



Join International Medicine Meeting 2007

'Health Security in the Tropics'

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Cryptosporidiosis: it is more than only an infection

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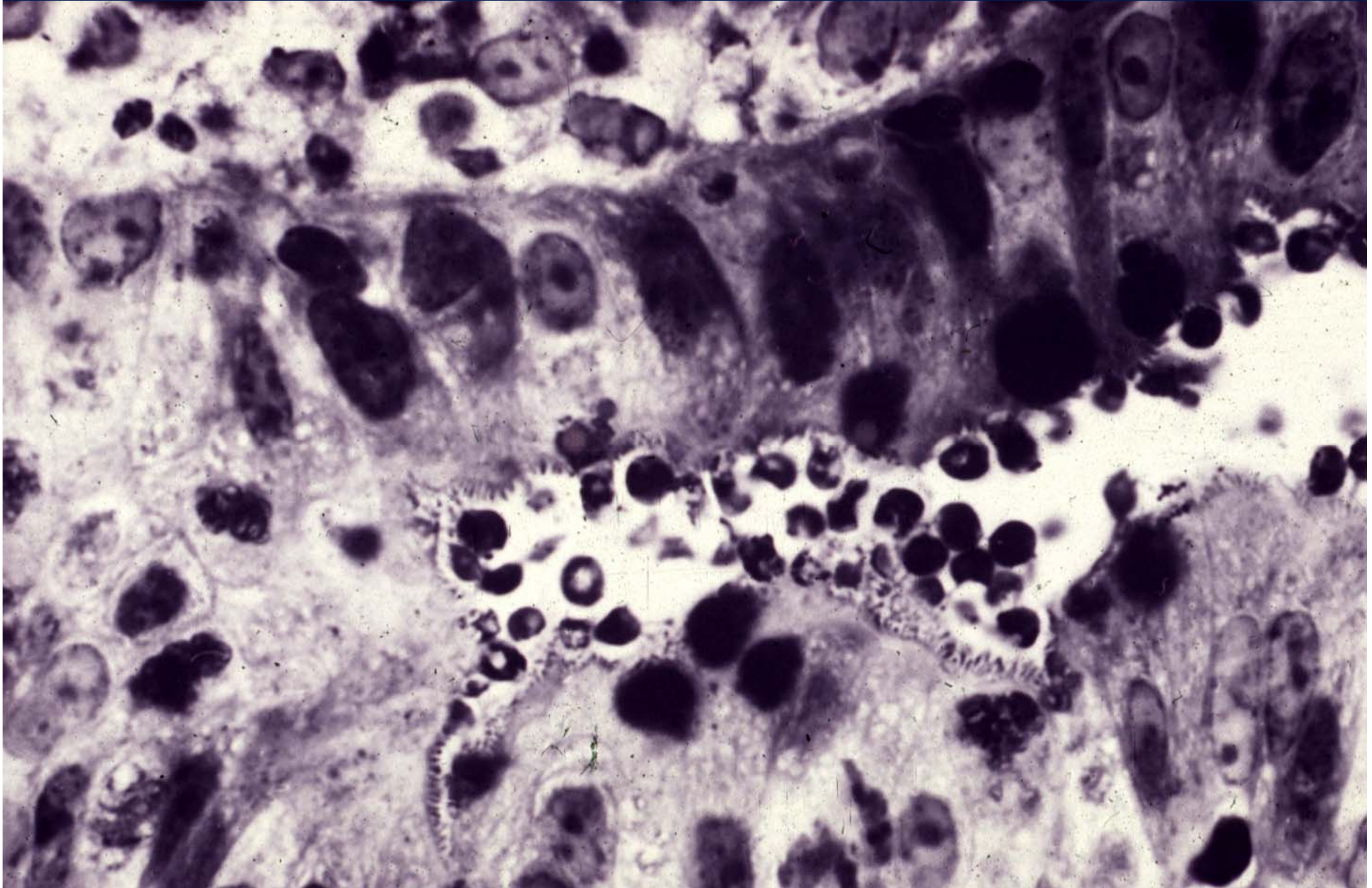
静岡畜産大学

原虫病研究センター

National Research Center
for Protozoan Diseases



Cryptosporidiosis/Pathogenesis: There is blunting or complete loss of villi; lamina propria infiltration with inflammatory cells may not correlate with clinical manifestations



Why *Cryptosporidium* research ?

- Cryptosporidiosis is an intestinal disease included in the 'NDI' by WHO. No drugs.
- *Cryptosporidium*: is water- and food-borne pathogen, ranked to the Category B Biodefense Pathogen.
- Is clinical and medical more relevant than previously believed.

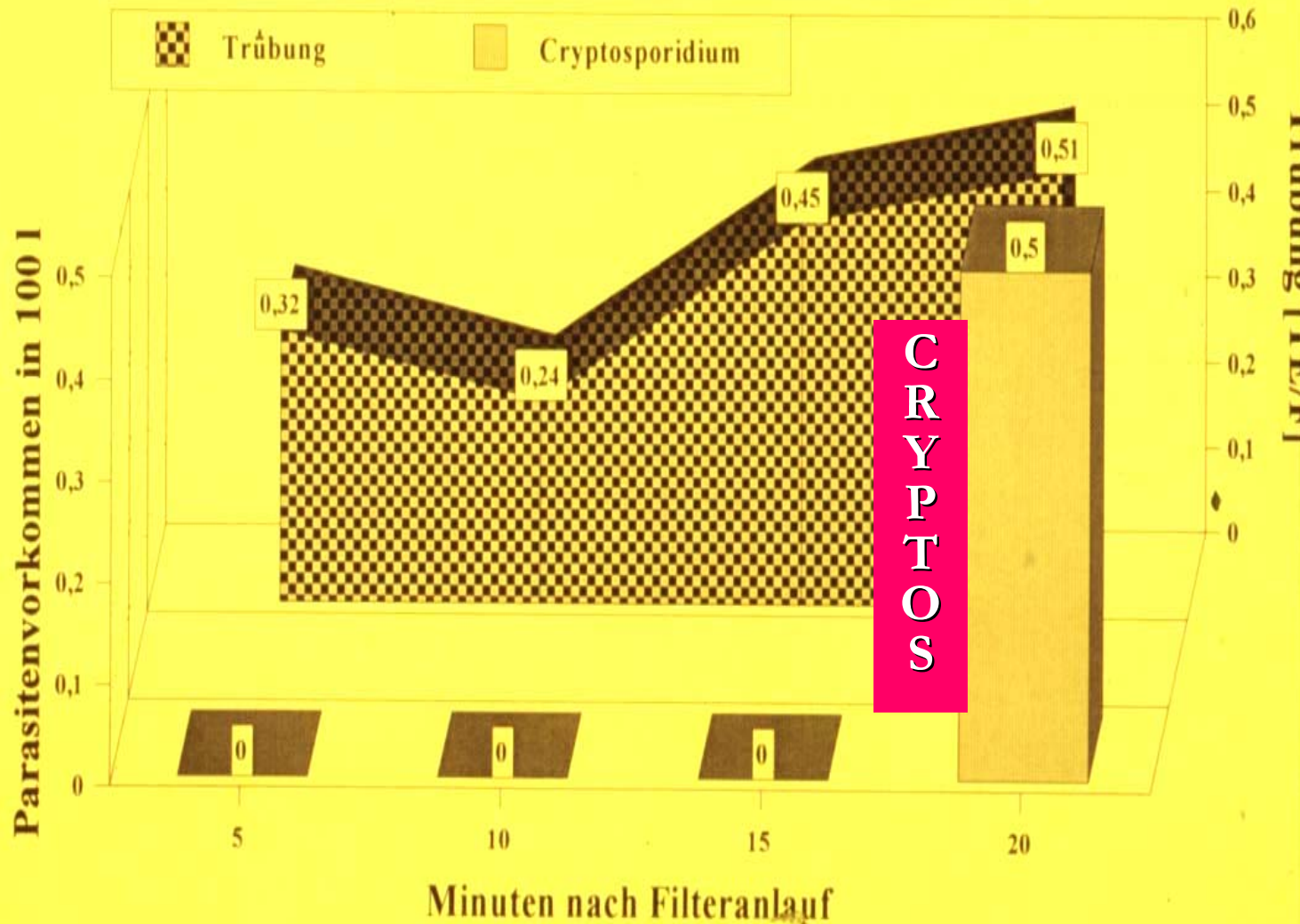
CRYPTOSPORIDIUM

- THE WATER AND FOOD CONTAMINATION

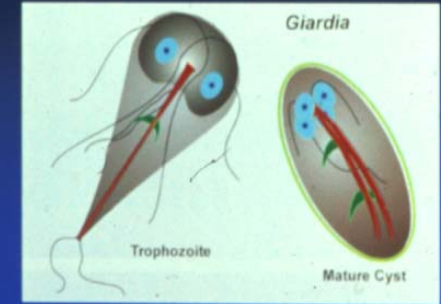
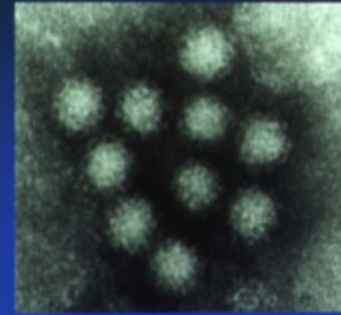
Correlation between turbidity and protozoan in the first filtrate of rapid sand filters after backwashing – 20 min

