

# Electron Microscopy in Diagnosis of Infectious Diseases

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**B**



Duke Medicine

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Society for  
Ultrastructural Pathology



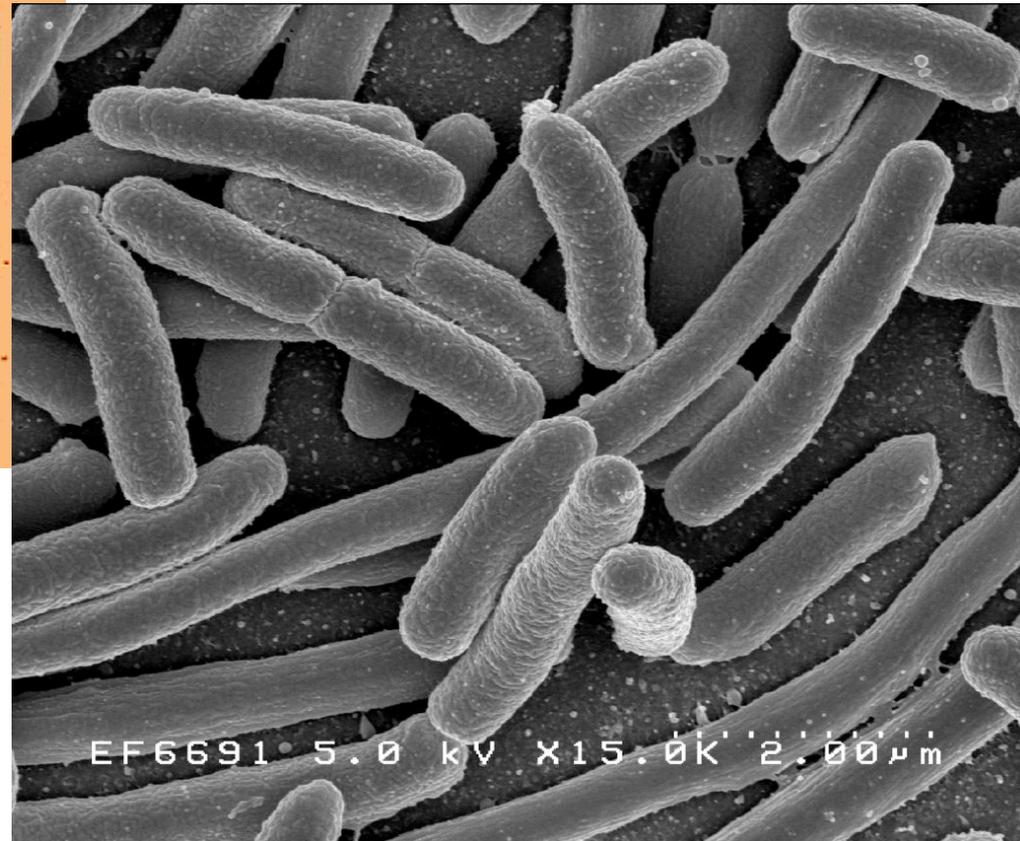
# Outline Part 1

## **B. Other organisms**

- 1. Bacteriology**
- 2. Mycology**
- 3. Photology (algae)**
- 4. Protozoology**

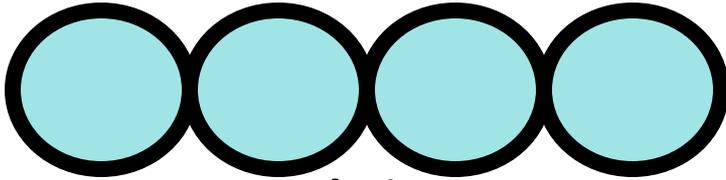
**Questions/Discussion**

# Bacteriology

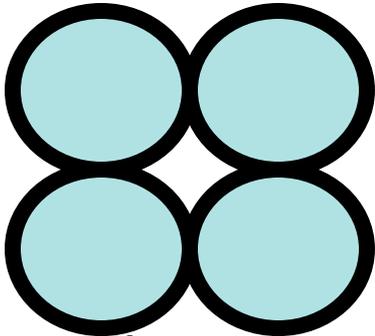


# Identification of Bacteria—Shape/Replication

**Spherical**

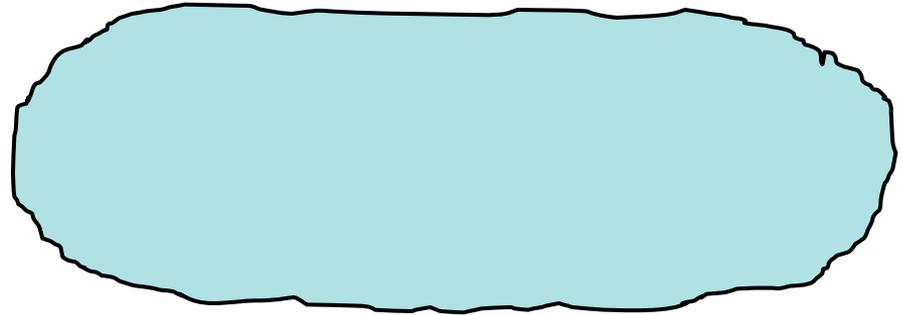


**Chains**

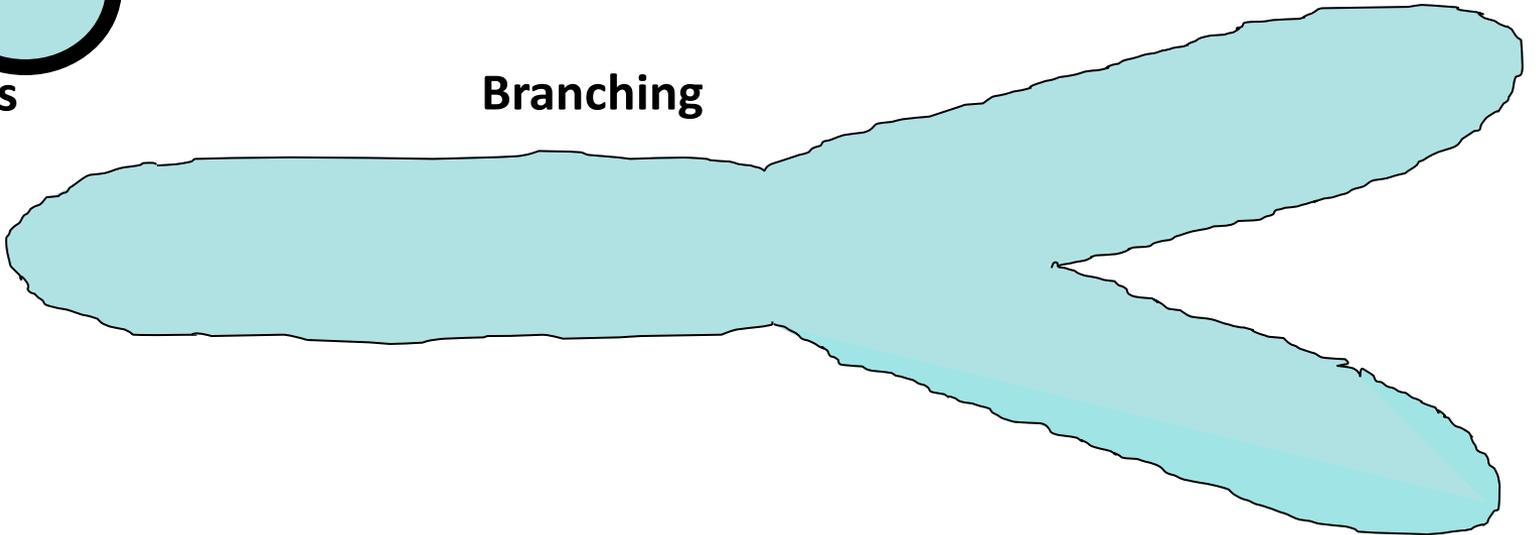


