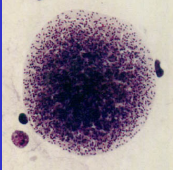



BioMedical Parasitology

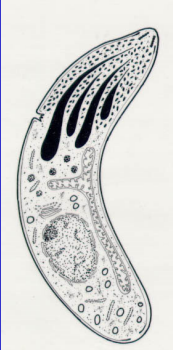
Protozoa
Coccidia

Prof Peter O'Donoghue

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Apicomplexa

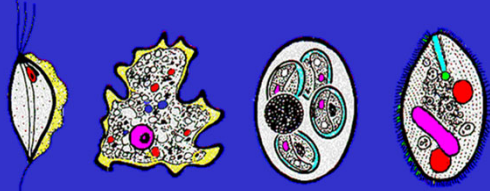





- cluster of anterior organelles designed to facilitate entry into host cell
- Apicomplexa are obligate intracellular parasites

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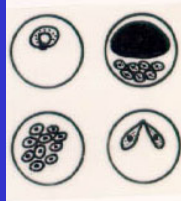
PROTOZOA

65,000 species
(31,250 extant + 33,750 extinct)

flagellates	amoebae	sporozoa	ciliates
			
6,900 species	11,550 species	5,600 species	7,200 species
5,100 free-living 1,800 parasitic	11,300 free-living 250 parasitic	all parasitic	4,700 free-living 2,500 parasitic




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APICOMPLEXA

Coccidia (conoid complete) (small intracellular gamonts)	Haematozoa (conoid absent) (motile kinete)
	
coccidia	haemosporidia/piroplasms


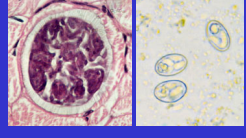
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SPOROZOA

Apicomplexa (apical complex) (oocysts)	Microspora (unicellular) (spores)	Myxozoa (multicellular) (spores)
		
All parasitic		

3

COCCIDIA

<ul style="list-style-type: none"> • enteric • monoxenous • fast cycle • acute 	<ul style="list-style-type: none"> • tissue cyst-forming • heteroxenous • slow cycle • chronic
	
<ul style="list-style-type: none"> • <i>Isospora</i> • <i>Eimeria</i> 	<ul style="list-style-type: none"> • <i>Toxoplasma</i> • <i>Sarcocystis</i>

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COCCIDIA

- undergo sexual development to form oocysts
- exogenous transmission stages
- infective zoites protected by double packing

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Enteric coccidia

<i>Eimeria</i> 1:4:2 herbivores fast monoxenous	<i>Isospora</i> 1:2:4 carnivores fast monoxenous
---	--

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Configuration

number sporozoites per sporocyst	1	2	3	4	8	16	n
0							
1							
2							
4							
8							
16							
n							

Cryptosporidium 1:0:4
Cyclospora 1:2:2
Isospora 1:2:4
Eimeria 1:4:2

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Eimeria

1:4:2

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Coccidiosis

faecal-oral transmission

enteric infection
enteritis, diarrhoea

contamination
of environment

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Isospora

1:2:4

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Parasite pathogenesis

Mucosal pathology

asexual schizogony sexual gamogony
all mature stages lyse cells

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Parasite biodiversity

Eimeria spp.

- hundreds of species described
- herbivorous/omnivorous mammals, birds, reptiles, fish
- most oioxenous
- 12 species in cattle
- 11 species in sheep
- 9 species in goats
- 7 species in chickens
- most in intestinal mucosa
- (some in liver, gall bladder, kidneys)

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Coccidiosis

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Parasite pathogenesis

Pathogenicity

- species vary markedly in virulence
- large species often exhibit slow growth, poor amplification, limited tissue invasion
⇒ low pathogenicity
- small species exhibit explosive growth, huge amplification, aggressive invasion
⇒ high pathogenicity
- need to differentiate pathogenic species

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Parasite pathogenesis

Pathology

- oedema, haemorrhage, inflammation
- villous atrophy, crypt hypertrophy, malabsorption
- diarrhoea, colic, weight loss, fever

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Parasite biodiversity

Isospora spp.