Korrelation zwischen Trübung und Parasitenvorkommen

im Erstfiltrat eines Mehrschichtfilters



The Evolution of Environmental Methods for *Cryptosporidium* - 1



The Emergence of *Cryptosporidium* as a Pathogen - 2



- 1993 - Milwaukee, WI waterborne OB, >400,000 cases

Europe

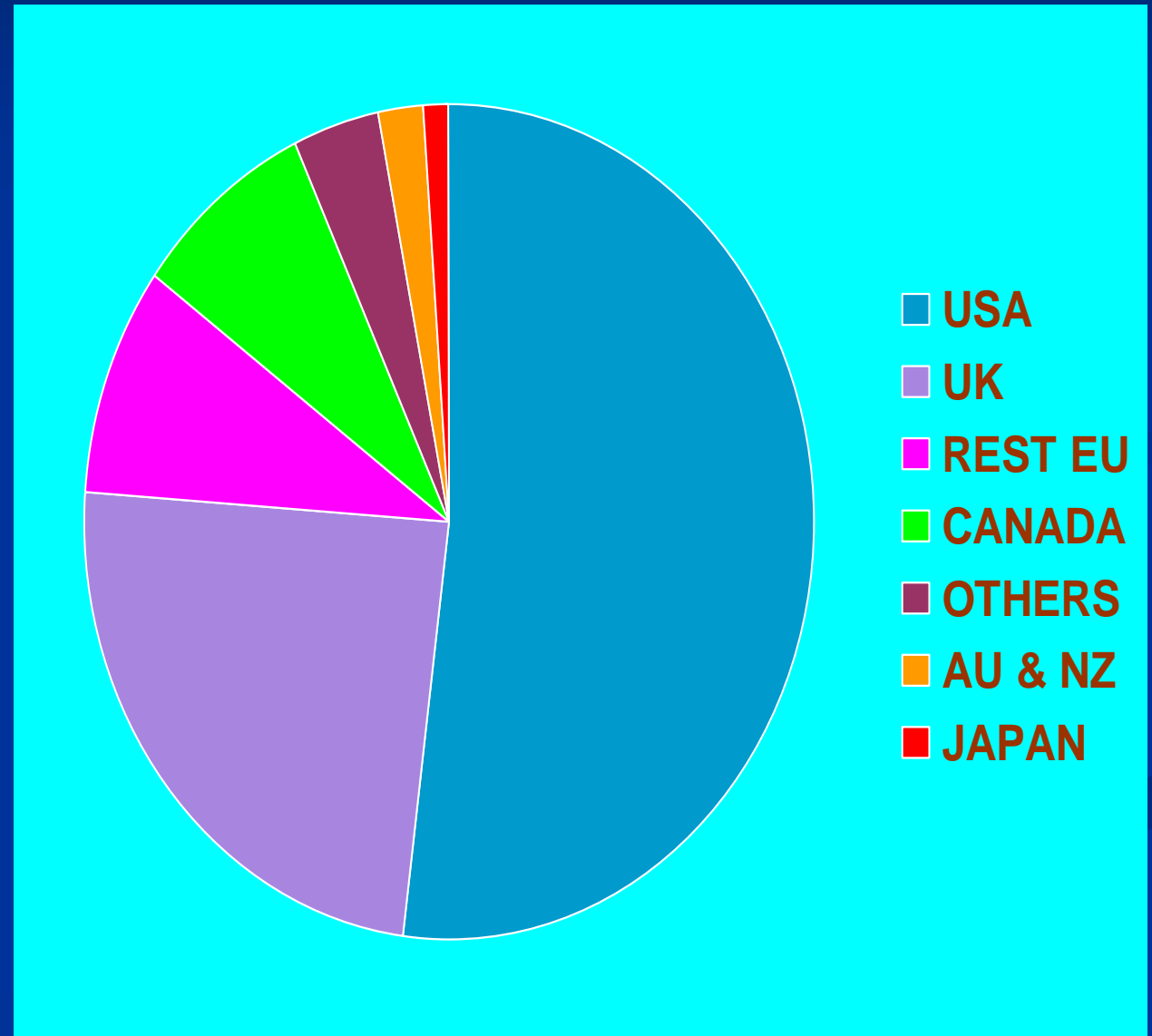


Asia



Worldwide review of outbreaks by parasite and country (J Water & Health 5: 1-38, 2007)

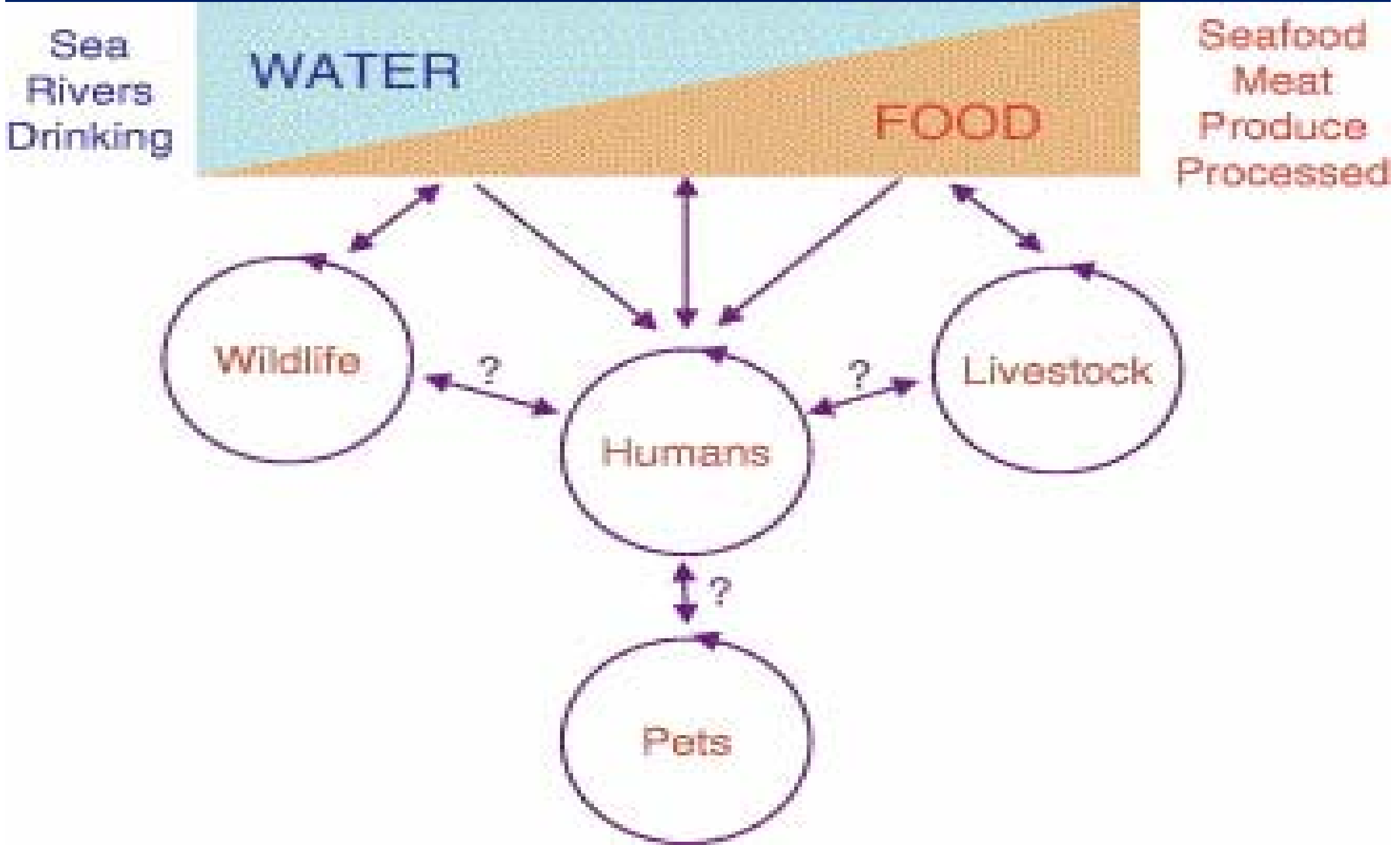
<i>Cryptosporidium</i>	165
<i>Giardia</i>	132
<i>Entamoeba</i>	9
<i>Cyclospora</i>	6
<i>Toxoplasma</i>	3
<i>Isospora</i>	3
<i>Blastocystis</i>	3
<i>Balantidium</i>	1
<i>Microsporidium</i>	1
<i>Acanthamoeba</i>	1
<i>Naegleria</i>	1
Total outbreaks	325

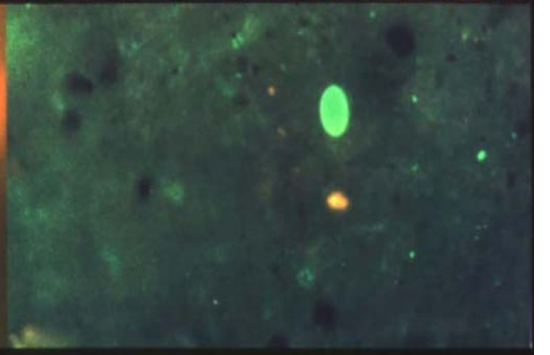
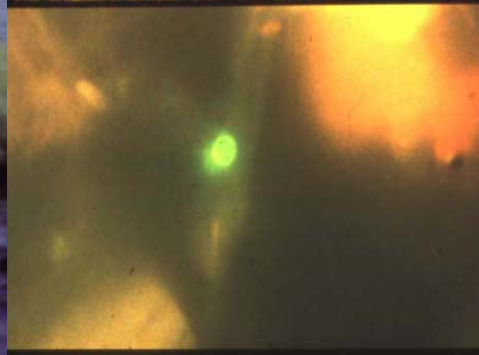
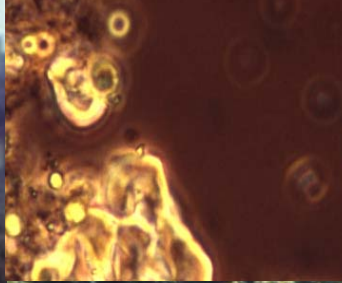
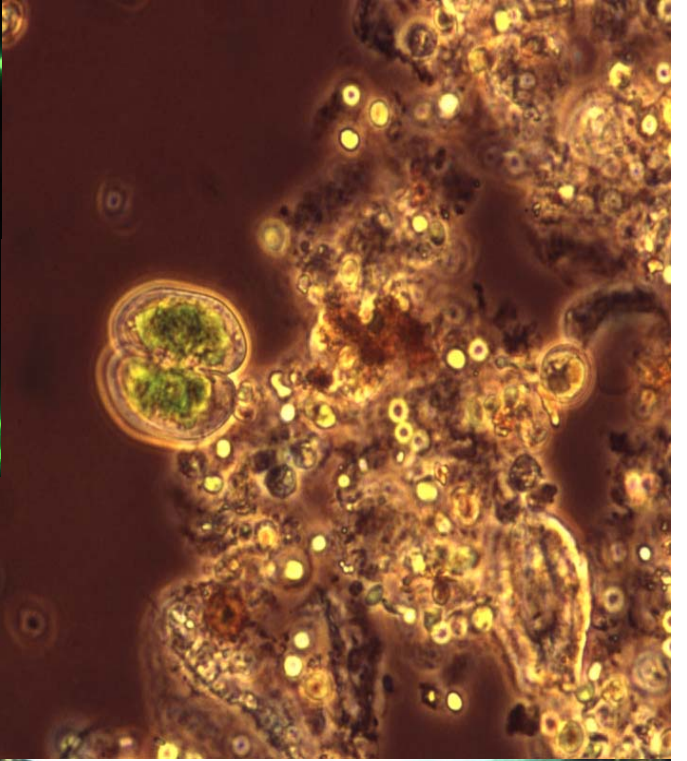
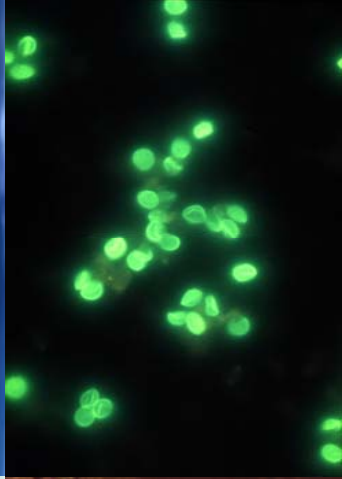
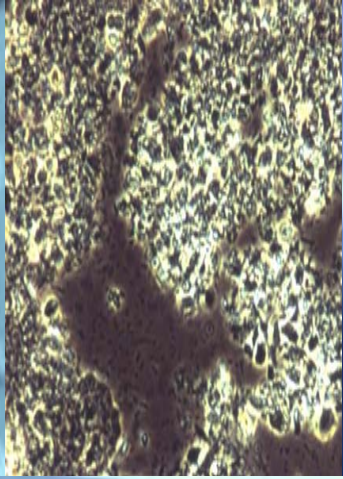
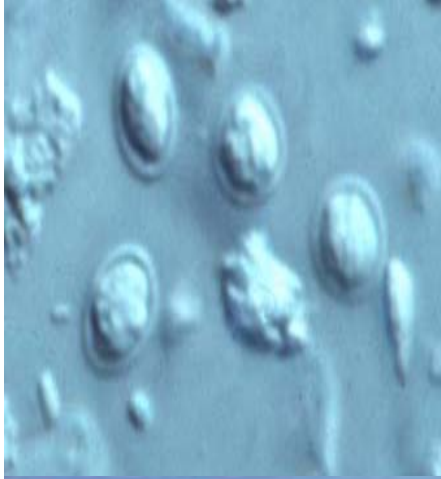


