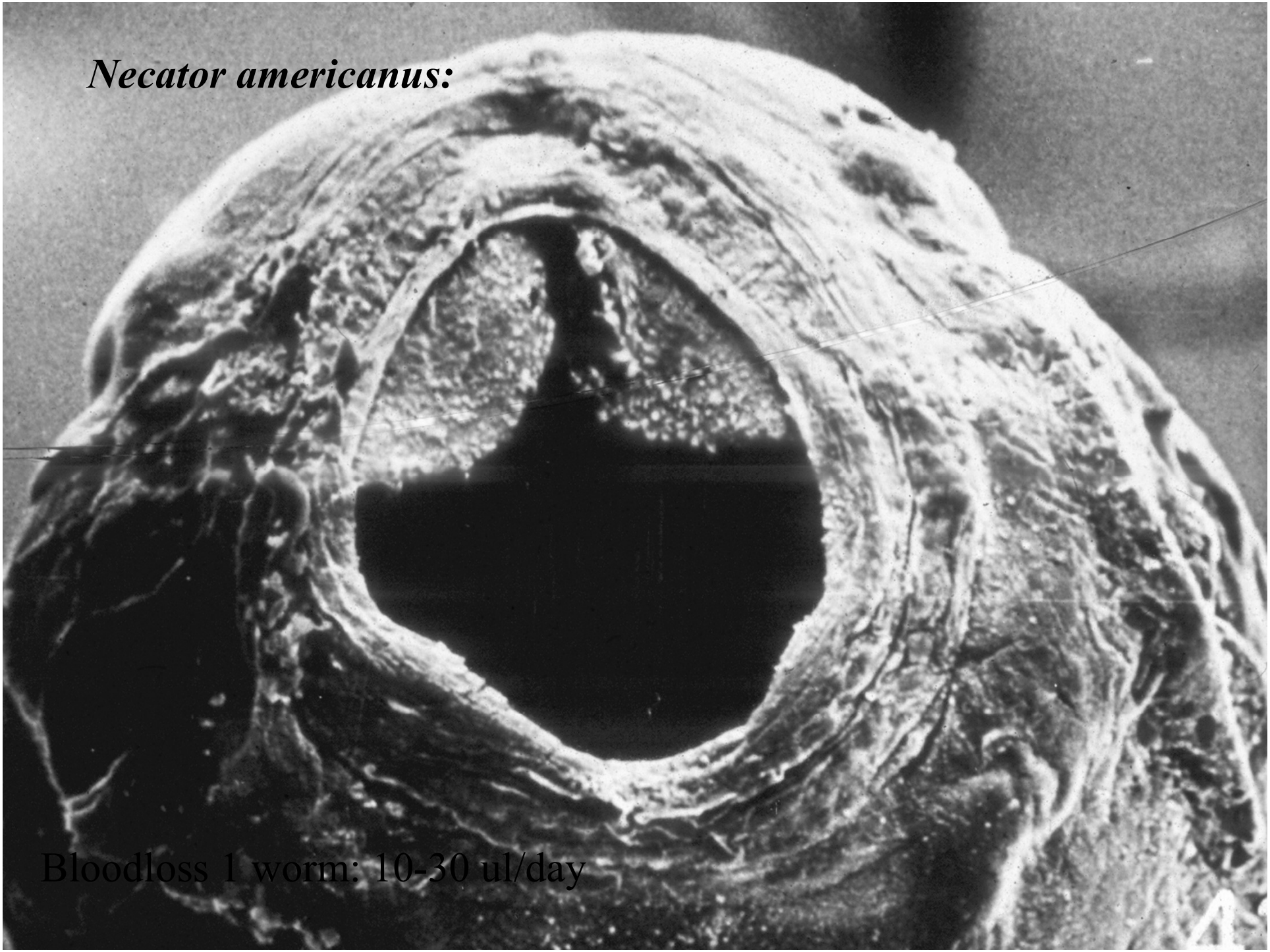
hierbij vorming van papels die na enkele dagen verdwijnen


Mijnwormen zitten vast aan wand van de dunne darm
Wormen kunnen 8-16 jaar overleven!

