

Retrospective species identification of microsporidian spores in diarrhetic fecal samples from HIV/AIDS patients by multiplexed fluorescent *in situ* hybridization (FISH)

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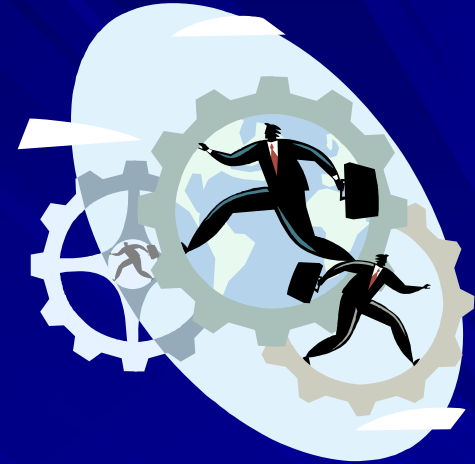
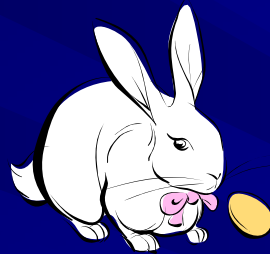
Source of Human Infection and Transmission

■ UNCERTAIN

■ Person-to-person transmission

■ Zoonosis

■ Environmental source (waterborne transmission?)

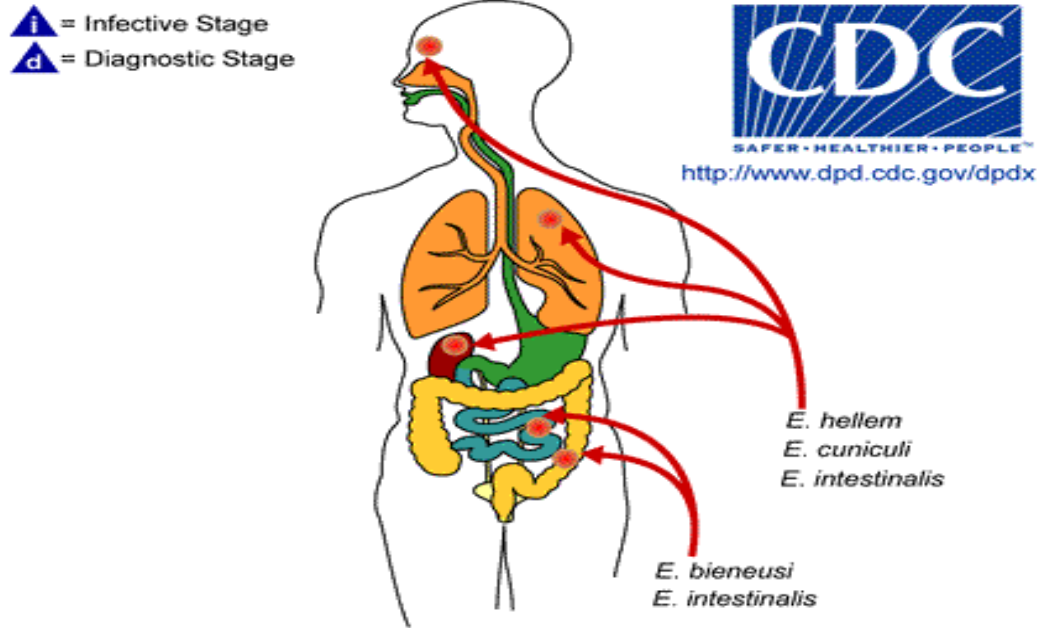


Microsporidia Species Associated with Infection in Man (7 genera)