**Clusters**

**Rod-shaped**



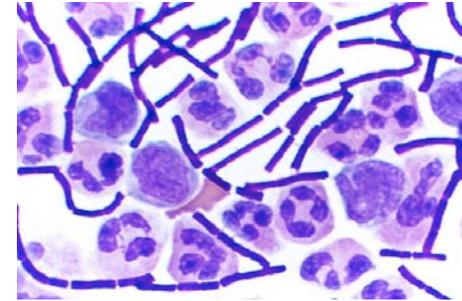
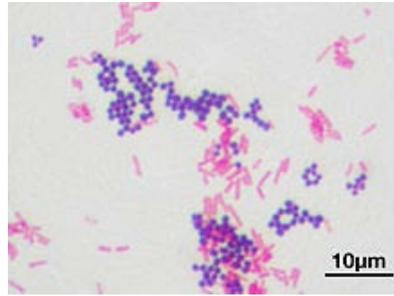
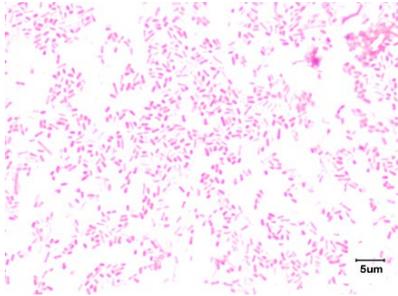
**Branching**



# Identification of Bacteria—Wall Structure

## Gram Negative Bacteria

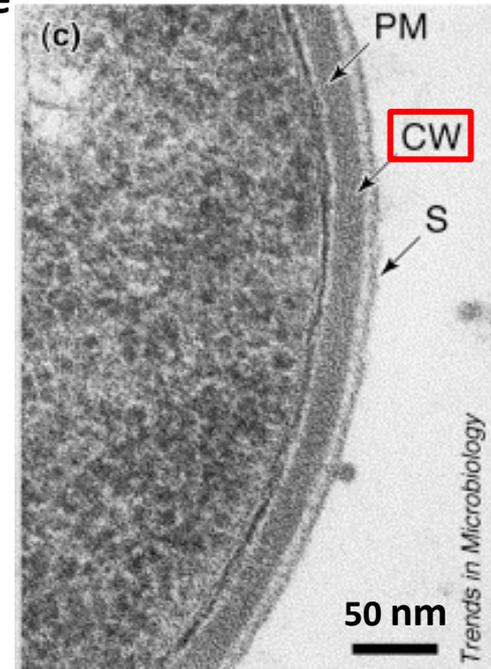
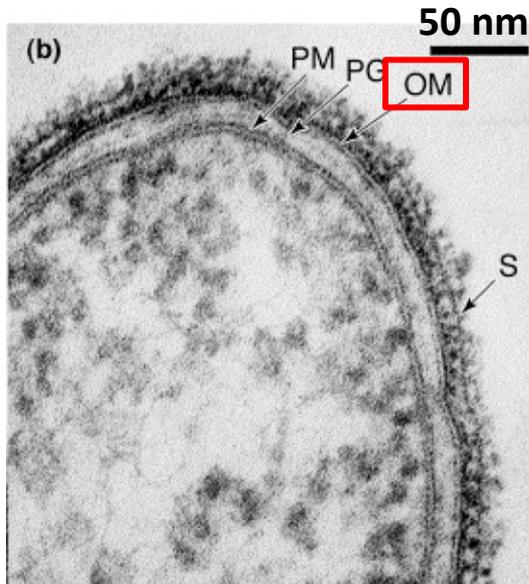
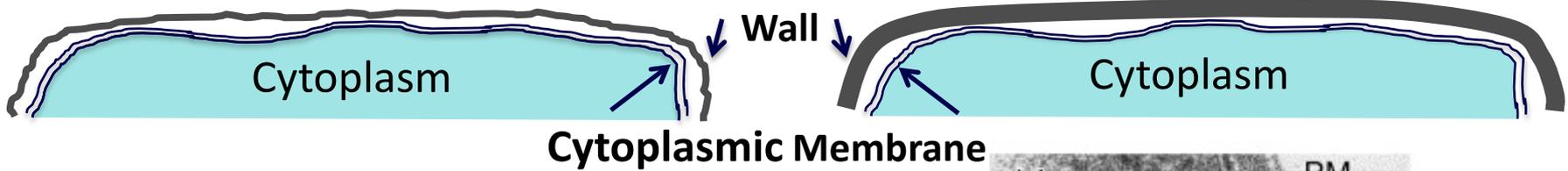
## Gram Positive Bacteria



7-8 nm

**Peptidoglycan**

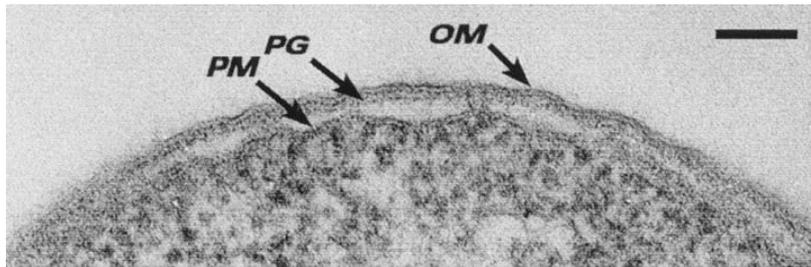
20-80 nm



Trends Microbiol.  
1999. 7(6):253-260.

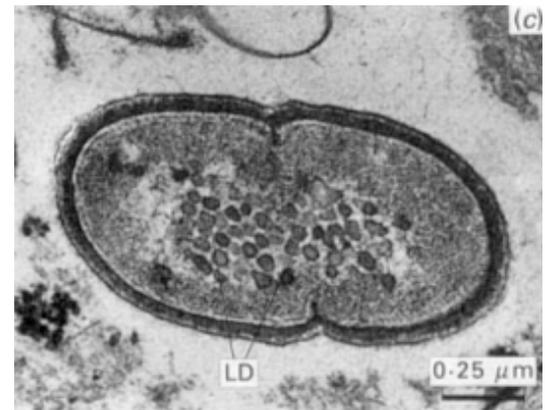
# Identification of Bacteria—Wall Structure

## Gram Negative Bacteria

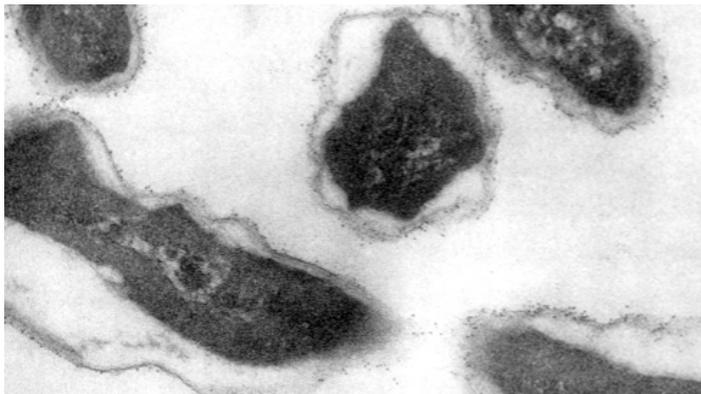


J Bacteriol. 1999. 181(16): 4725–4733.