Sewage treatment and agriculture runoff is a significant source of contamination for marine animals – “Biological Pollution”

- - discovery of *Cryptosporidium* variants in phocid host (Appelbee et al. , TP 2005).
- - susceptibility to infection with terrestrial strains of *Cryptosporidium*.
- - potential transmission from seals to humans.
- - demonstrates the potential for anthropogenic activities such as contaminations sources.
- - “biological pollution” refers to the widespread introduction of non-native flora and fauna into new areas resulting in a loss of diversity.

Most important cycles of transmission for maintaining *Cryptosporidium*.
Questionmarks indicate uncertainty regarding the frequency of interaction
between cycles (Int. J. Parasitology, 2005).





Cryptosporidium research
(Karanis & collaborators)

EUROPE

(Germany, Greece, Hungary, Bulgaria, Russia)

■ ASIA

■ (Japan, China, Mongolia, Malaysia, Thailand)

■ AFRICA

■ (South Africa, Cameroon)

Cryptosporidium & Water

Water treatment; Detection methods

Multiple barrier system (protection, treatment, disinfection)

Develop new methods (e.g. LAMP)

Species identification in clinical and environmental samples

Identify sources of contamination

Implementations

EU Water Framework Directive

Diffuse Agricultural Sources

WBPD

Training in Water-Borne-Parasitic-Diseases (WBPD)

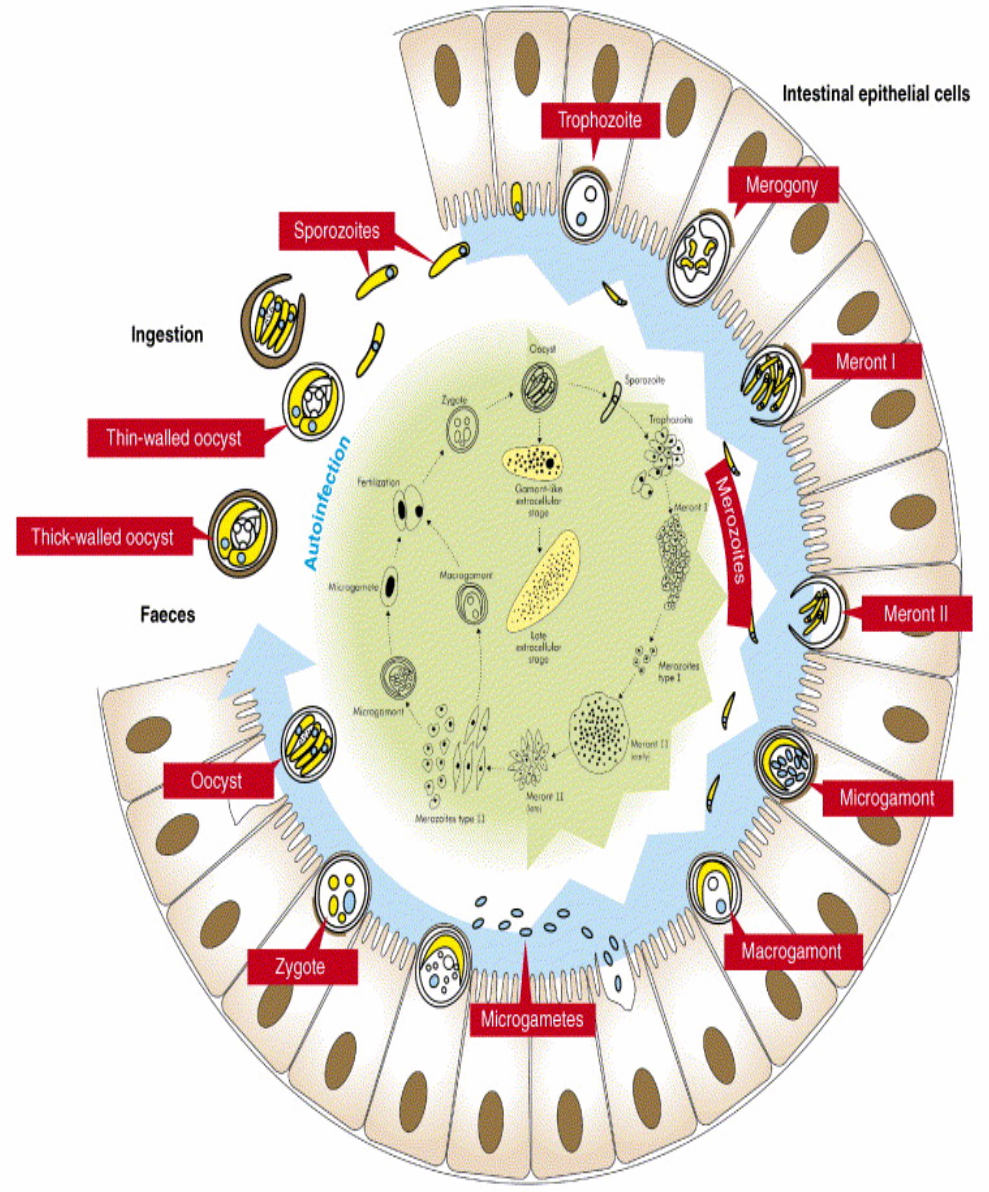
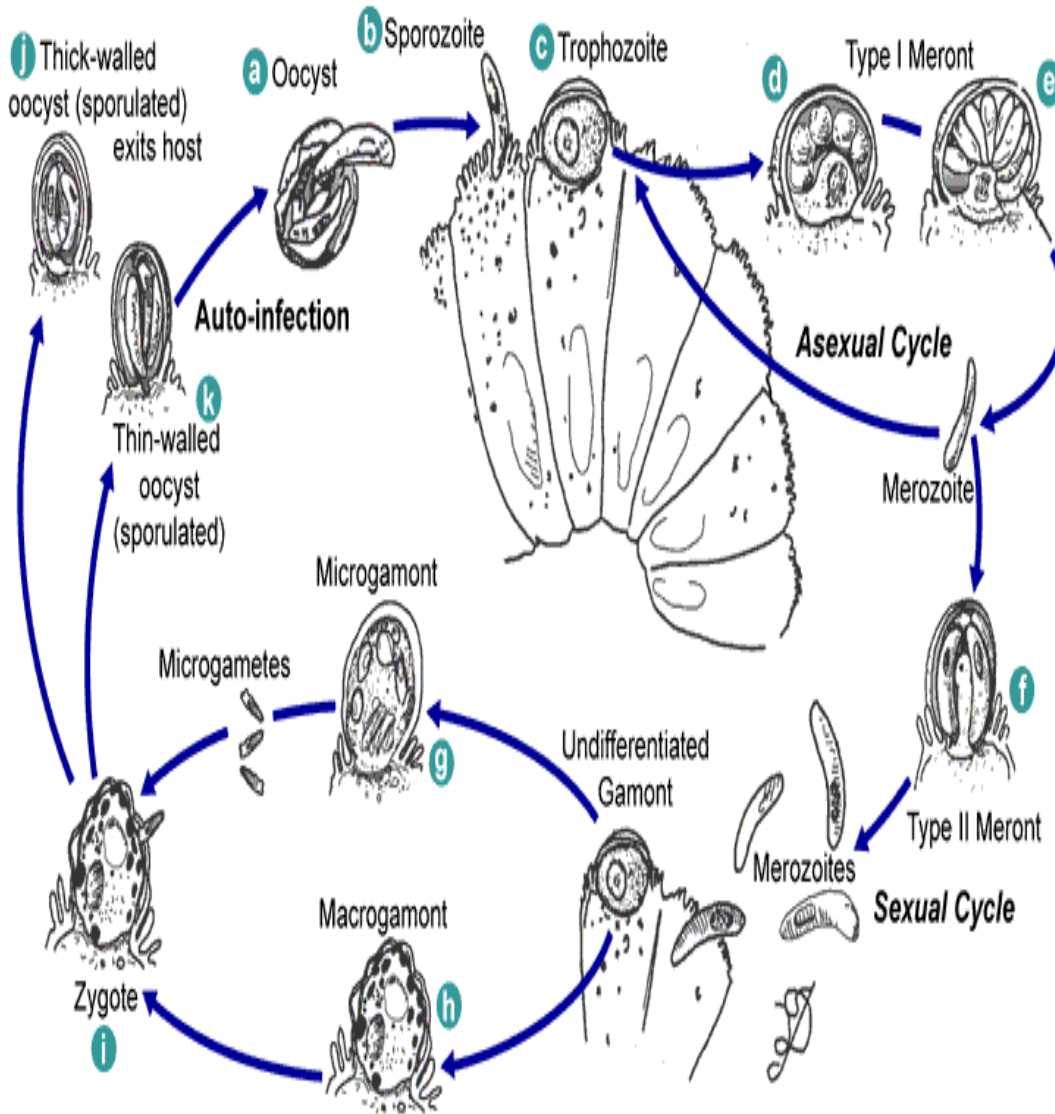
Unusual *Cryptosporidium*

- *Cryptosporidium* has an unusual resistance to antimicrobial agents
- In vitro axenic culture
- The *Cryptosporidium* pathogen, which can be found in the faeces of both humans and animals, is difficult to work with.