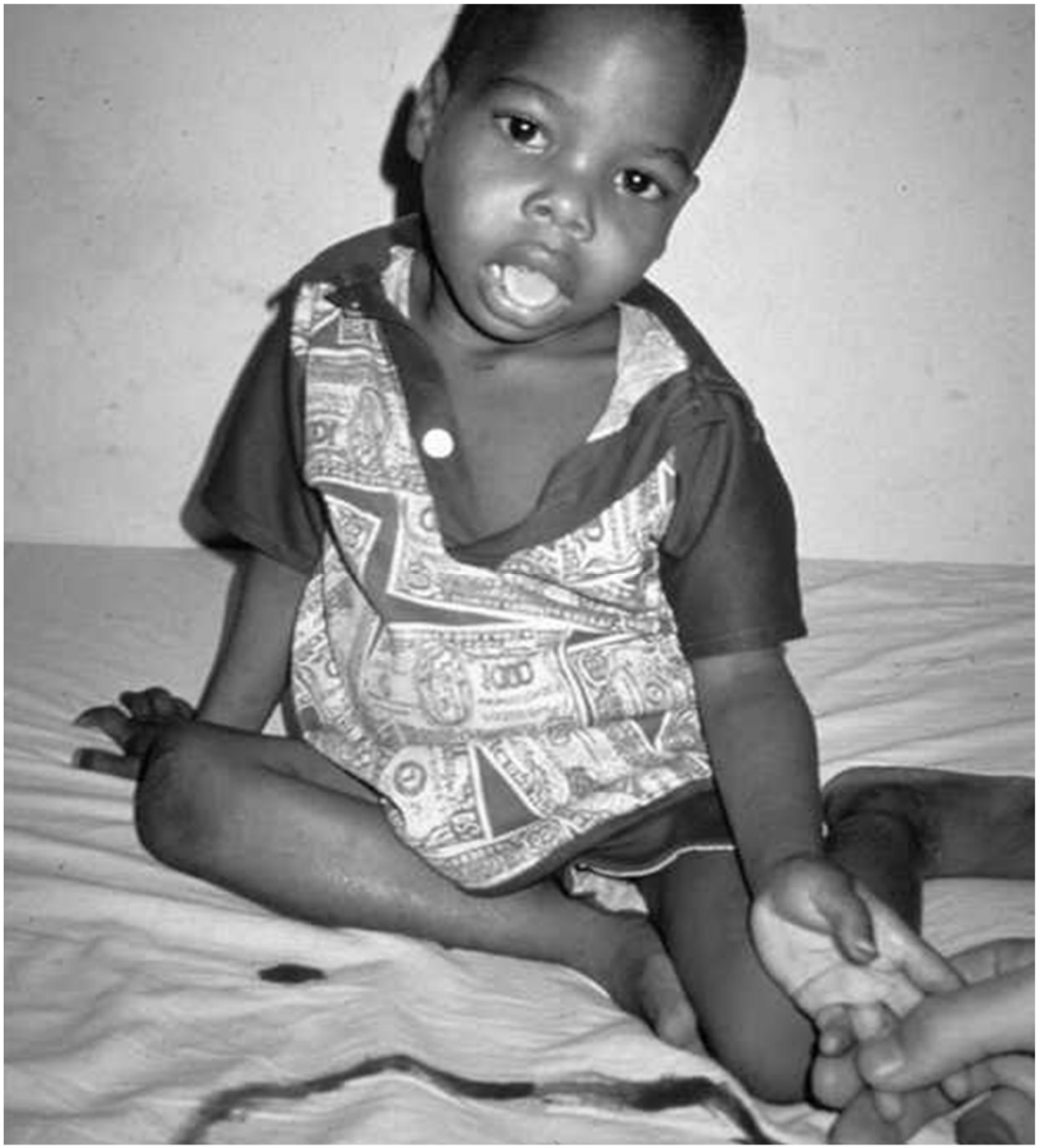


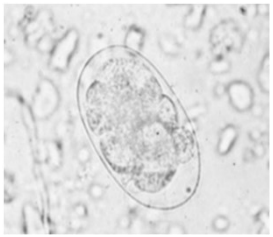
Kop van Ancylostoma duodenale (Old World Hookworm)

Bloed verlies 1 worm: 140 -400 ul/dag


Necator americanus:
Bloodloss 1 worm: 10-30 ul/day

Belangrijkste symptoom mijnworminfecties: ernstige anaemie





Welke mijnworm-soort bij de patiënten?

Afmetingen van eieren van wormen in feces van vrouw en man

Afmetingen eieren:	*Ancylostoma duodenale*	L 60	x B 40 um
(literatuur)	*Necator americanus*	L 64 -75	x B 40 um

M: Gem: L: 59,25 um en B: 38,95 um

V: Gem: L: 57,6 um en B: 36,0 um

Grootte van eieren passen *mogelijk* bij een infectie met *A. duodenale*. Maar: grootte van eieren is niet bewijzend voor de soort!!