## Gram Positive Bacteria



Brit J Nutrit. 1990;63;563-578



J Bacteriol. 1978;135;687-702

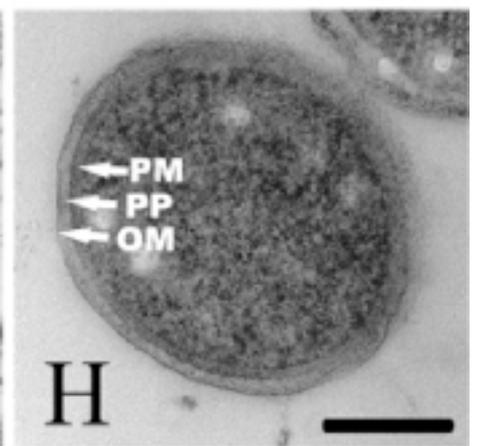
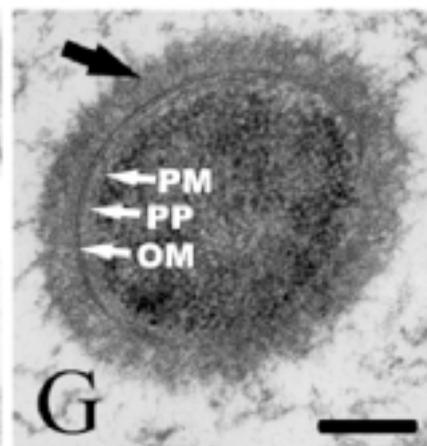
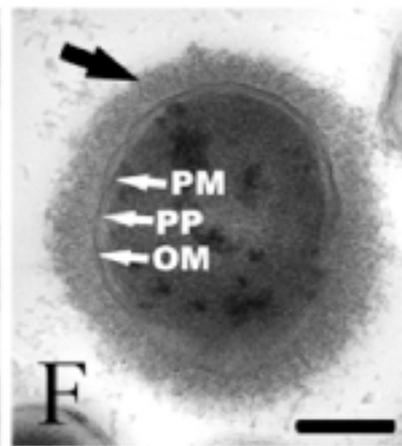
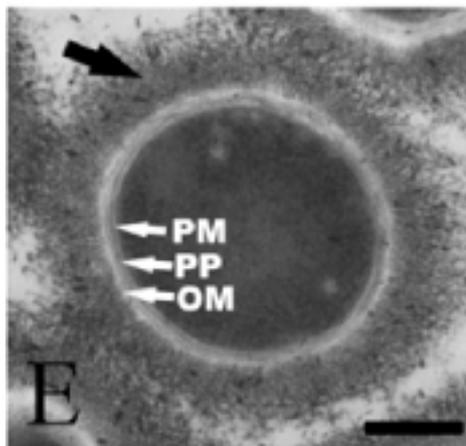
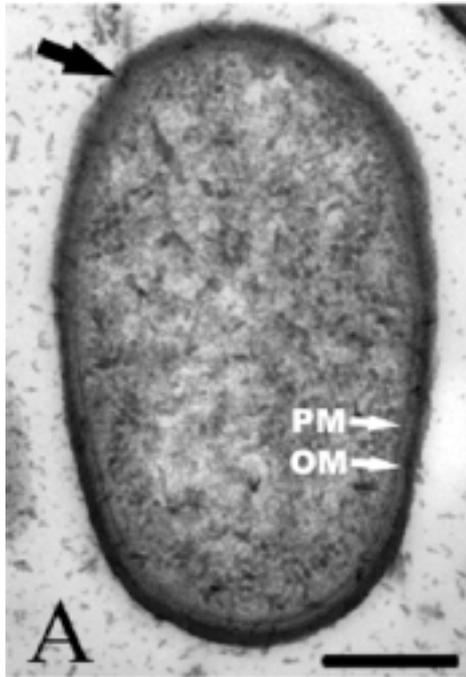
# Identification of Bacteria—Capsule