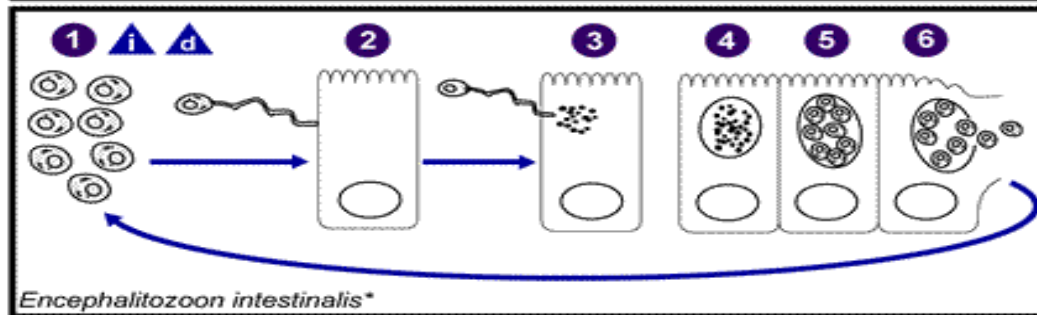
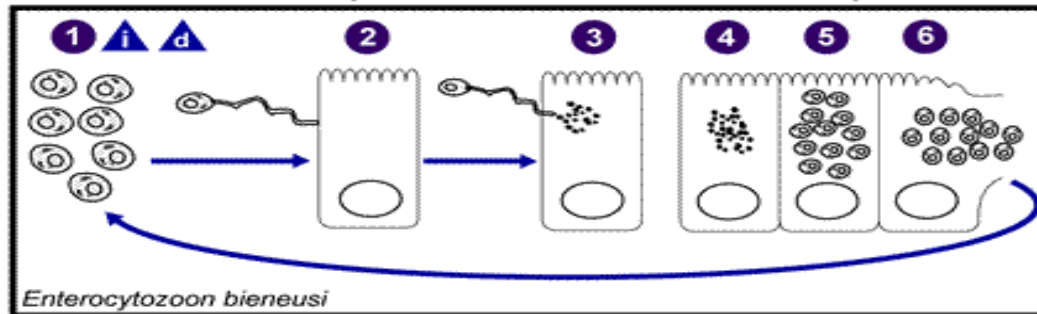
- *Encephalitozoon cuniculi* - peritonitis, hepatitis, disseminated infection (**HIV/AIDS, non-HIV/AIDS**)
- *E. hellem* - pneumonia, disseminated infection, keratoconjunctivitis (**HIV/AIDS**)
- *E. intestinalis* – diarrhea, disseminated infection (**HIV/AIDS**)
- *Enterocytozoon bieneusi* – diarrhea (**HIV/AIDS, non-HIV/AIDS**)
- *Nosema corneum* – keratitis (**non-HIV/AIDS**)
- *N. ocularum* – keratitis (**non-HIV/AIDS**)
- *Vittaforma corneae* – keratitis (**non-HIV/AIDS**)
- *Brachiola (Nosema) algerae* – keratitis, skin infection, myositis (**non-HIV/AIDS**)
- *Brachiola vesicularum* – myositis (**non-HIV/AIDS**)
- *Pleistophora sp.* – myositis (**non-HIV/AIDS**)
- *Trachipleistophora hominis* – myositis (**non-HIV/AIDS**)
- *T. anthropophthera* – myositis (**non-HIV/AIDS**)
- *Microsporidium ceylonensis* – corneal ulcer (**non-HIV/AIDS**)
- *Microsporidium africanum* – corneal ulcer (**non-HIV/AIDS**)