Het lijkt een duidelijke zaak….

echter….. de huidbeelden bij man en vrouw zijn ernstiger dan normaliter bij infectie met humane mijnwormen (*A. duodenale en N. americanus)* !!

Is er een mijnworm infectie waarbij zowel een heftig huidbeeld als de *productie van eieren in de darm* voorkomt?

Huidafwijkingen bij de mens door worm infecties

Larva currens
Strongyloides stercoralis

Cercariën dermatitis
Zoönotische schistosomen

Creeping eruption (larva migrans)
<u>Zoönotische</u> <u>mijnwormen</u>
A. braziliensis, A. canium, A.ceylanicum

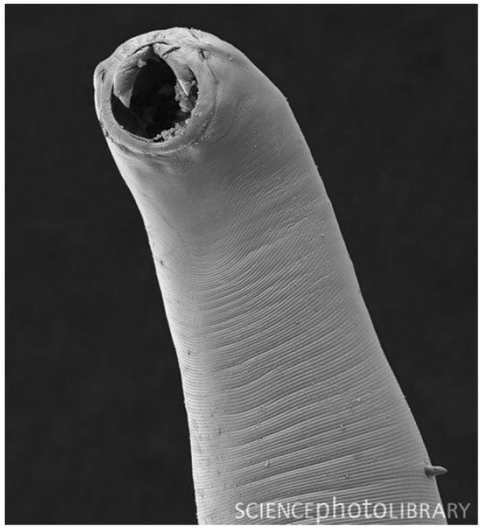
Zoönotische mijnworminfecties ?

A. braziliensis en A. canium minder waarschijnlijk: bij infectie bij de mens: geen patente infectie (= eiproductie), en huidafwijkingen bij patiënten lijken niet op creeping eruption.

Resteert: Ancylostoma ceylanicum

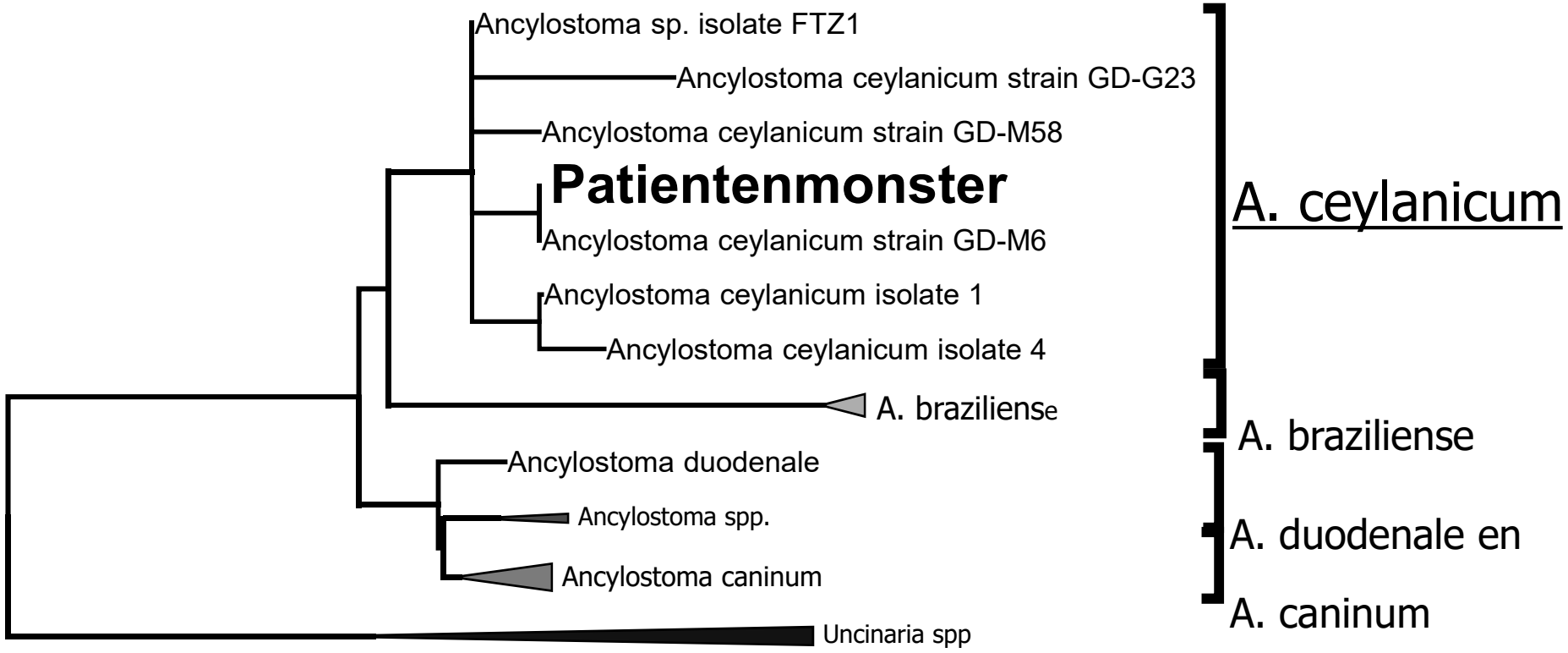
- *mijnwormen van (vooral) honden en katten*
- *komt ook voor bij mens, mét patente infectie (eiproductie)*
- *bij mens vaak heftige (allergische) reactie op zich ontwikkelende wormen: buikpijn (bekend) en huidafwijkingen* (*minder bekend*)