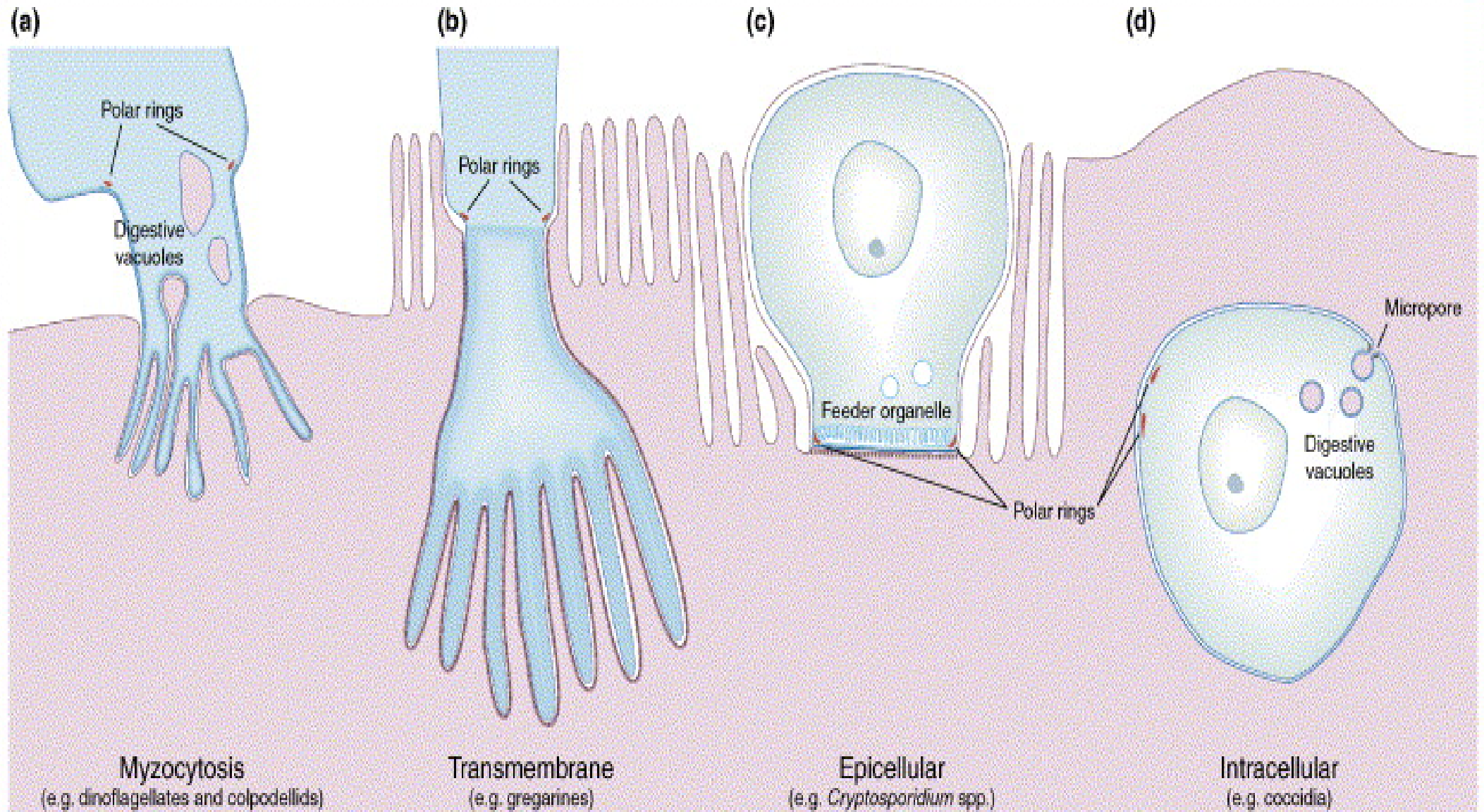
CRYPTOSPORIDIUM

- THE LIFE CYCLE

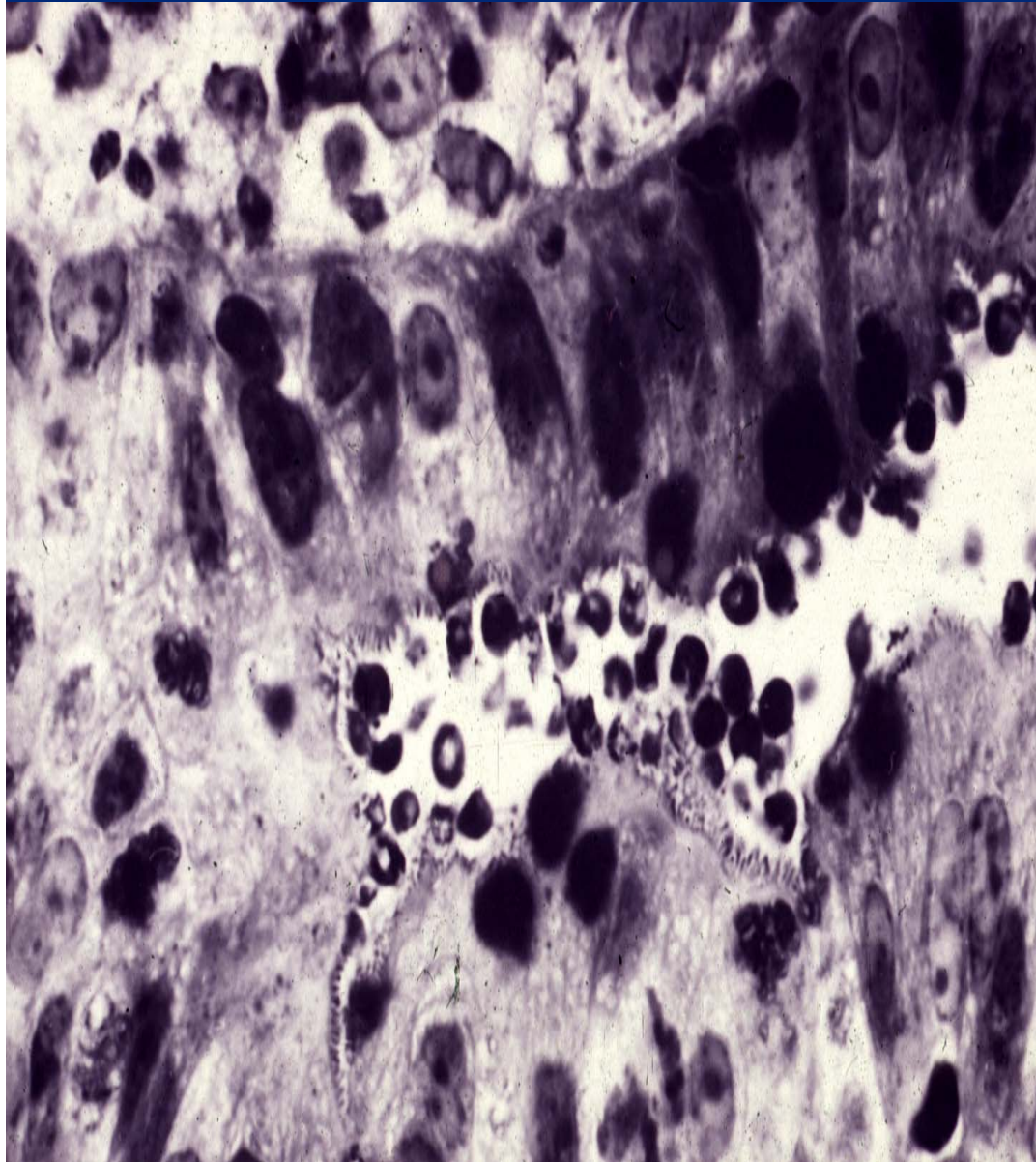
Life cycle of *Cryptosporidium parvum*



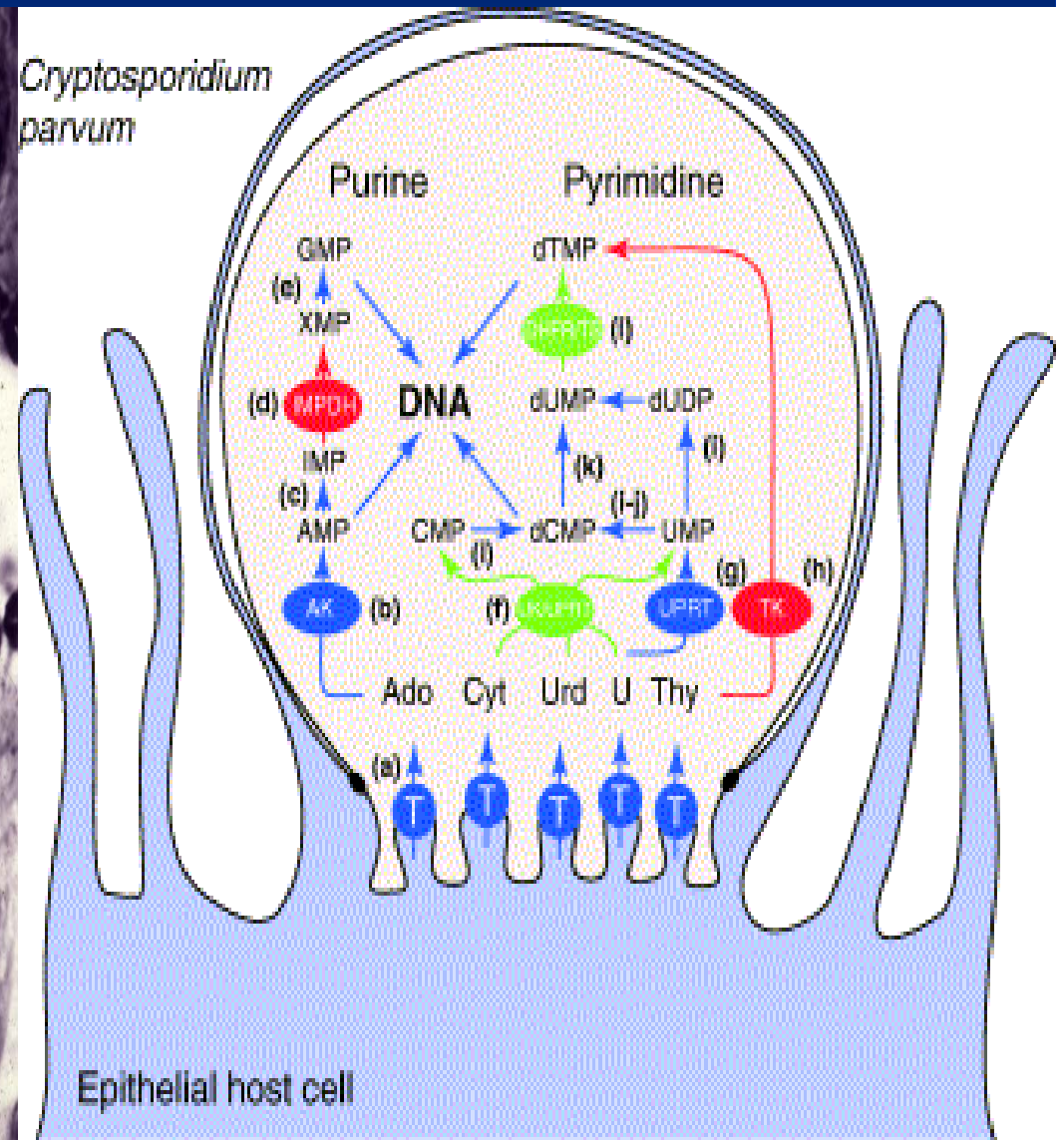
Host-parasite interactions in (a) dinoflagellates, (b) gregarines, (c) *Cryptosporidium* species and (d) coccidia
(Trends Parasitology 2006)



The *Cryptosporidium* nucleotide biosynthetic pathway is a phylogenetic mosaic.