Life Cycle

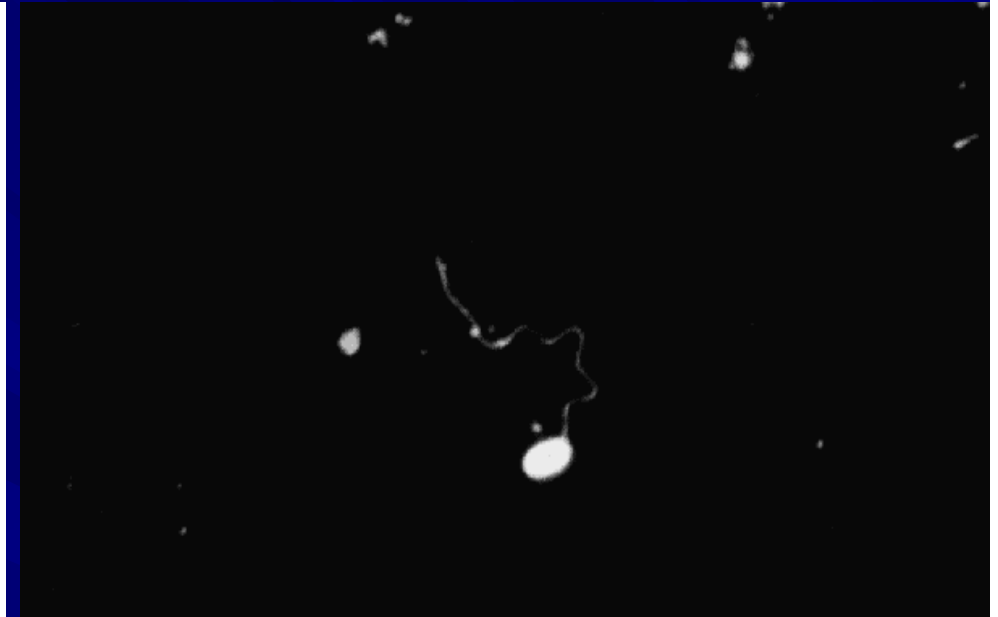
Microsporidian spores



Intracellular development of *E. bienersi* and *E. intestinalis* spores.



*Development inside parasitophorous vacuole also occurs in *E. hellem* and *E. cuniculi*.



- ✓ **Identification of human-virulent microsporidian spores represents a challenge because microsporidia can infect a variety of non-human hosts, and spore morphology is insufficient for species identification**
- ✓ **Until recently, microsporidia species have rarely been considered in differential diagnosis of opportunistic infections in HIV/AIDS patients**
- ✓ **Species-specific identification of microsporidian spores is essential for advising HIV/AIDS patients on how to avoid exposure, as the epidemiology of microsporidia varies considerably**
- ✓ **Identification of microsporidian spore species is essential for prompt and proper pharmacological therapy in order to reduce the risk of progression to disseminated infection with fatal outcome since different treatments may be indicated depending on the species identified**

Species Identification Options

PCR

and

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Simultaneous Detection of Four Human Pathogenic Microsporidian Species from Clinical Samples by Oligonucleotide Microarray

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The Graczyk's Lab: Multiplexed Fluorescent *In Situ* Hybridization (FISH)

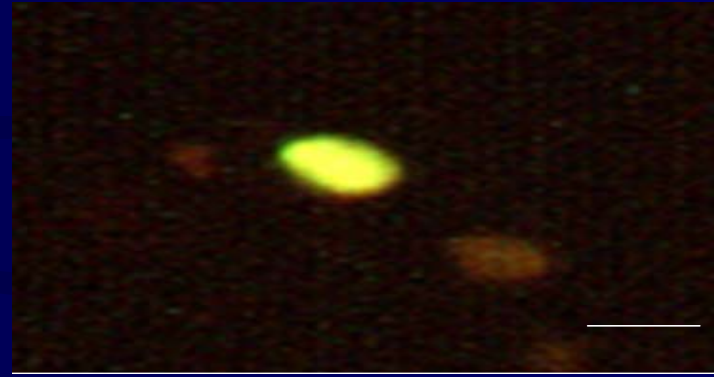
E. hellem Hester et al. (2000) *J Eukaryot Microbiol* 47:299-308.