*E. coli*

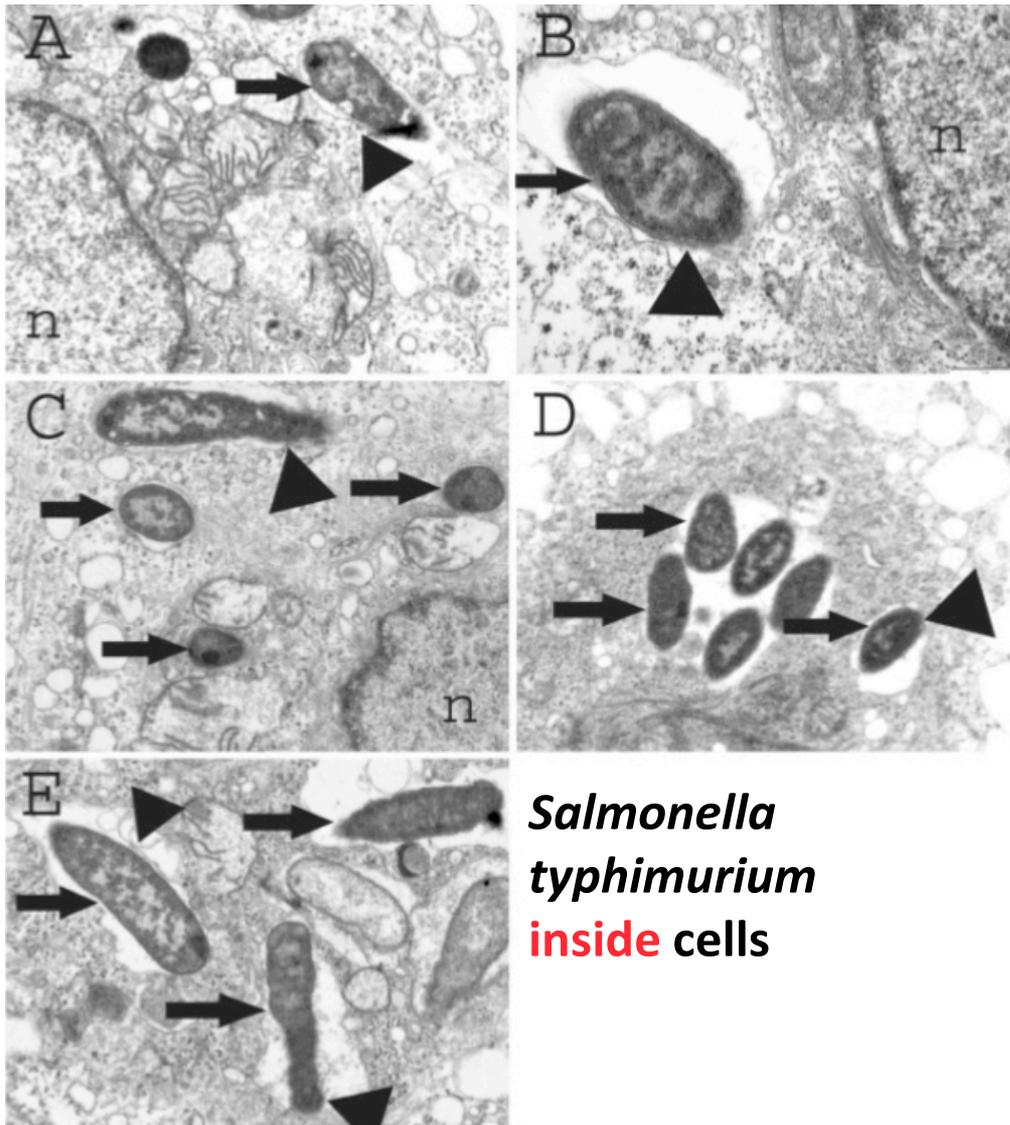
*P. aeruginosa*

*S. oneidensis*

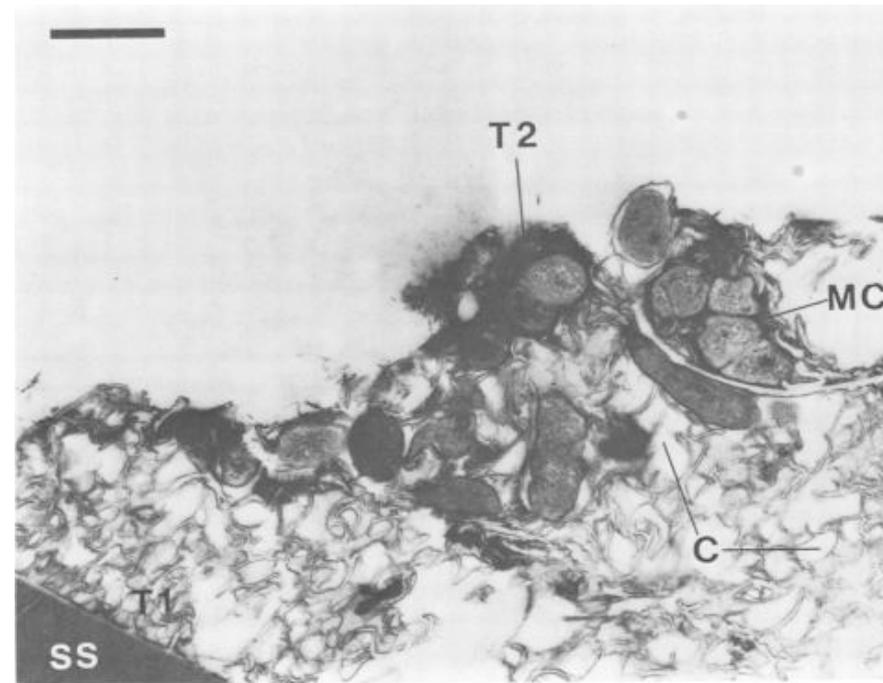
*G. sulfurreducens*



# Identification of Bacteria—Location



*Salmonella typhimurium*  
inside cells



Surface bacteria

# Summary:

## Identification of Bacteria

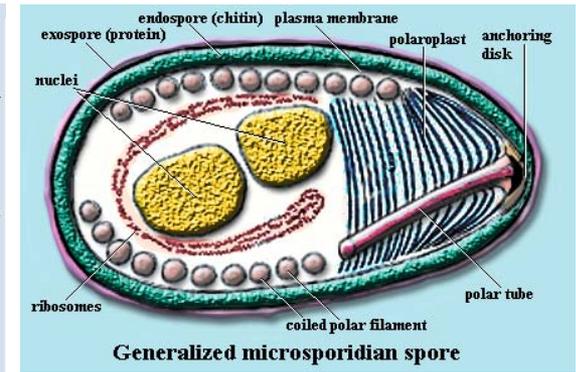
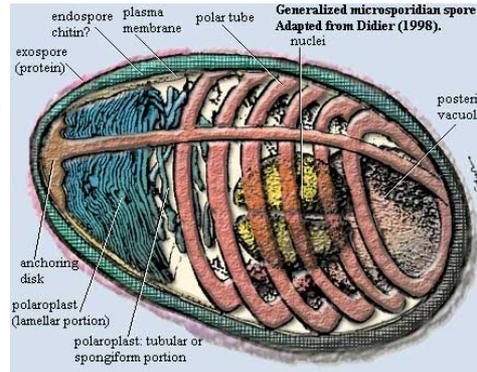
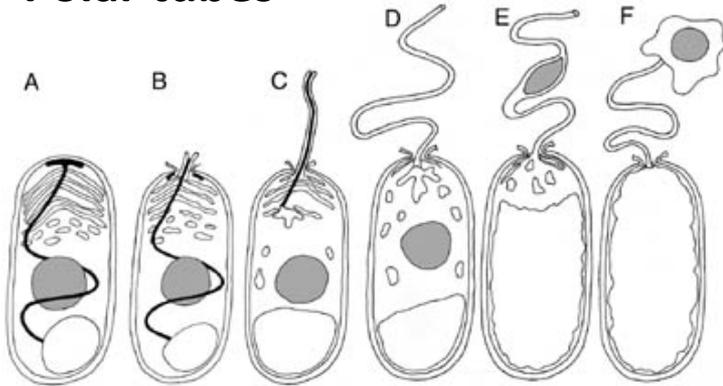
- **Shape: coccoid/baciliform (rod-shaped)**
- **Size: diameter/length**
- **Cell wall type: Gram negative/positive**
- **Location: intracellular/extracellular**
- **Variety: all one kind or mixed**
- **Replication: clusters, chains**
- **Numbers: many, few**

# Mycology (Fungi)



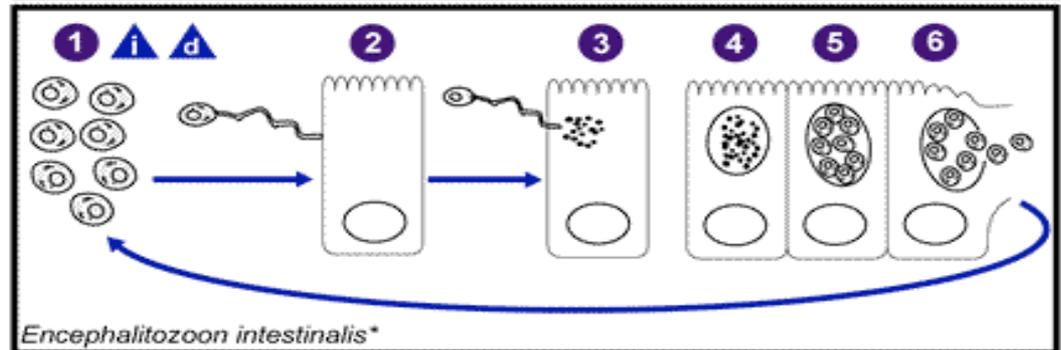
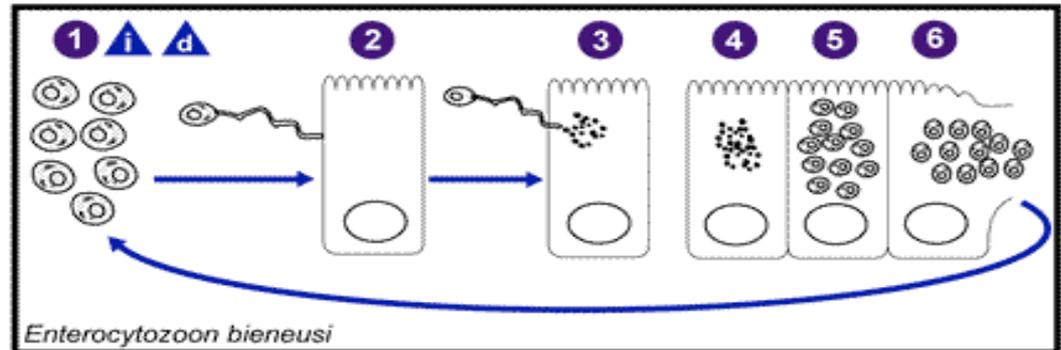
# Identification of Microsporidia

