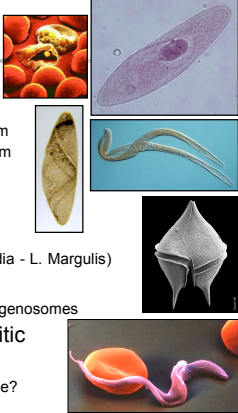


## Protist Diversity

- **Size**
  - Plasmodium merozoites - 2-5  $\mu\text{m}$
  - Paramecium (free-living) - 200-500  $\mu\text{m}$
  - Spirostomum (free-living) - up to 4 mm
- **Morphology - diverse!**
- **Protection - Cyst formation**
- **Locomotion**
  - Pseudopodia, flagella, cilia (undulipodia - L. Margulis)
- **Subcellular organelles**
  - Glycosomes, acidocalcisomes, hydrogenosomes
- **Feeding - free-living vs parasitic**
- **Reproduction**
  - Asexual vs. Sexual - genetic exchange?




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## History

- **Robert Hook - 1665**
  - Developed microscope with 30x magnification
- **Anton van Leeuwenhoek**
  - Began grinding lenses
  - First descriptions bacteria, protozoa and spermatozoa
  - "animalcules"
  - Series of letters to Royal Society of London
  - Whole new world
  - Rain water, canal water, saliva, his own feces



Modern day reconstruction - Al Shinn  
<http://www.mindspring.com/~alshinn/>

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## Early Parasitologist

■ My excrement being so thin, I was at diverse times persuaded to examine it; and each time I kept in mind what food I had eaten, and what I had drunk, and what I found afterwards. I have sometimes seen animalcules a-moving very prettily...

A. van Leeuwenhoek



**Most common flagellate of human intestinal tract**

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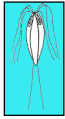

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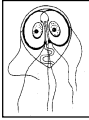

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## Order Diplomonadida

- **Family Hexamitidae**
  - parasitic
- **Giardia, Hexamita**
  - Bilateral symmetry
  - Defining family feature - 2 identical nuclei
  - Forms cysts
  - Binary fission - no sexual reproduction detected

Hexamita meleagridis

Giardia lamblia

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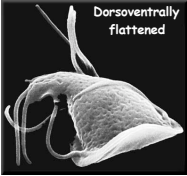
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## Giardia lamblia


- worldwide distribution (cosmopolitan)
  - higher prevalence in tropical or developing countries (20-30%)
  - 1-6% in temperate countries
- most common protozoa in stools
  - ~200 million cases/yr
- Giardiasis - beaver fever
  - often asymptomatic
  - acute or chronic diarrhea



Dorsoventrally flattened

Taxonomy

- one human species, aka:
  - *G. duodenalis*
  - *G. intestinalis*
- morphologically similar forms in other mammals



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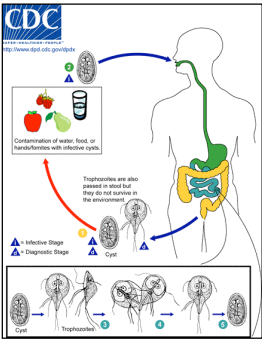
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## Giardia Life Cycle - Direct

- Fecal/Oral transmission
- **Cyst** - Infective stage
  - Resistant form
- **Trophozoite** - feeding, binary fission

Ingest cysts - pass through stomach, excystation in duodenum (small intestine).  
 Inhabit lumen of small intestine  
 As feces dehydrate in colon trophozoite encysts.



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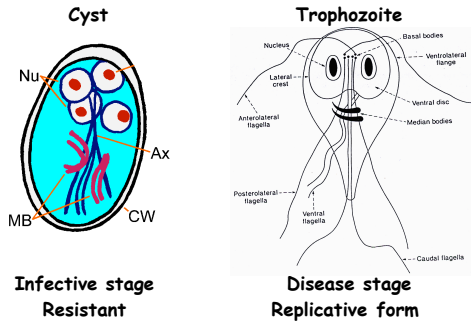
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## Giardia Life Forms




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## Keys Features of Cysts



- oval shape
- 11-14 x 6-10  $\mu\text{m}$
- distinct cyst wall (CW) set apart from cytoplasm
- 4 nuclei at anterior end
- fibrils (axonemes) evident
- median bodies (MB)
- Resistant to chlorine

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## Key Features of Trophozoites



- pear shape
- 12-15 x 5-10 x 2-4  $\mu\text{m}$
- Dorsoventrally flattened
- 2 nuclei
  - Morphologically identical
  - Transcriptionally active
- fibrils (axonemes) evident
  - bilateral symmetry
- pair of median bodies
- 4 pair flagella
  - motility like a falling leaf
- adhesive disk (not always evident)

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## Clinical Features and Symptoms

**Range of Outcomes**

- asymptomatic/latent
- acute short-lasting diarrhea
- chronic/nutritional disorders

**Subacute/Chronic**

- recurrent diarrheal episodes
- cramps common
- sulfuric belching, anorexia, nausea frequent
- can lead to weight loss and failure to thrive