Graczyk et al. (2007) *J Clin Microbiol* 45:1255-60

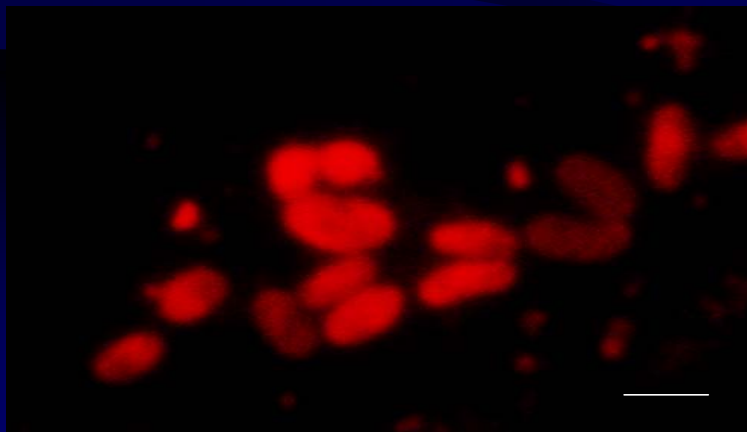
E. hellem



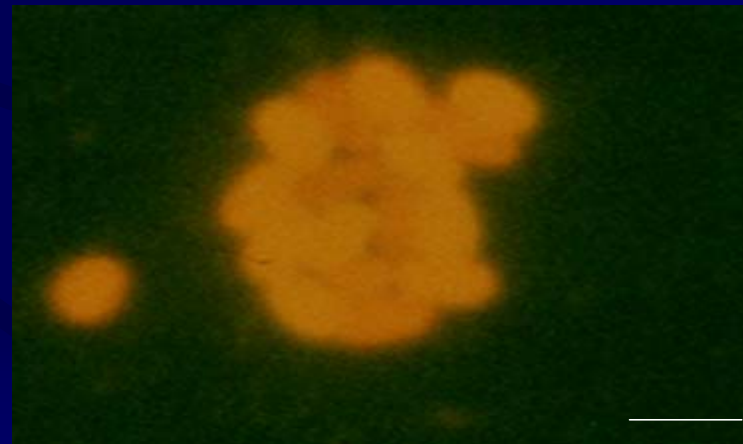
E. bieneusi



E. intestinalis



E. cuniculi

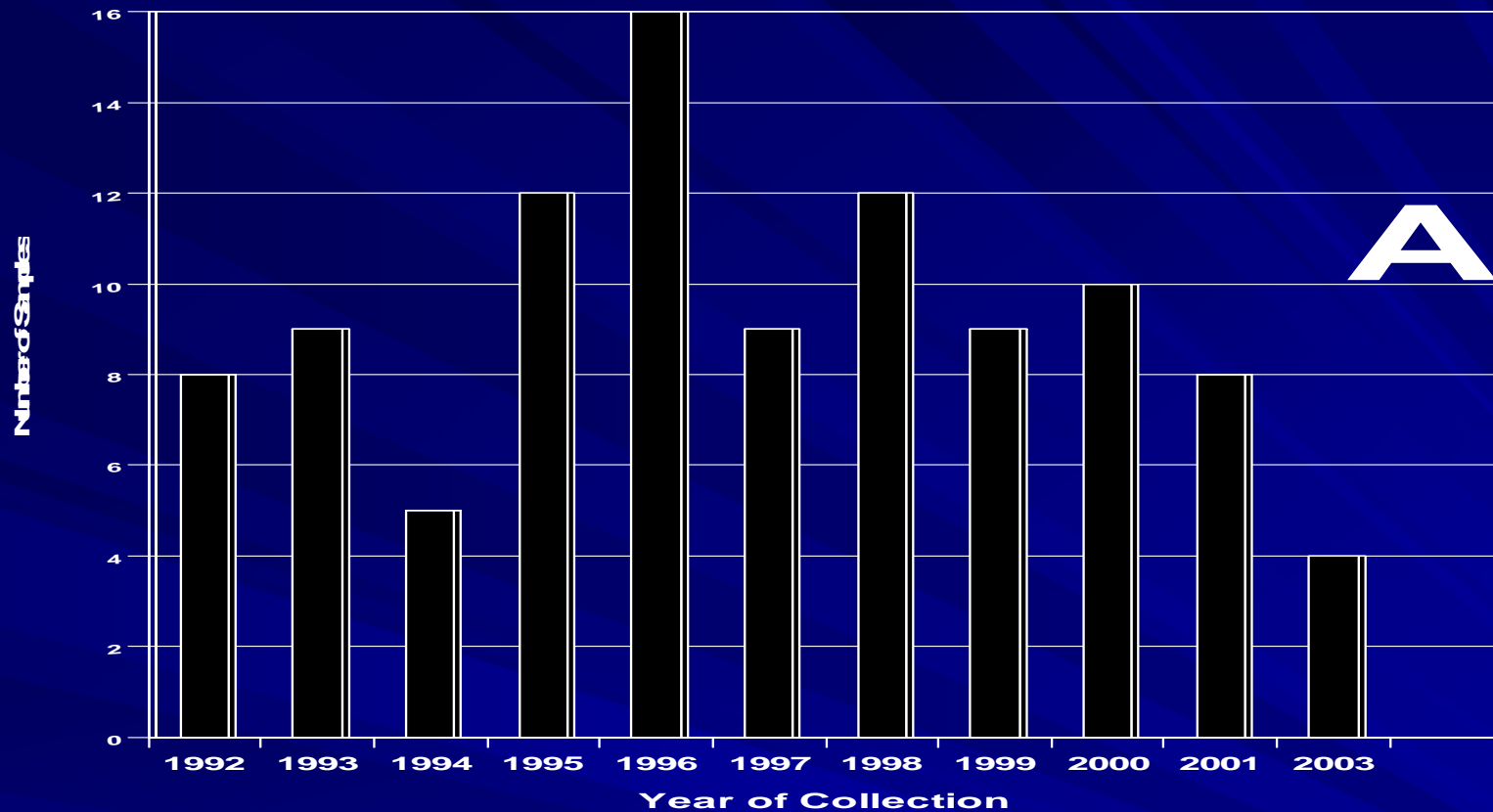




Encephalitozoon intestinalis spores

Fluorescence *In Situ* Hybridization

110 diarrhetic HIV/AIDS fecal samples



**Concentration
and Purification**

Chromotrope-2R

**CalcoFluor White
2MR**

**Multiplexed FISH
assay**

Procedure

1. Permeabilize in Acetone
2. Wash w/ Hybridization buffer
3. Hybridize with probe for 3hrs at 57°C
4. Wash w/H₂O
5. Make slides
6. Examine with an epifluorescent microscope

RESULTS

77 of 110 (70.0%) direct fecal smears were positive for microsporidian spores by Chromotrope-2R and CalcoFluor White M2R stains ($P < 0.05$). Conversion to a spore-negative status was observed among the oldest samples, i.e., from 10 to 14-yr old

94 of 110 (85.5%) samples were positive by multiplexed FISH assay

- ✓ 49 - *Enterocytozoon bienersi*
- ✓ 43 - *Encephalitozoon intestinalis*
- ✓ 2 - *Encephalitozoon hellem* (confirmed by PCR)
- ✓ 9 - *E. bienersi* and *E. intestinalis*

Spore Concentration

E. bienersi 3.5×10^3 - 4.4×10^5 / ml (mean: 8.8×10^4 / ml) ($P < 0.001$)

E. intestinalis 2.3×10^2 - 7.8×10^4 / ml (mean: 1.5×10^4 / ml)

E. hellem 1.8×10^2 - 3.6×10^2 / ml (mean: 2.7×10^2 / ml) ($P < 0.02$)

Conclusions