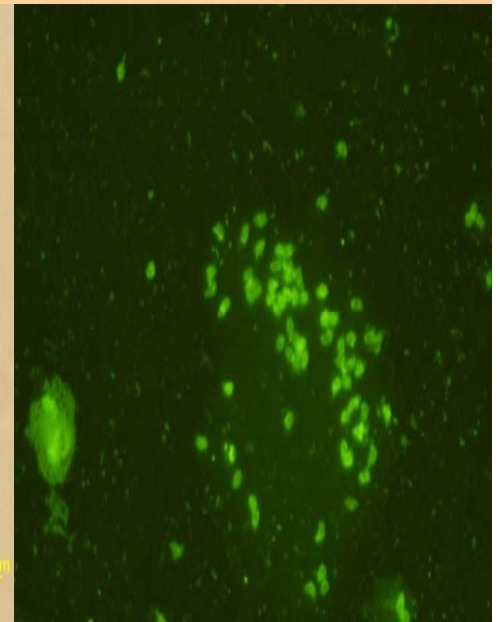
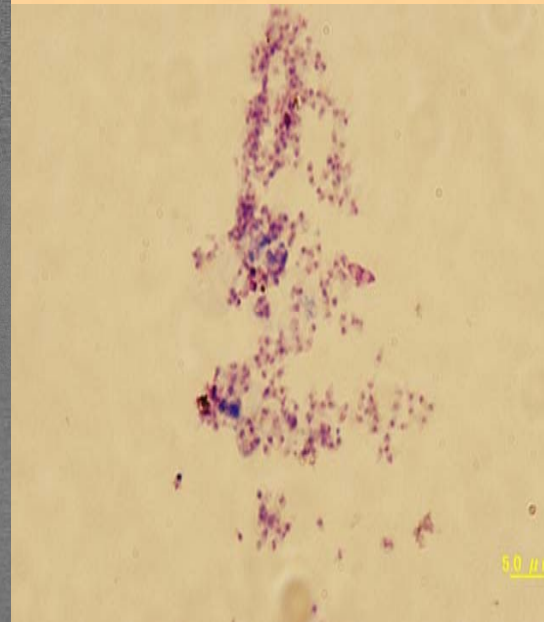
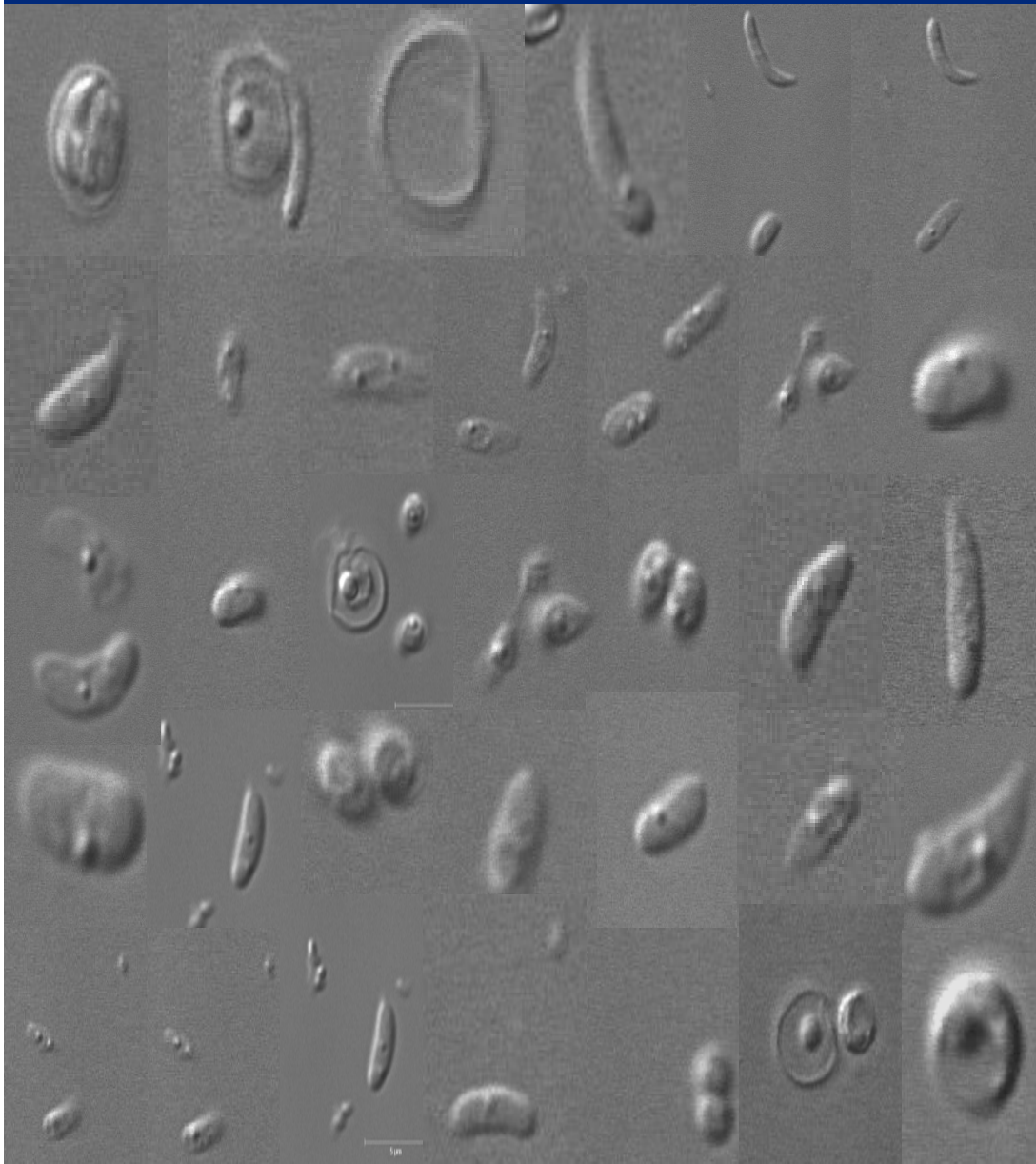


Cryptosporidium parvum



Developmental biology

Cryptosporidium stages in vitro axenic culture



Cryptosporidium genome sequence

- Genome sequence of *Cryptosporidium parvum*: Science 304, pp. 441-445, Abrahamsen et al., April 2004.
- Genome sequence of *Cryptosporidium hominis*: Letters to Nature, pp. 1107-1012, Xu et al., October 2004.