- many species described
- carnivorous/omnivorous mammals, birds, reptiles
- most oioxenous
- 1 species in humans
- 1 species in pigs
- 4 species in dogs
- 2 species in cats
- most in submucosa


Oocysts		DOG	CAT
Large, unsporulated		<i>I. canis</i>	<i>I. felis</i>
Medium, unsporulated		<i>I. ohioensis</i> -like	<i>I. rivolta</i>
Small, unsporulated		<i>H. heydorni</i> <i>N. caninum</i>	<i>H. hammondi</i> <i>B. arloingi</i> <i>T. gondii</i>
Small, Sporulated		<i>Sarcocystis</i>	<i>Sarcocystis</i>

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Parasite diagnosis

Diagnosis

- **symptomatology**
 - clinical signs (nonspecific)
- **direct demonstration of parasite**
 - coprology (numerous oocysts)
- **indirect indication**
 - antibodies (acute infection too fast)
 - antigens (copro-tests)
 - DNA (PCR)



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Parasite pathogens

Treatment/Control

- **pre-medicated food (coccidio-stats/-cides)**
- **high levels of drug resistance**
- **effluent disposal/litter management**
- **vaccine development**
 - live vaccines
 - nonpathogenic species (cross-protection)
 - precocious strains (low virulence)
 - attenuated vaccines (irradiated)
 - subunit vaccines (extracts, recombinants)
 - maternal transfer
 - egg immunoglobulins (vaccinate hen)
 - hyperimmune colostrum (suckling)

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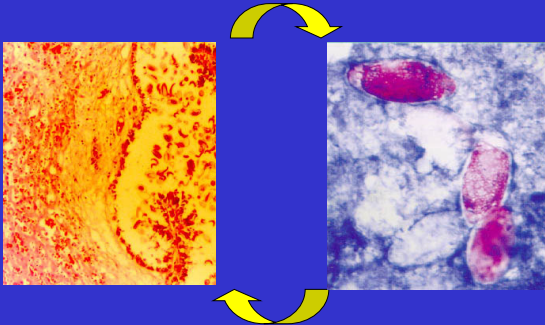
Isospora belli oocysts

unsporulated (sporoblast)	sporulated (1:2:4 configuration)
	

Treatment with coccidiostats/coccidiocides
(trimethoprim-sulfamethoxazole)

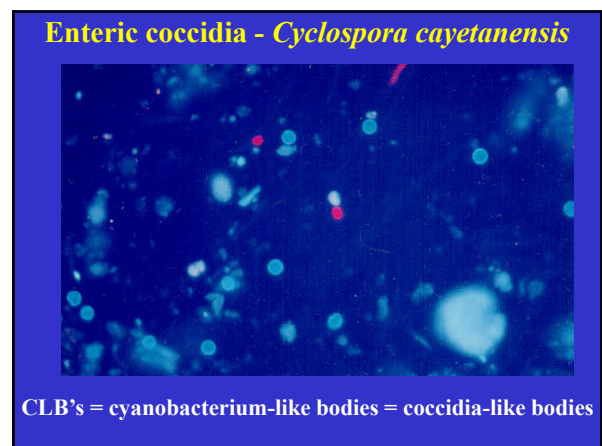
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Enteric coccidia - *Isospora belli*