A. ceylanicum



Aanvullende moleculaire diagnostiek op microscopisch positieve feces

PCR Strongyloides spp (incl S. fulleborni): negatief
PCR mijnwormen: positief, obv sequentieanalyse
Ancylostoma ceylanicum !



AB11

Ancylostoma ceylanicum

- Voor het eerst beschreven in 1911

- Van 1930 tot ca 1950 discussie over status (dd A. b*razilien*sis)

- Microscopisch (eieren) niet goed te onderscheiden va*n*
  A. duodenale / N. americanus: decennia "neglected parasite"

- Infectie via de huid of oraal, longpassage, en ontwikkeling in darm

  Bij mens vaak heftige allergische reacties op ontwikkeling parasiet o.a. met buikpijn

AB11 A. Bart; 20-05-2019

AB11

Ancylostoma ceylanicum

- *Met moleculaire identificatie: komt zeer frequent voor in ZO Azië*

- *Schatting: 19-73 miljoen patiënten geïnfecteerd met de parasiet in Thailand, Laos en Maleisië.*

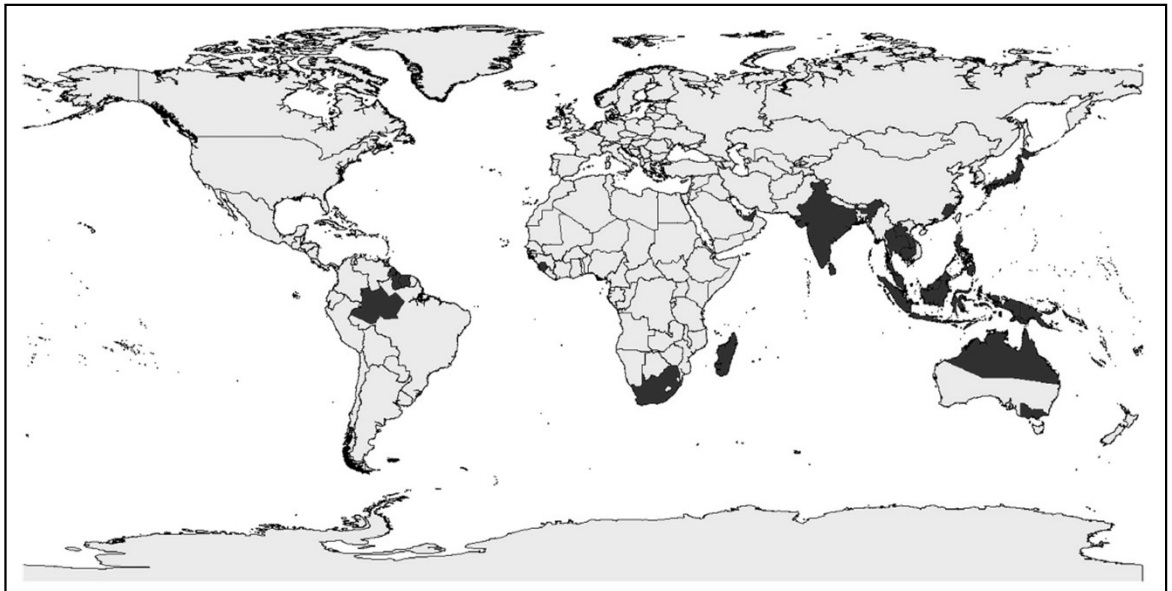


Fig. 1. The reported geographical distribution of *Ancylostoma ceylanicum* (1911-present). This map was created using ArcGIS® software by Esri© (http://www.esri.com).

Traub, Int. J. Parasit. 2013

AB11 A. Bart; 20-05-2019

Met dank voor de attentie …en

Zoekt en gij zult vinden!!