## Polar tubes



<http://www.palaeos.com/Eukarya/Units/Microsporidia/Microsporidia.000.html>

## Pattern of growth inside cells



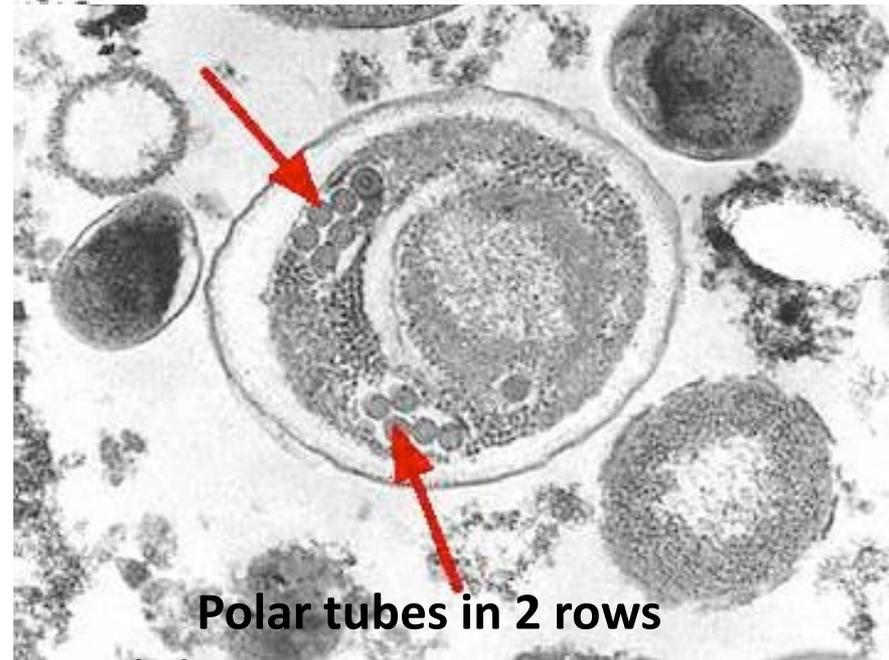
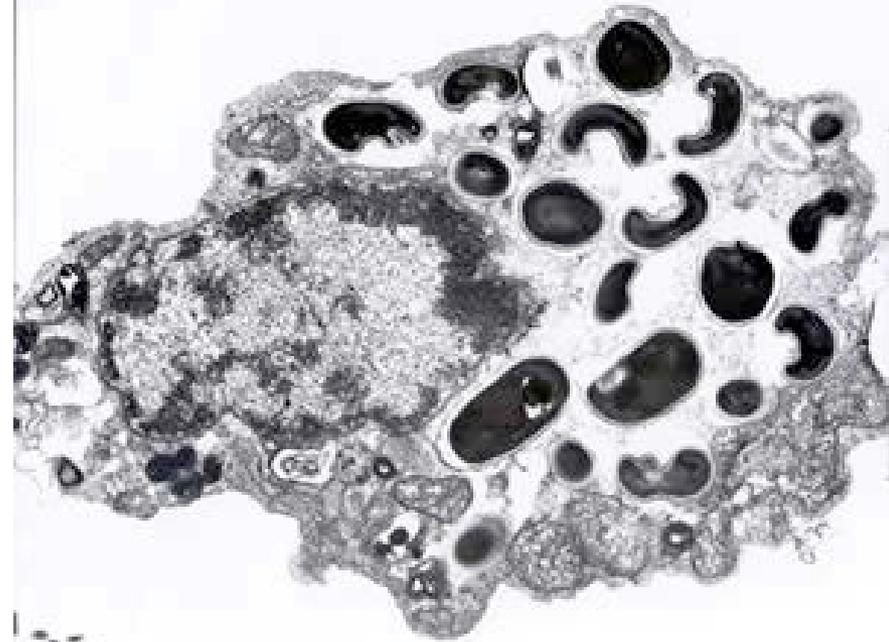
[www.dpd.cdc.gov/dpdx](http://www.dpd.cdc.gov/dpdx)

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

**Septated parasitophorous vacuoles of *Encephalitozoon intestinalis* in jejunum**



J Clin Microbiol. 2002 June; 40(6): 1892–1901.  
doi: 10.1128/JCM.40.6.1892-1901.2002



**Polar tubes in 2 rows**

[http://dpd.cdc.gov/dpdx/HTML/Frames/M-R/Microsporidiosis/body\\_Microsporidiosis\\_EM.htm](http://dpd.cdc.gov/dpdx/HTML/Frames/M-R/Microsporidiosis/body_Microsporidiosis_EM.htm)