**Acute Symptoms**

- 1-2 week incubation
- sudden explosive, watery diarrhea
  - bulky, frothy, greasy, foul-smelling stools
  - no blood or mucus
- upper gastro-intestinal uneasiness, bloating, flatulence, belching, cramps, nausea, vomiting, anorexia
- usually clears spontaneously (undiagnosed), but can persist or become chronic
- Propensity for multiple reinfections

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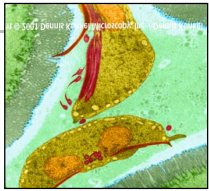
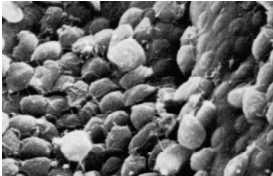
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## Pathogenesis

- Epithelial damage
  - Microvillus blunting
- Malabsorption
  - Large numbers of trophozoite

**Possible Mechanisms**

- mechanical irritation
- obstruction of absorption

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## Variable Symptomology

- Antigenic Variation - clonal phenotypic variation
  - Trophozoites express a single VSP covering the surface.

**Table 1.** Characteristics of antigenic variation in *Giardia lamblia*.

1. A property of all *Giardia lamblia* isolates
2. The varying-specific surface proteins (VSPs) are a family of related proteins
3. Occurs spontaneously in culture and *in vivo* in humans and laboratory animals
4. Rate of VSP change is isolate and VSP-dependent and ranges from one switch every 5–13 generations
5. Repertoires of VSPs may differ among isolates
6. Identical epitopes can be present in molecules of varying sizes
7. Monoclonal antibodies to VSPs are cytotoxic at lower dilutions and inhibit growth at higher dilutions
8. Switching during encystation–excystation occurs in some *Giardia*

Nash, TE. Mol Micro (2002) 45: 585

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## Variable Symptomology

**Table 2. Characteristics of variant-specific surface proteins of *Giardia lamblia*.**

1. Vary in size from about 20 to 200kDa
2. Cysteine-rich with numerous CXXC motifs
3. Conserved hydrophobic tail
4. Hydrophilic carboxyl-terminal five amino acids are CRGKA
5. One or more conserved GGCY motifs
6. Zn finger motif
7. Contains O-linked glucose and N-acetylglucosamine
8. Palmitate residue at carboxyl terminus
9. Amino terminus most peripheral at parasite-host interface

- VSPs are Cysteine-rich - 11-12% cysteine (disulfide linkages)
  - CXXC motifs usually associated with DNA binding proteins
  - VSP repertoire of 235 - 275 possible variants.
  - Clonal success depends on fighting host immunity and proteases.
  - Possibly all VSP genes are transcribed, but ONLY 1 protein is stable.
  - RNA interference regulates this expression!
  - If RNAi system is disturbed - parasite expresses all VSPs on the surface.

Prucca C.G. et al. Nature (2008) 456: 750

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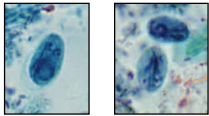
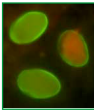
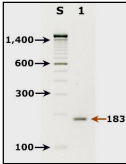
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## Giardia Diagnosis

- Microscopic
  - Detection in stool samples
    - Multiple samples tested
  - Concentration of samples
  - Classical stains
    - Iron-hematoxylin stain
  - Immunofluorescence
- Molecular
  - PCR-based methods
  - Detection from a single cyst

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## Control/ Prevention

- Highly contagious - cysts are quite resistant
- avoid fecal-oral transmission
- improve personal hygiene
  - especially institutions - day care centers, cafeterias
- treat asymptomatic carriers
  - eg, family members
- health education
  - hand-washing
  - sanitation
  - food handling
- protect water supply - breakdown in water treatment facilities
- treat water if questionable
  - boiling
  - iodine
  - not chlorine!

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## Treatment

- Metronidazole (Flagyl)
  - Actually an antibiotic
  - mode of action covered in biochemistry
  - 4X/day for 7 days
- Tinidazole (Fasign)
  - Newest addition - more expensive
  - shorter course of treatment
  - Single dose or two day course
- Quinacrine hydrochloride (Atabrine)
  - Antiprotozoal and anthelmith
  - Mode of action uncertain - cell membrane
- Furazolidone (Furoxone)
  - broad-spectrum anti-infective; effective against most gastrointestinal tract pathogens.
- Paromomycin (Humaitin)
  - aminoglycoside antibiotic with antibacterial and antiprotozoal activity.