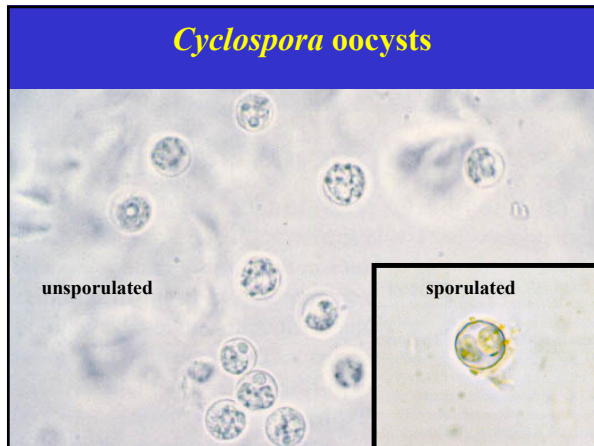


endogenous stages
faecal-oral
exogenous oocyst

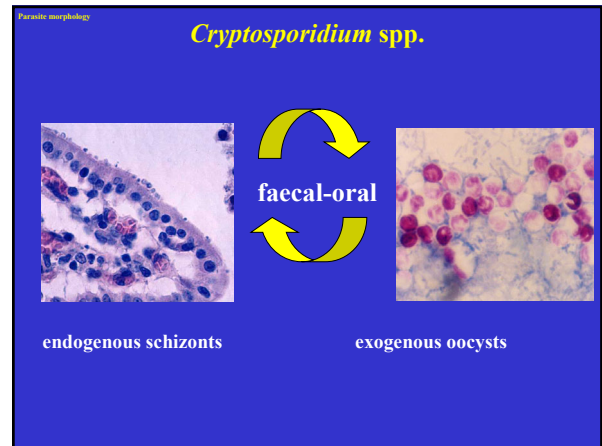
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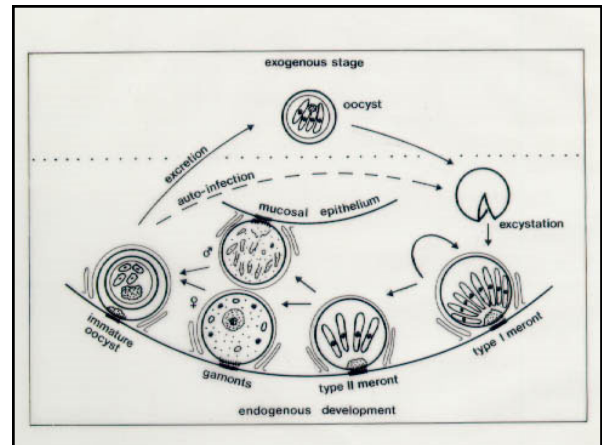


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Cyclospora

- associated with diarrhoea
- world-wide (esp. SE Asia & Central America)
- flu-like illness = nausea, vomiting, anorexia, explosive diarrhoea for 1-3 weeks
- contaminated water?
- contaminated raspberries?
- now discovered to be another coccidian but with 1:2:2 configuration

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Cryptosporidium

Parasite problem

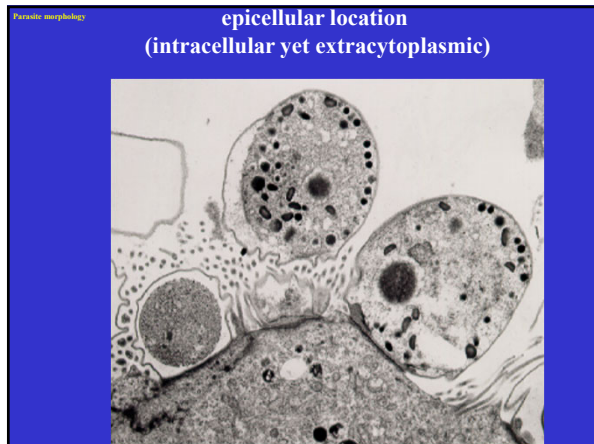
Very small oocysts
Overlooked for years,
but now....

- newly recognized enteropathogen
- protozoan parasite similar to coccidia
- causes significant morbidity, some mortality
- anthroponotic, zoonotic, water-borne

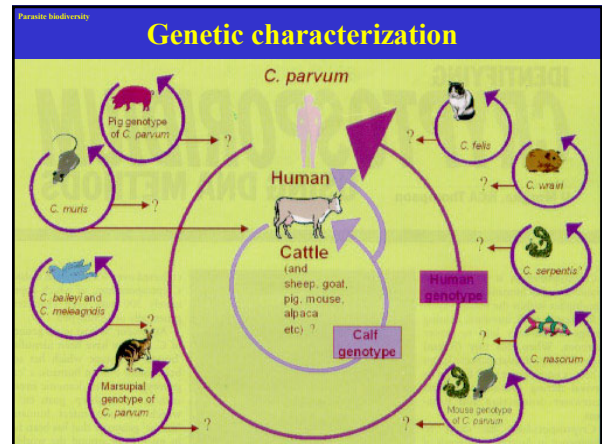
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Pathogenesis

Cryptosporidiosis

- villus atrophy
- microvillus destruction
- impaired glucose and electrolyte transport
- impaired carbohydrate and protein digestion
- malabsorptive and maldigestive disease
- pernicious (acute)
- auto-infection (chronic)
- no treatment (nitazoxanide?)
- variable sources (H/C)