# Identification of Yeasts

Size: ~5  $\mu$ m

Ovoid

Thick cell wall

Budding

Encapsulated



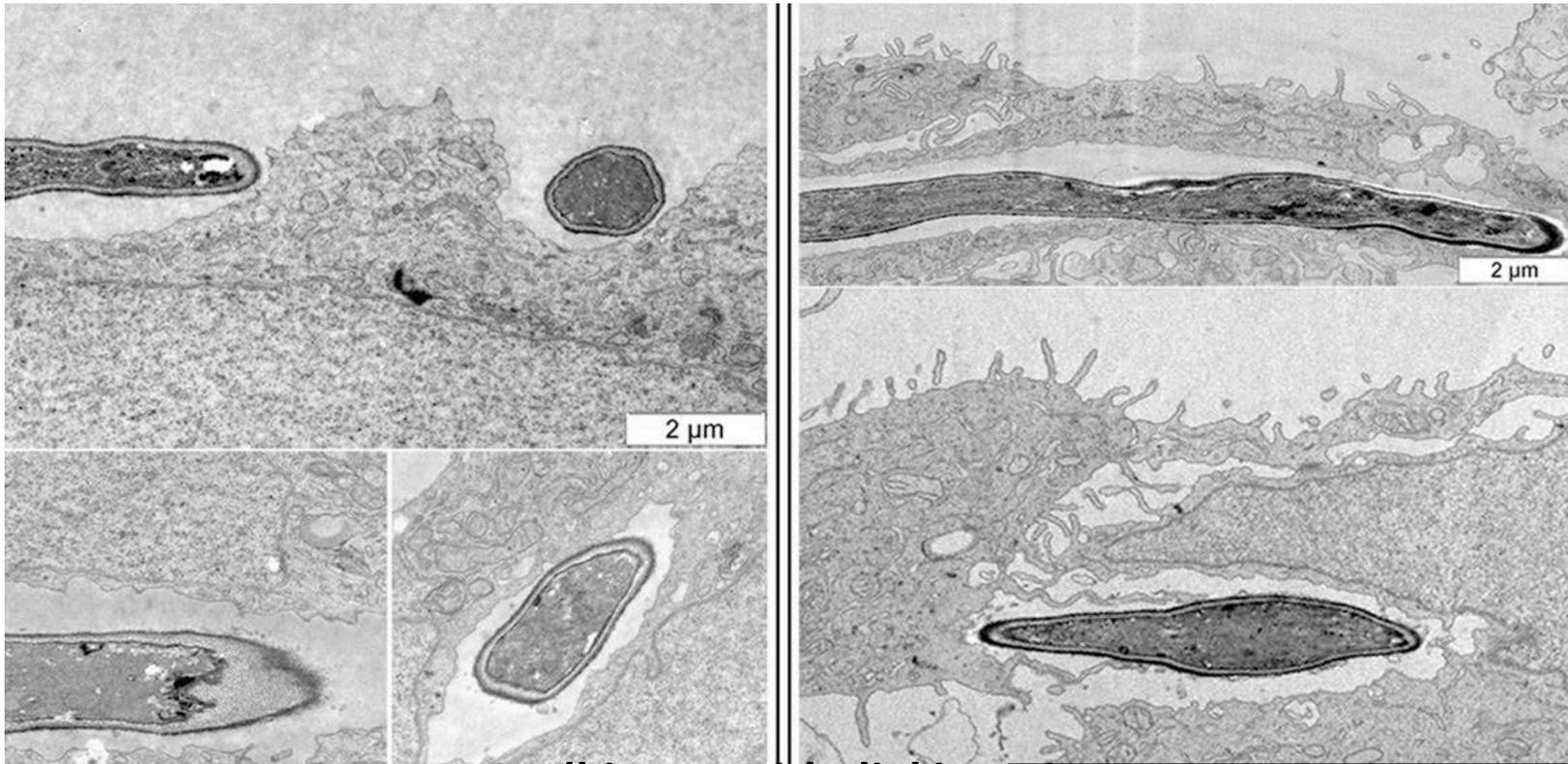
**Yeast invading cells**

From Dr. Soumitra Ghoshroy  
Univ So Carolina



**Yeasts in BAL**

# Identification of Yeasts-Location



***C. albicans* epithelial invasion**

PLoS One. 2012; 7(5): e36952.

Published online 2012 May 14. doi: 10.1371/journal.pone.0036952

# Photology (Algae)

Division Chlorophyta Class Chlorophyceae



*Chlamydomonas* (X2500)



*Ulothrix* (X500)



*Ulva* (X0.1)

Division Chrysophyta  
Class Bacillariophyceae



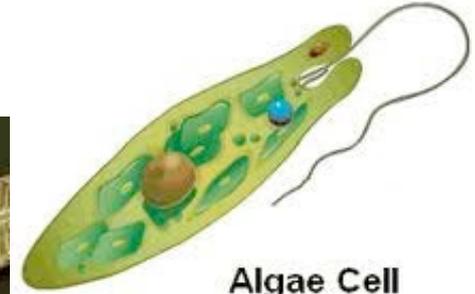
*Melosira* (X600)



*Ceratium* (X200)



*Polysiphonia* (X0.25)



Algae Cell

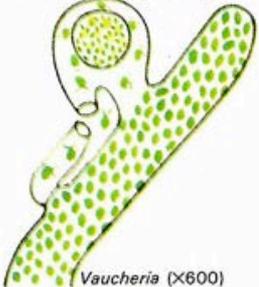
Division Pyrrophyta  
Class Pyrrophyceae

Division Rhodophyta  
Class Rhodophyceae

Division Chrysophyta  
Class Xanthophyceae

Division Phaeophyta  
Class Phaeophyceae

Division Euglenophyta  
Class Euglenophyceae



*Vaucheria* (X600)



*Nereocystis* (X0.05)



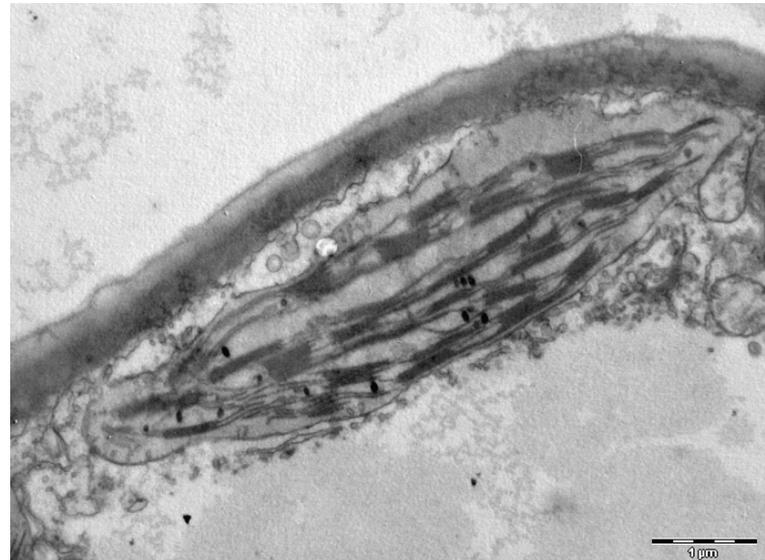
*Euglena* (X600)



# Algae Pathogens

- Most algae with chloroplasts are not pathogenic, except to those with immunosuppression.
- Most common pathogenic alga is *Prototheca*.
- *Prototheca* does not have chloroplasts.