CRYPTOSPORIDIUM

- THE DIAGNOSIS

Methods for Identification

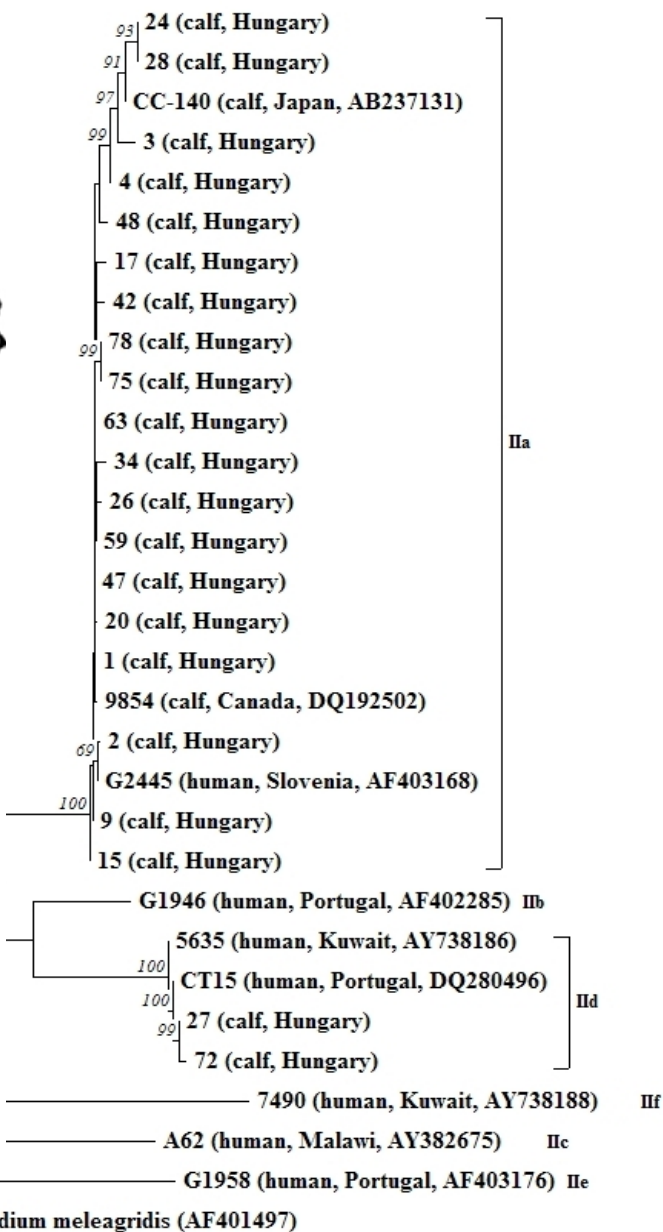
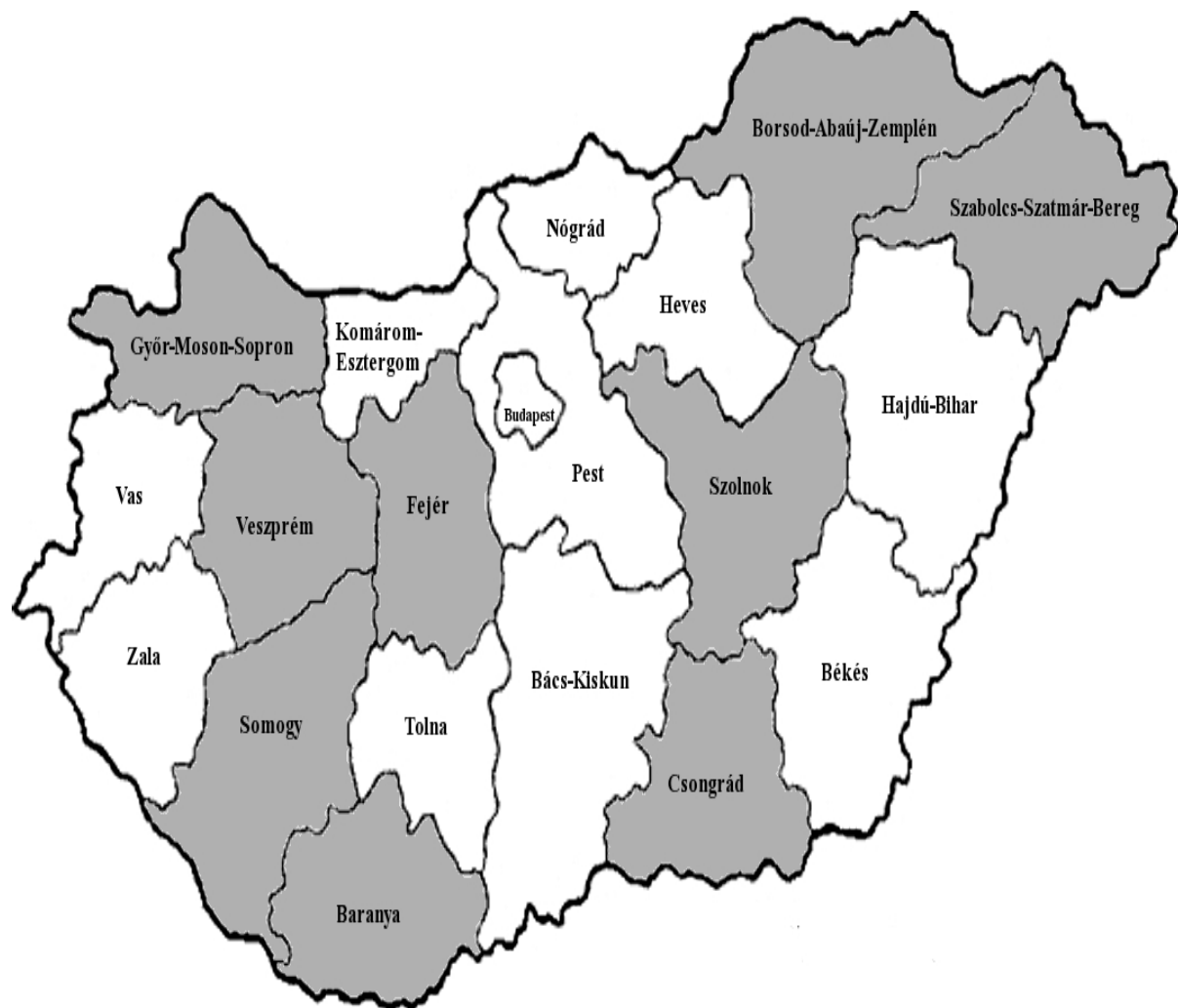
- Microscopy (IFT, DAPI, DIC, LSM)
 - PCR, PCR-RFLP, Sequence
 - LAMP

DETECTION OF *CRYPTOSPORIDIUM*

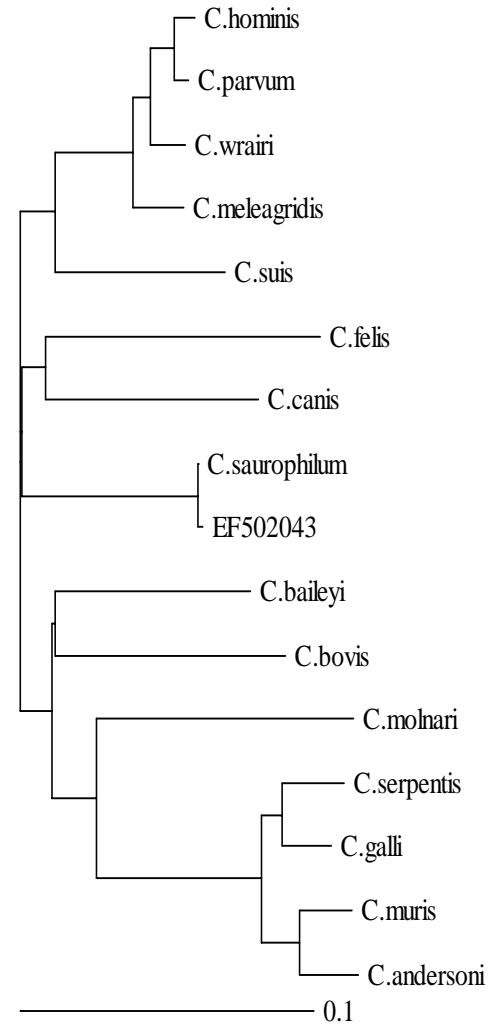
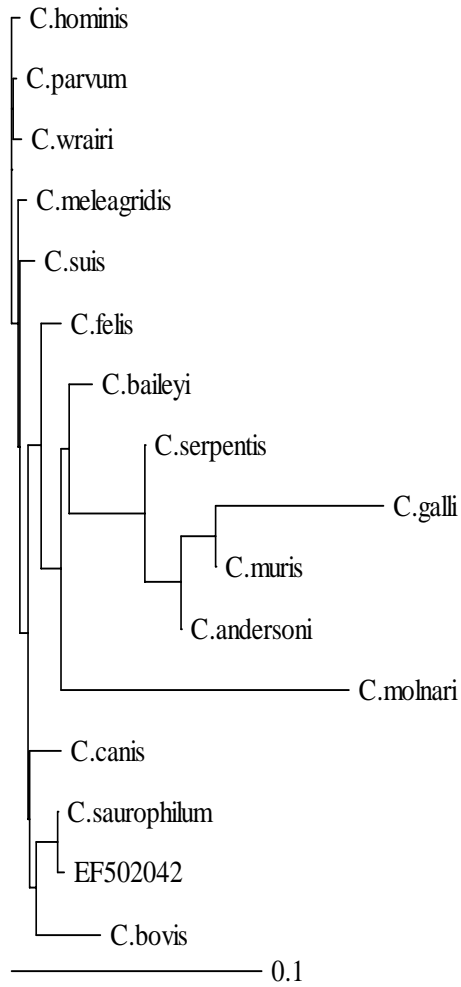
Genotyping techniques

- Techniques for detection, species determination, and genotyping of *Cryptosporidium* have greatly advanced.
- Techniques need to be strictly evaluated and more widely applied to improve clinical diagnosis.
- Outbreaks or bioterrorism events to identify sources of contamination.
- Genetic polymorphism reflects the extend of diversity of subpopulation within the genotype. Genetic markers for subpopulation level identification needed.
- Molecular biology provides insights into epidemiology and taxonomy, host specificity and transmission routes.
- PCR protocols on detection and genotyping needs carefully and detailed evaluation.