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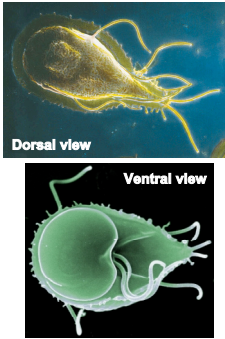
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## Giardia - Early branching eukaryote

- Eukaryotic properties
  - Genome sequenced 2007.
  - Nucleus - albeit 2!
  - Ribosomes
    - 60S/40S vs. 50S/30S
  - Multiple linear chromosomes
    - 11.7 MB genome
    - 5 chromosomes - 6470 ORFs
  - Lysosomal vesicles




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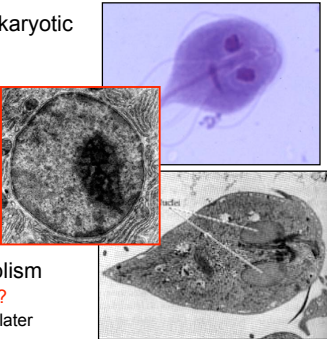
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## Interesting Biological Features

- "Missing link" - eukaryotic evolution
- Ventral adhesive disc
- No nucleoli - now
- No peroxisomes
- No obvious stacks
- No mitochondria
  - Amitochondriate??
    - to be discussed later




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## Giardia does have nucleoli

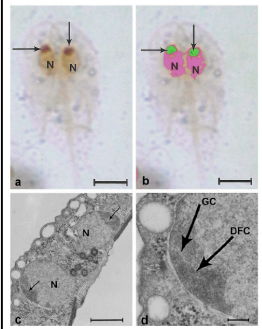
- Previous techniques - light, fluorescence, EM - no nucleoli
- Previously recognized anteronuclear zone
- Silver staining of nucleus organizing regions
- Distribution of markers, rRNA fibrillar, ribonuclear protein component (CBF5)

Available online at www.sciencedirect.com  
ScienceDirect  
International Journal of Parasitology 36 (2006) 1047–1054  
www.elsevier.com/locate/ijpara

Rapid Communication

**Identification of nucleoli in the early branching protist *Giardia duodenalis***

Luis Felipe Jiménez-García<sup>a</sup>, Guadalupe Zavala<sup>a</sup>, Bibiana Chávez-Munguía<sup>a</sup>, María del Pilar Ramírez-Gómez<sup>a</sup>, Gabriel López-Villanueva<sup>a</sup>, María de Lourdes Segura-Velazco<sup>a</sup>, Cecilia Montalvo<sup>a</sup>, Adrián B. Held<sup>b,c</sup>, Raúl Argüello-García<sup>a</sup>, Guadalupe Ortega-Pereira<sup>a</sup>



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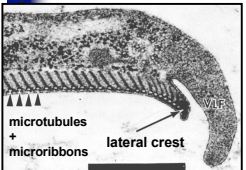

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## Adhesive Disk Components

- Microtubules
  - Tubulins ✓
- Microribbons
  - Giardins ✓
- Lateral crest
  - Actin ✓
  - Myosin ???

**Immunofluorescence techniques for identification**

**Cross-reacting antibodies!**

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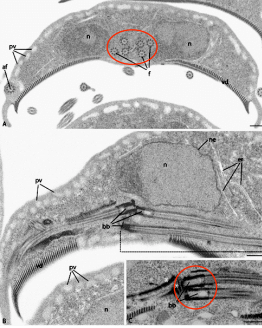
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## Cytoskeleton - rich in microtubules

- Microtubule structures
  - Flagella
  - Basal body
  - Median body
  - Ventral disk
- Composed of Tubulin
  - $\alpha$ -tubulin - 2
  - $\beta$ -tubulin - 3
  - $\gamma$ -tubulin - 1



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## Microtubule structure

- Protofilaments
  - $\alpha$ -tubulin- $\beta$ -tubulin dimer
- 9 + 2 arrangement
  - Central pair - singlets
  - Outer pair - doublets
- Basal body
  - Triplet arrangement
  - $\gamma$ -tubulin

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## Specific localization

- Tubulin modifications
  - C-terminus
  - Polyglutamylation
  - polyglutamylolation
  - Phosphorylation
  - acetylation
- Monoclonal antibodies
  - $\alpha$ -tubulin - polyglut
    - Proximal Flagella
  - $\alpha$ -tubulin/ $\beta$ -tubulin - polyglyc
    - Flagella, median body

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## Specific localization

- Giardins (~25-30 kDa)
  - Ventral disk
- Originally identified by 2D gel electrophoresis
  - $\alpha$ -giardin (19) - annexins
  - $\beta$ -giardin (1) - SF-assemblin
  - $\gamma$ -giardin (1) - no homology
  - $\delta$ -giardin (1) - no homology
- Classification is ongoing!

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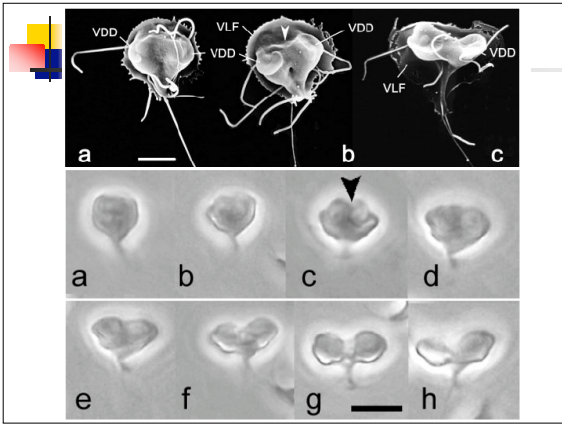
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