Chloroplast



## **Prototheca species**

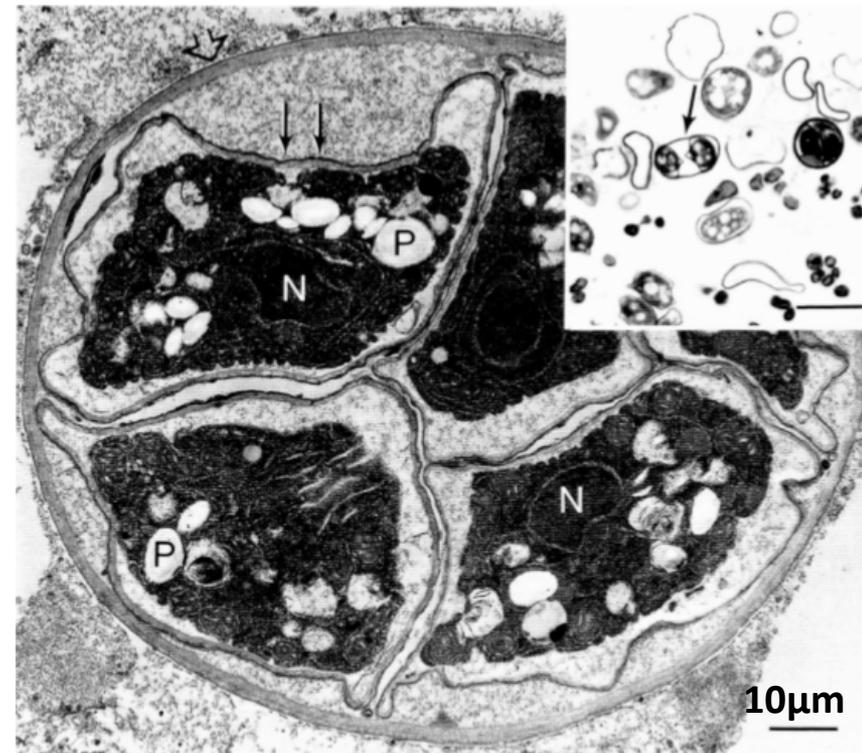
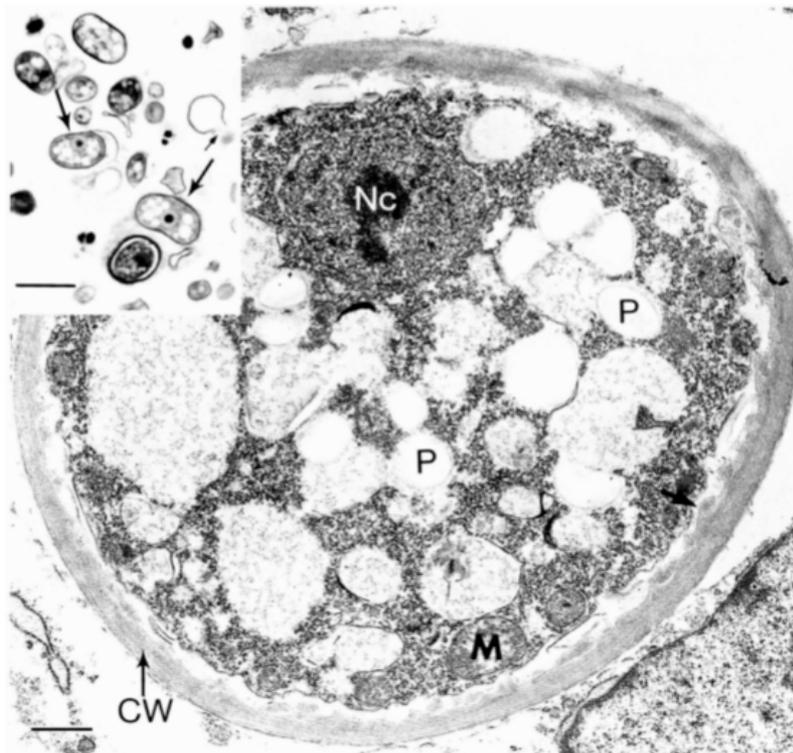
- **Wide range of infections in humans, including cutaneous infections, olecranon bursitis, and disseminated disease.**
- **Can occur in both immunocompetent and immunosuppressed patients.**
- **More severe and disseminated infections tend to occur in immunocompromised individuals.**
- **Found in water; seen in water-contaminated wounds.**

# *Prototheca wickerhami*

6-10  $\mu\text{m}$

No chloroplasts

Internal septations



Vet Pathol 21: 61-66 (1984)

# Protozoology

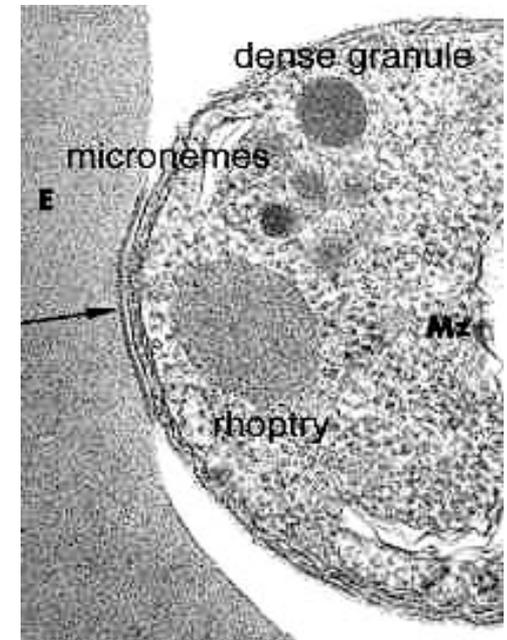
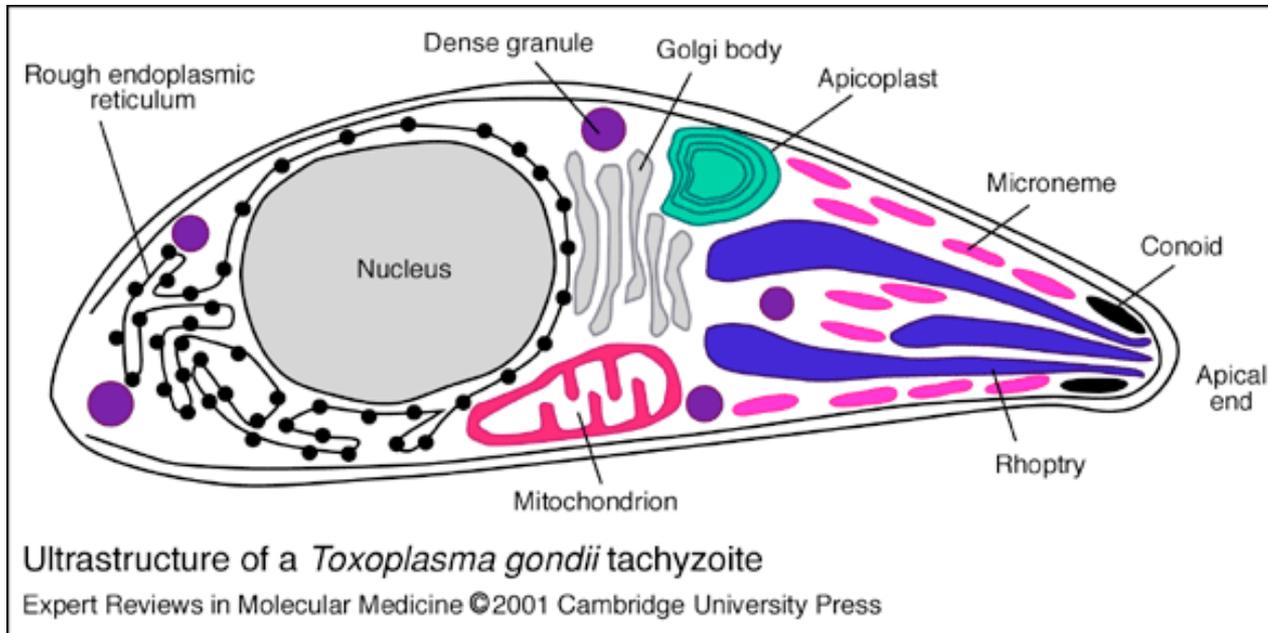
**Big: 10 to 52  $\mu\text{m}$ , up to 1 mm**