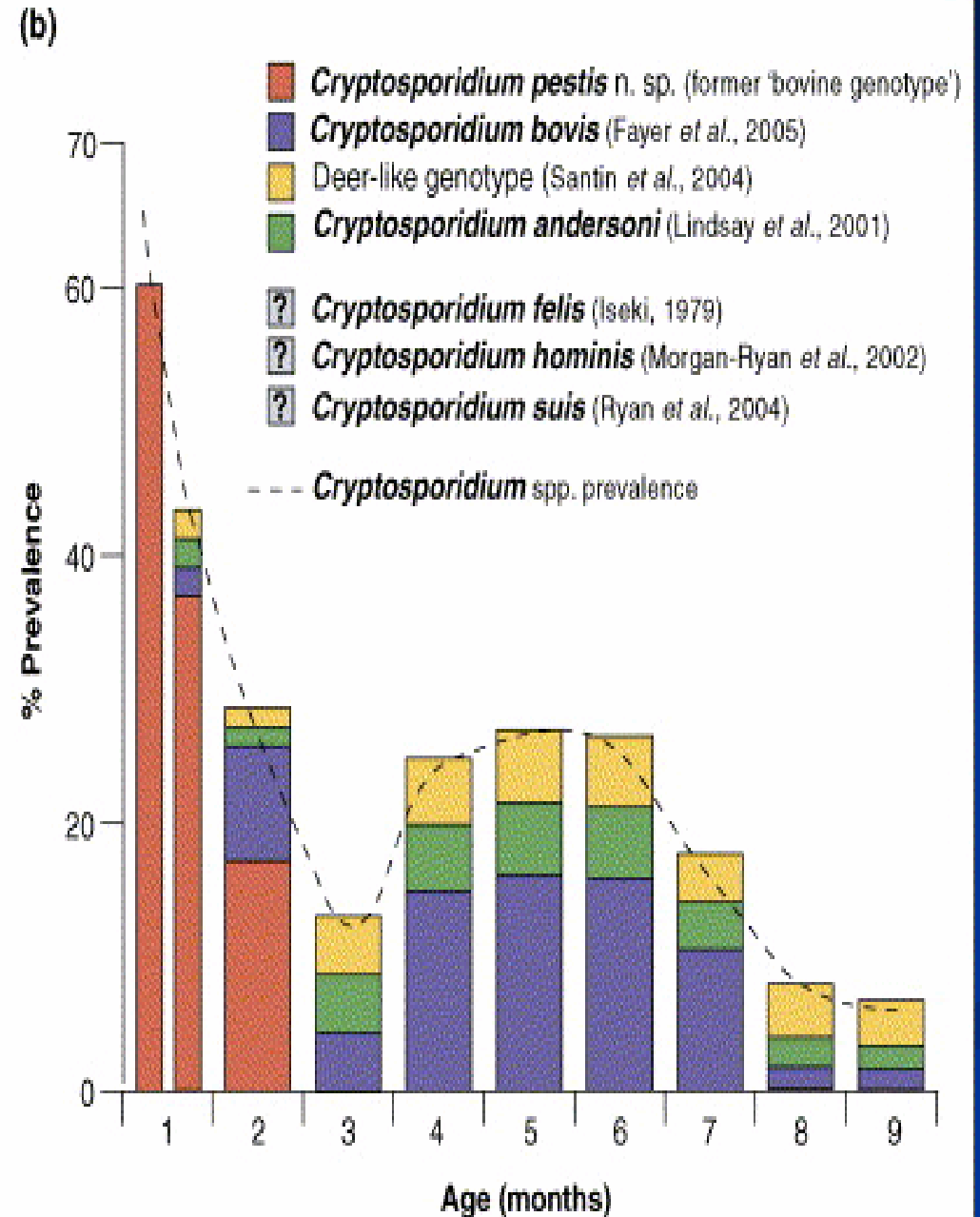
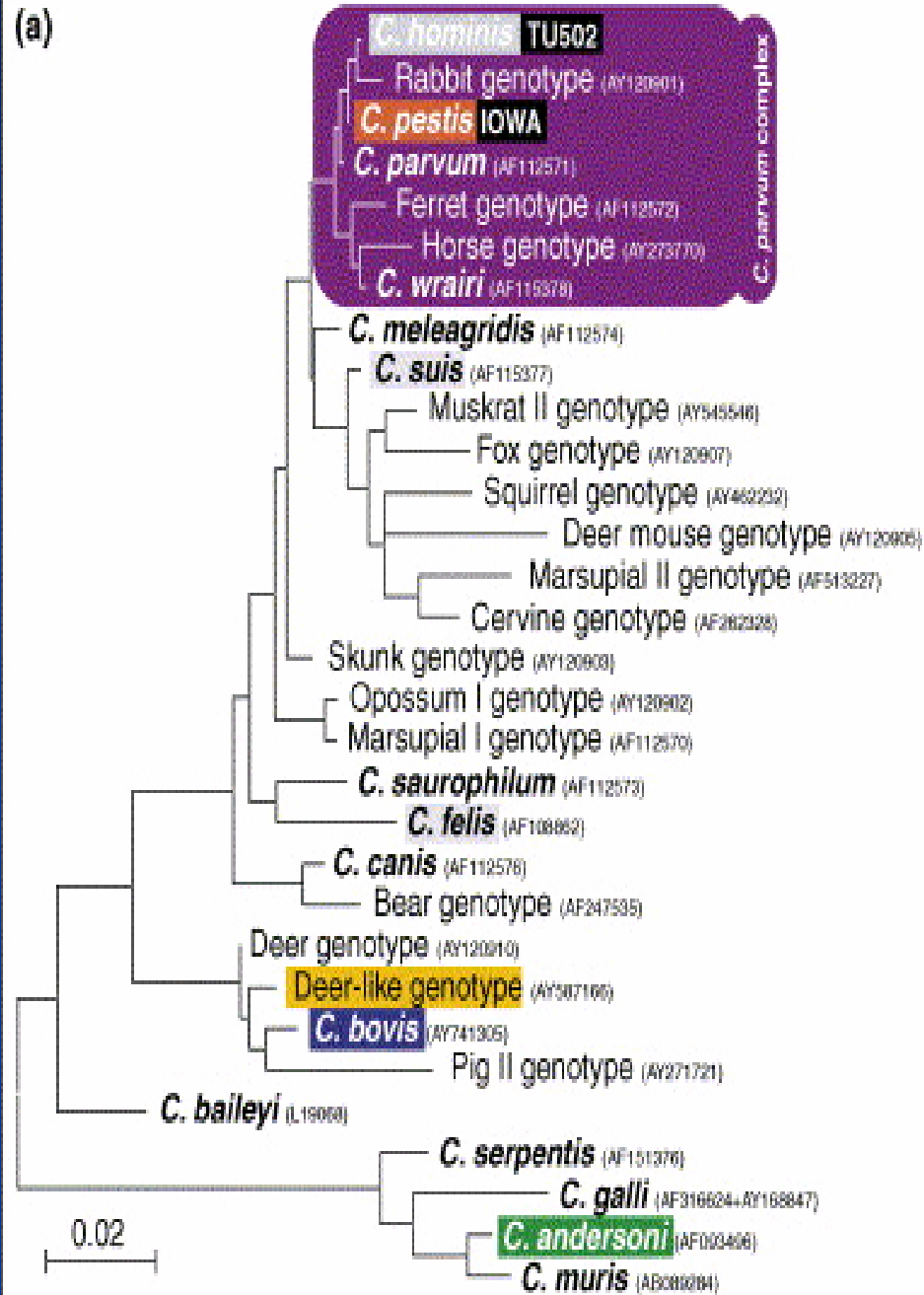
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Cryptosporidium spp. from *Elaphe guttata guttata* and other *C.*-species.
(e.g. SSU rRNA gene, actin gene)



Diverse assemblage of *Cryptosporidium* species affecting cattle, based on small subunit rDNA, (Slapeta, TP 2006)



Objective

Development of LAMP for diagnosis of protozoan infections.

LAMP

**LAMP is the abbreviation for:
Loop-Mediated Isothermal Amplification (of DNA)**

(LAMP) is a novel method that amplifies DNA with high specificity, efficiency and rapidity under isothermal conditions and relies on autocycling strand displacement DNA synthesis by a *Bst* DNA polymerase.

LAMP has been already developed for the detection of protozoan infections including African Trypanosomiasis, canine Babesiosis, Cryptosporidiosis, Giardiasis, Malaria, Toxoplasmosis.

LAMP Reagents

4 LAMP Primers

LAMP Buffer (RM)

DDW

Bst DNA Polymerase

Isothermal Reaction

63~65°C

1 hour

Loopamp DNA Amplification Kit

(Eiken Chemical Co. Ltd, Japan)



Materials for Amplification

1. Water-bath



2. Laboratory Heat-block

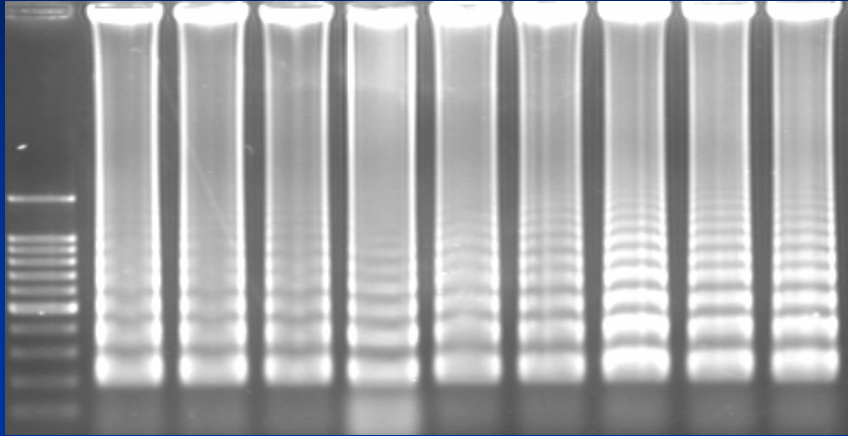


3. LAMP Heat-block



Visualization of LAMP Results

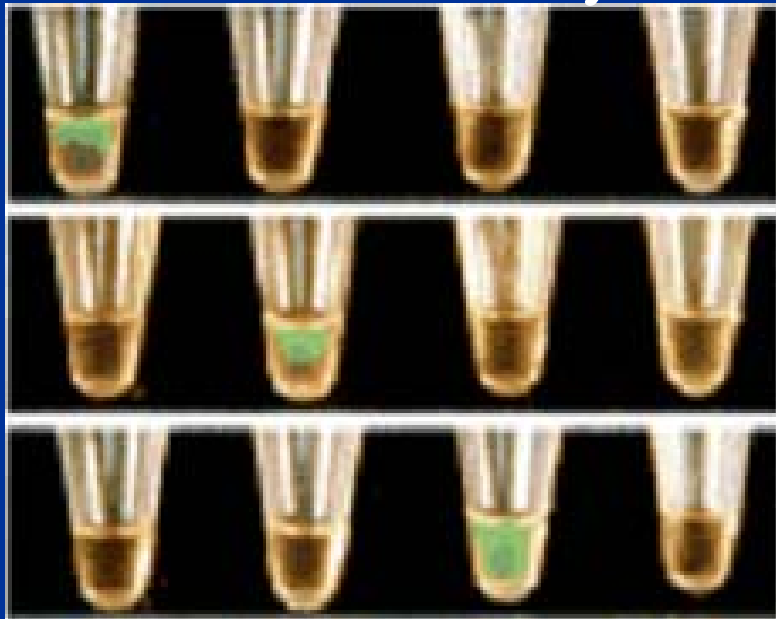
1. Agarose Gel Electrophoresis



2. Turbidity



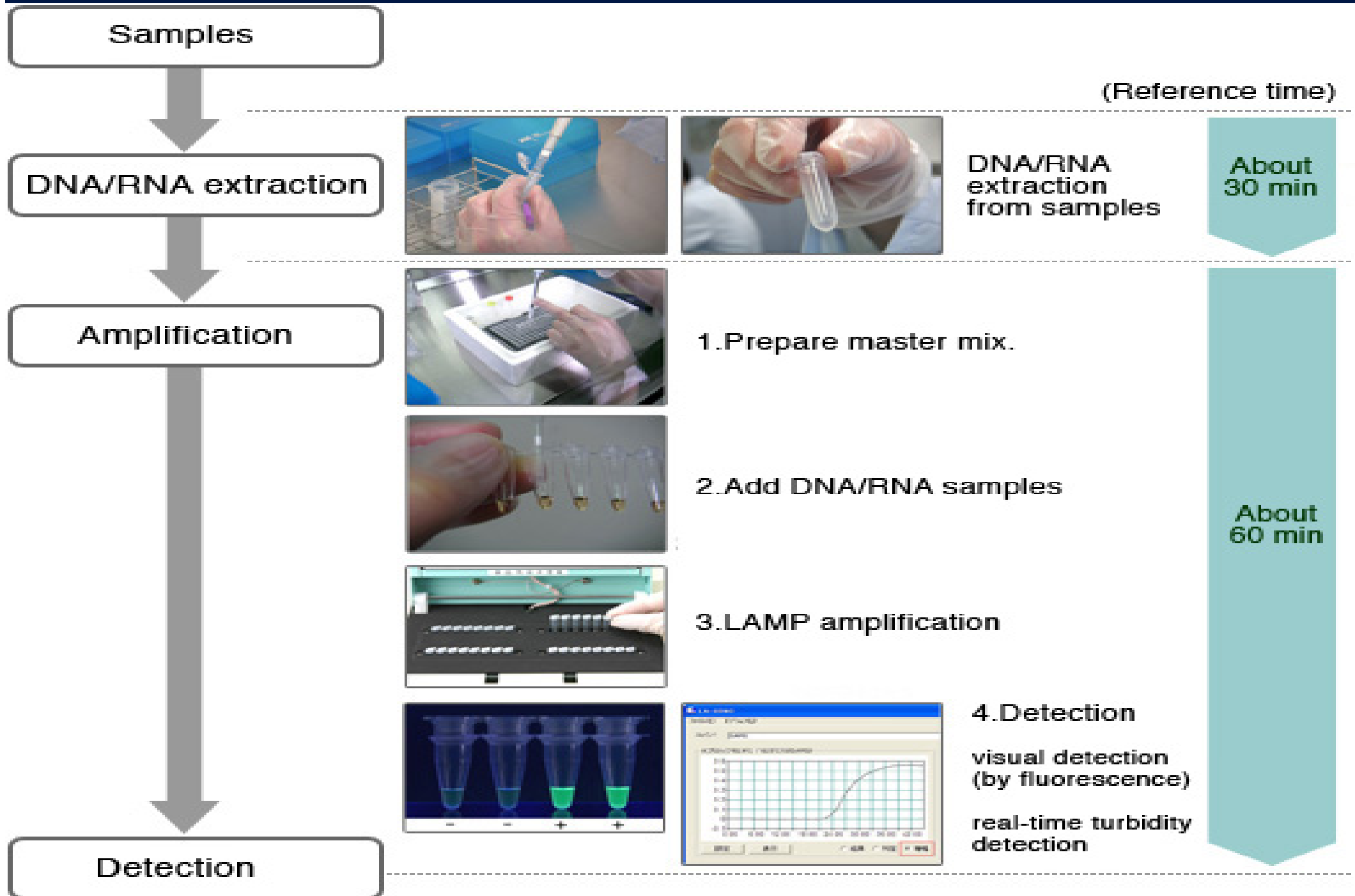
3. Modified Visual Detection SYBR Green I dye