- ✓ The reasons why multiplexed FISH assay did not identify microsporidian spores in 20 of 110 (18.2%) fecal samples verified to have spores at the time of collection, are not clear. The spores could be present in low concentrations and may have been lost during the purification and concentration steps. Alternatively, their rRNA could have been altered by the long-term formalin storage (i.e., 10 to 14 years)
- ✓ *Enterocytozoon bienewisi* was a predominant species in HIV/AIDS diarrhetic fecal samples
- ✓ Identification of *E. hellem* in two HIV/AIDS diarrhetic fecal samples is surprising as this species is predominantly reported in HIV/AIDS patients from extraintestinal, i.e., respiratory, infections. It is quite possible that patients with respiratory *E. hellem*-associated microsporidiosis swallow the spores which then can be present in their fecal samples
- ✓ Concentration of *E. bienewisi* spores observed in the present study (range; 3.5×10^3 - 4.4×10^5 /ml; mean; 8.8×10^4 /ml) falls within the reported ranges

Multiplexed FISH assay is a reliable, quantitative, molecular fluorescent microscopy method for:

- ✓ simultaneous detection of *E. bienersi*, *E. intestinalis*, *E. hellem*, and *E. cuniculi* spores in fecal samples of HIV/AIDS patients
- ✓ quantifying spore shedding intensity in intestinal microsporidiosis
- ✓ simultaneous detection of *E. bienersi*, *E. intestinalis*, *E. hellem*, and *E. cuniculi* spores in environmental samples

Graczyk TK, M Johansson, L Tamang, GS Visvesvara, LS Moura, AJ DaSilva, AS Girouard, O Matos. 2007. Retrospective species identification of microsporidian spores in diarrhetic fecal samples from HIV/AIDS patients by multiplexed fluorescent in situ hybridization (FISH). *Journal of Clinical Microbiology*: 45; 1255-60.