**Specialized organelles:**

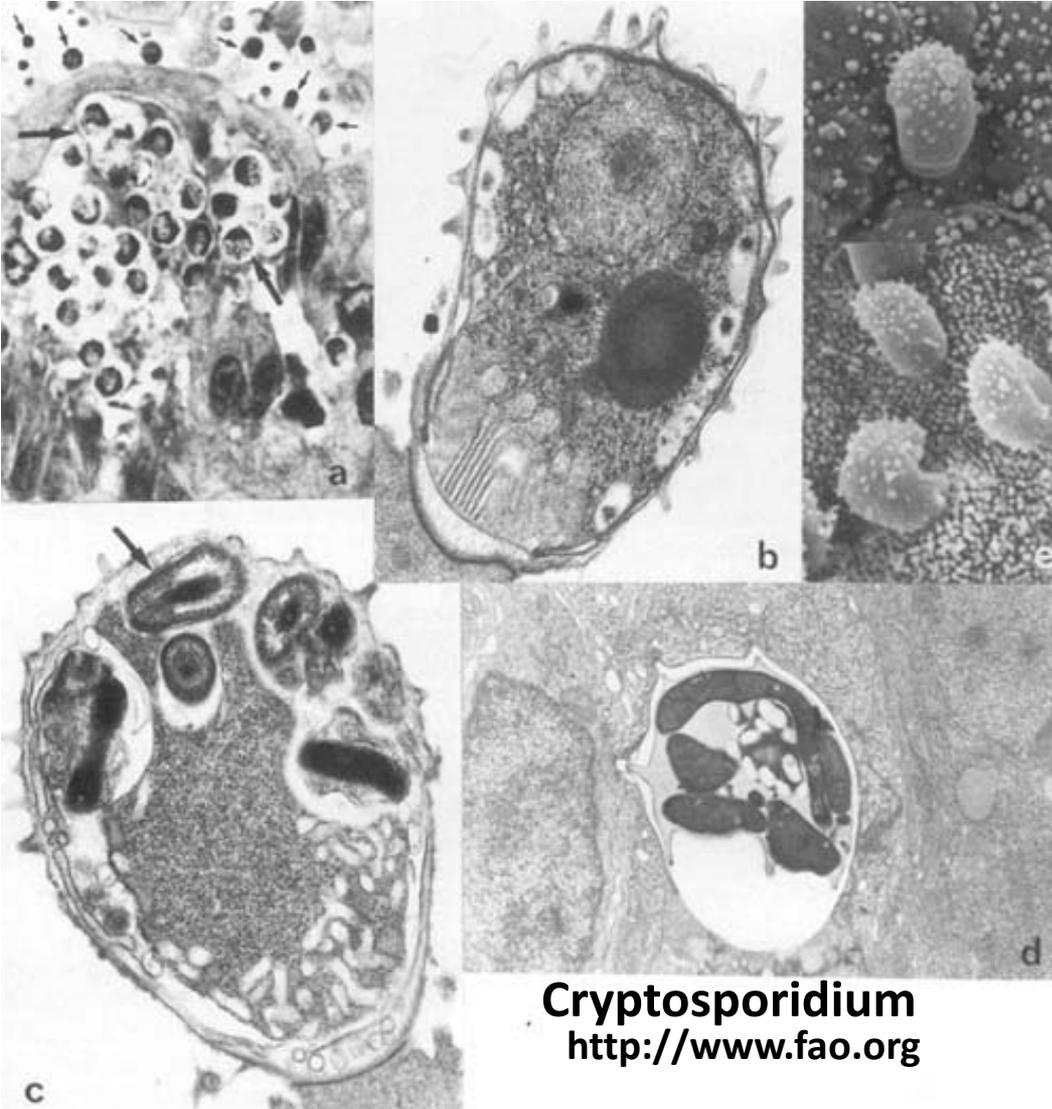
**Rhoptries**

**Micronemes**

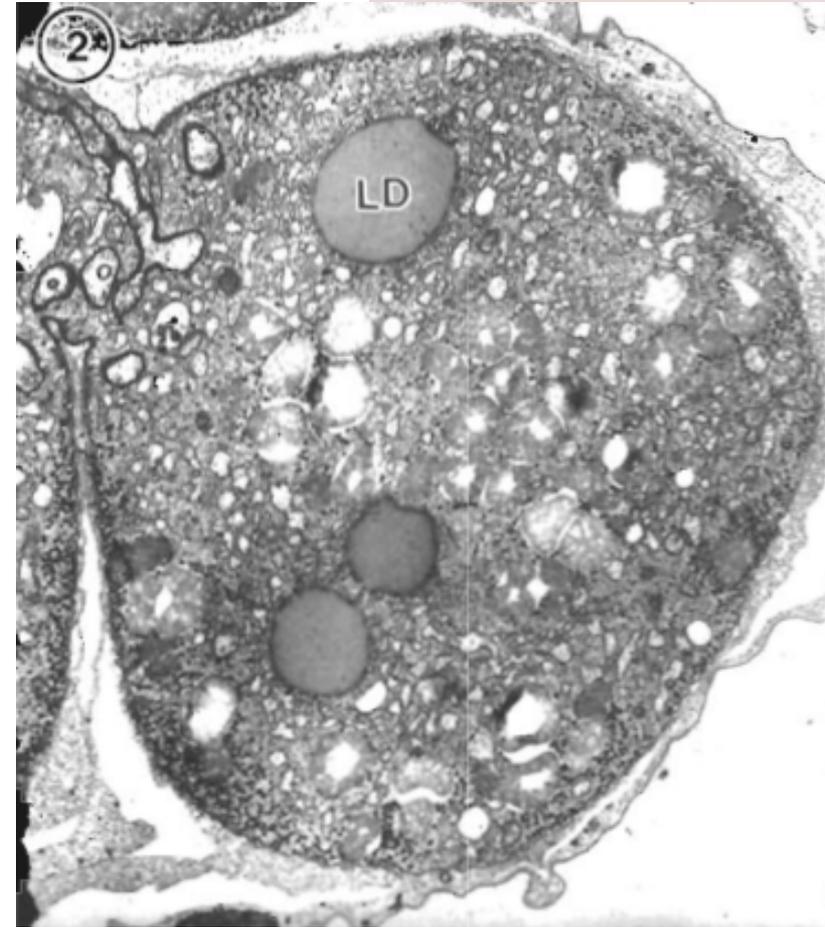
**Conoids**



# Protozoology



**Cryptosporidium**  
<http://www.fao.org>



**Coccidium**

# Summary:

## Higher Organisms

- Is it big?
- Does it have a nucleus?
- Is it a yeast? (ovoid, budding)
- Does it have hyphae? (filamentous)
- Does hyphae have segments, branches?
- Does it have polar tubes, rhoptries, chloroplasts, other identifying organelles/characteristics?
- Where is it? extracellular/intracellular; lung; how specimen obtained?
- Is it a mixed infection?



**Characteristics for Identification**

## References for Protocols:

**Negative Staining Electron Microscopic Protocol for Rash Illness.**

<http://www.bt.cdc.gov/labissues/>

Then click on title above.

**Electron Microscopy for Rapid Diagnosis of Emerging Infectious Agents.**

[http://wwwnc.cdc.gov/eid/article/9/3/02-0327\\_article.htm](http://wwwnc.cdc.gov/eid/article/9/3/02-0327_article.htm)

**Bioterrorism and electron microscopic differentiation of poxviruses from herpesviruses: dos and don'ts.**

[Ultrastruc Pathol. 2003;27:133-140.](#)

**Modern uses of electron microscopy for detection of viruses.**

[Clin Microbiol Rev. 2009;Oct;22\(4\):552-63. doi: 10.1128/CMR.00027-09.](#)

[Review](#)

**Detection and identification of viruses by electron microscopy. J**

[Electron Microsc Tech 4:265-301;1986.](#)