4. Fluorescent Detection Reagent



LAMP Reaction



The strategy: Development and Application of LAMP

- **1. Select the target genes and design the specific primers**
- **2. Evaluation of specificity**
- **3. Evaluation of sensitivity**
- **4. Application of LAMP in water, food, clinical and environmental samples.**

Loop-mediated isothermal amplification (LAMP)

C. parvum: fig.1) specificity, fig. 4) Fluorescence detection
(Appl. Environ. Microbiol, in press, 2007)

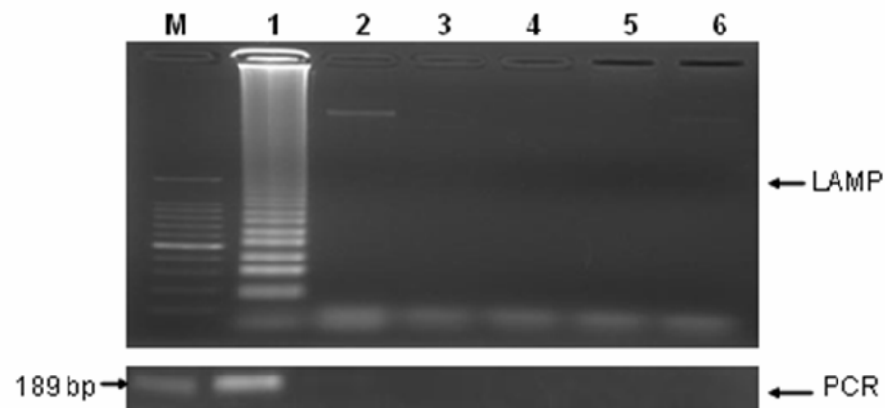


Fig.1.

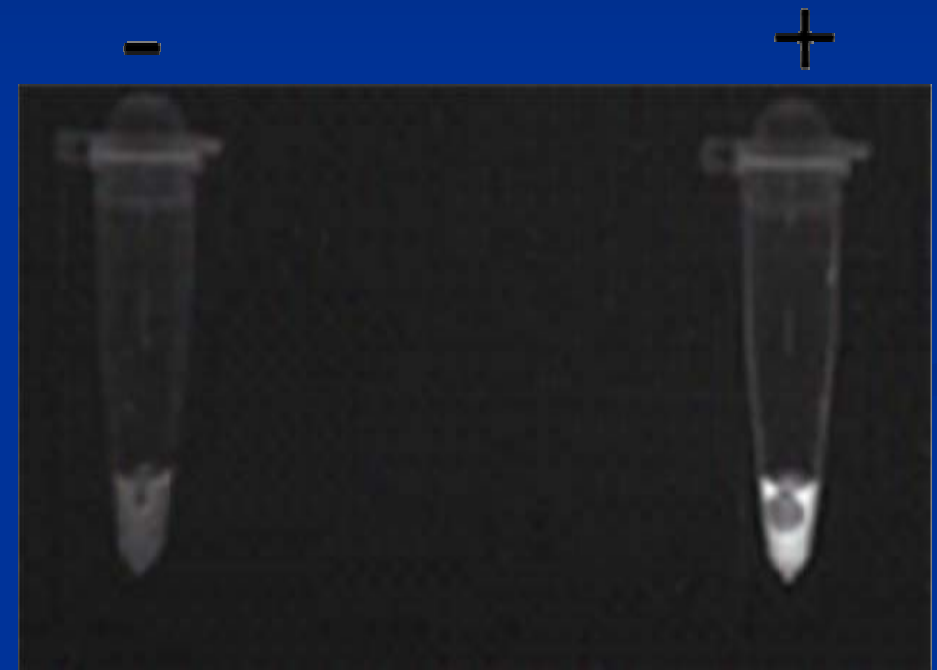


Fig. 4.

CRYPTOSPORIDIUM

- THE CLINICAL & MEDICAL IMPORTANCE

Further complications with Cryptosporidiosis

- **Extra-intestinal sites: infections of gall bladder, bile duct epithelium (acalculous cholecystitis & sclerotic cholangitis).**
- **Other tissues: pancreas, lungs and stomach, ren.**
- **Renal transplant recipients with Cryptosporidiosis**
- ***Cryptosporidium* and adenocarcinoma**
- **The parasite's life cycle should be reconsidered.**

Species and genotypes of *Cryptosporidium*

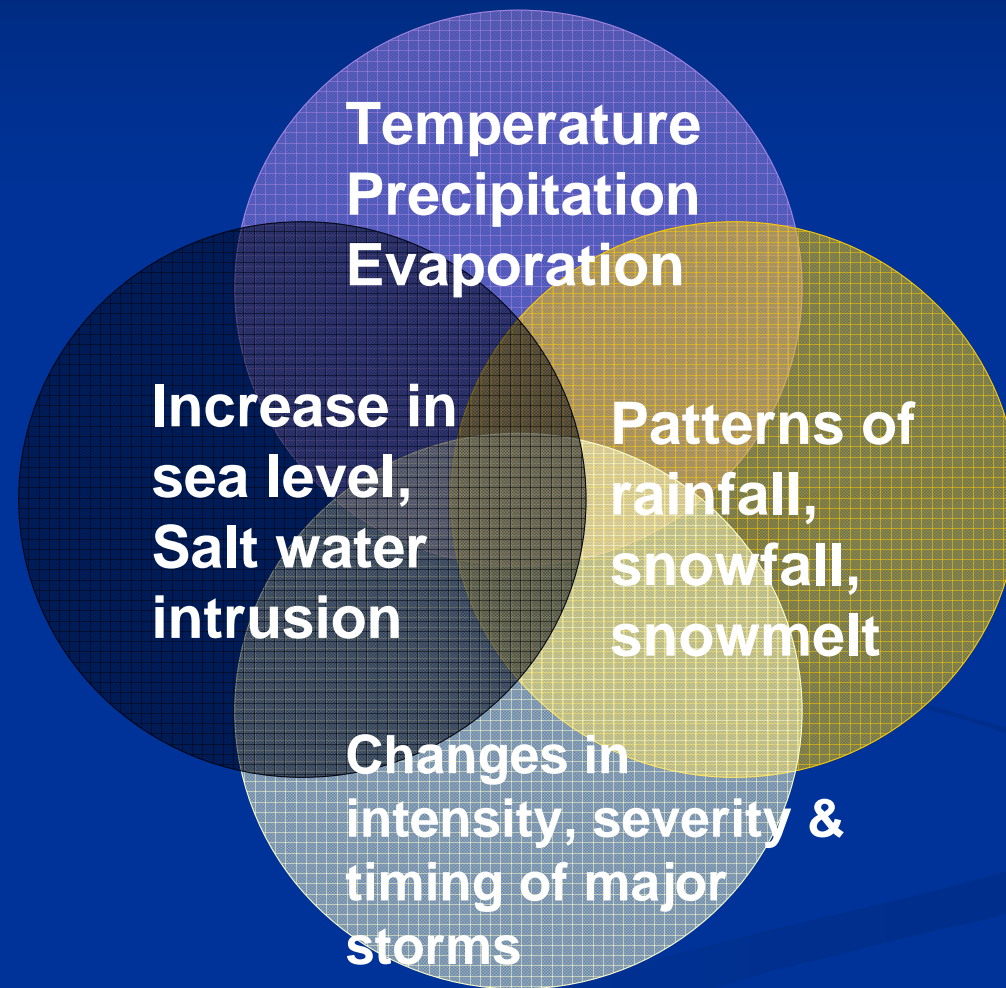
- *C. parvum** Livestock, Humans
- *C. hominis** Humans, monkeys
- *C. muris** Rodents
- *C. meleagridis** Birds
- *C. wrairi* Guinea pig
- *C. serpentis* Snakes
- *C. baileyi* Birds
- *C. saurophilium* Reptiles
- *C. galli* Chickens
- *C. andersoni** Cattle (abomasum)
- *C. canis** Dogs
- *C. molnari* Fish
- *C. suis** Pigs
- *C. felis** Cats
- Cervine genotype* Deer

Genetic analysis of *Cryptosporidium* from 2414 humans with diarrhoea (Leoni et al., 2006)

■ <i>Cryptosporidium parvum</i>	56.1%
■ <i>Cryptosporidium hominis</i>	41.7%
■ <i>C. parvum</i> and <i>C. hominis</i>	0.9%
■ <i>C. meleagridis</i>	0.9%
■ <i>C. felis</i>	0.2%
■ <i>C. andersoni</i>	0.1%
■ <i>C. canis</i>	0.04%
■ <i>C. suis</i>	0.04%
■ <i>C. cervine</i> type	0.04%



Potential effects of climatic changes in water resources are:



Should they occur, these changes could alter water demand, supply and quality

Cryptosporidium
research program
(2008-2011)

Material (Oocysts)

- IFT, IMS,
- DNA extraction
- PCR, LAMP
- Genotyping
- In vitro axenic culture

■ **In vitro culture**

- -gene expression in developmental stages
- - surface proteins identification
- - proteomic analysis
- - proteins & host cell manipulation

Plant-made vaccine

- Selection of sporozoites' surface antigens
- expression in plants
 - animal oral immunization
 - protection evaluation

"In the arena of human life the honors and the rewards fall to those who show their good qualities in action"

(Aristotle)

As the proverb says, to see the future is good, but to prepare for it is better.