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Treatment

- chemotherapy ineffective
- conventional drugs do not work
- supportive therapy (treat symptoms)
- many molecular/biochemical studies searching for novel targets
- some recent success with nitazoxanide (PFOR inhibitor)

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Parasite biodiversity

Parasite species

• mammals	<i>C. hominis</i>	intestines	acute
	<i>C. parvum</i>	intestines	acute
	<i>C. muris</i>	stomach	chronic
	<i>C. wrairi</i>	intestines	chronic
• birds	<i>C. meleagridis</i>	intestines	acute
	<i>C. baileyi</i>	trachea	acute
• reptiles	<i>C. serpentis</i>	stomach	chronic
• fish	<i>C. nasorum</i>	stomach	chronic

Diagnosis: host occurrence, parasite morphology, site of infection, proteins, nucleic acids (rDNA, HSP, COWP)

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Host susceptibility/resistance

Age-related

- clinical infections most common in neonates
- rapid development of resistance in animals

Acquisition of mature intestinal flora

- severe infections in germ-free/gnotobiotic animals

Malnutrition

- depleted iron status, low protein diet

Immunological competency

- immature - senescent
- immunodeficiencies (congenital + acquired)
- immunosuppression (iatrogenic + concomitant)

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Humoral immunity

Serum antibodies (acute-convalescent)

- serological tests (IFAT, ELISA)
- transient IgM, IgA, IgE (weeks)
- prolonged IgG (months)

Copro-antibodies (patent infections)

- local/secretory IgA, IgM, IgG 5-16 dpi

B cell deficiencies

- hypo-, a-gammaglobulinaemia
- selective immunodeficiencies

Antibodies alone not protective (strong responses in AIDS patients with chronic infections)

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Immuno-modulation

CD4 (helper) limit duration
 IFN γ limit severity

Led to concept of cytokine immunotherapy
 IFN γ ; therapeutic application truncated infection
 (but oocyst shedding recommenced after treatment)
BUT,
 adverse effects unknown and therapy cost-prohibitive

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Cell-mediated immunity

inflammation/infiltration

- neutrophils, macrophages, lymphocytes, plasma cells

T-cell deficiencies

- low CD4 (helper)/AIDS patients - chronic infections
- CD4 depletion in animals - chronic infections
- CD4 restitution - limits duration of infection
- CD8 (cytotoxic) modulation - no effect
- NK (natural killer) cell modulation - no effect
- SCID mice, nude mice/rats - chronic infections

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Lactogenic immunity

Observations from surveys of neonates

- fewer infections in breast-fed children than in bottle-fed children
- more severe infections in colostrum-deprived calves, lambs

Passive transfer studies

- colostrum neutralizes sporozoites
- colostrum protects against severe disease
- colostrum helps resolve symptoms



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Cytokines

IFN γ (interferon-gamma)

- selective depletion by neutralizing mAb's leads to severe infections
- restoration reduces severity of infection
- deficient C57/BL6 mice develop non-resolving fatal infections compared to asymptomatic self-limiting infections in normal wild type
- PBMC (peripheral blood mononuclear cells) produce IFN γ in immunocompetent patients but not in AIDS patients

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Colostrum

- maternal milk produced post-partum
- nutritionally-rich (protein/fat)
- immunologically-rich (antibodies)
- plentiful source (dairy industry)

Source

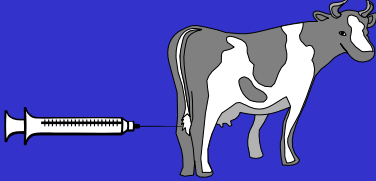
- uninfected cows - low titre (1:100)
- infected cows - medium titre (1:1000)
- devise immunization schedule to improve titre

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Immunobiology

Immunization

Route - intramammary infusion
 Dose - soluble oocyst-sporozoite extracts
 Schedule - weekly pre-partum
 Collection - two days post-partum



HIBC = hyper immune bovine colostrum

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HIBC immunotherapy

- similar strategy used for other enteropathogens e.g. rotavirus
- protective activity of colostrum well known in animal industries/veterinary science as prophylaxis against neonatal diarrhoea esp. in piggeries

Alternative strategies

- mouse monoclonal antibodies
- hyperimmune egg yolks

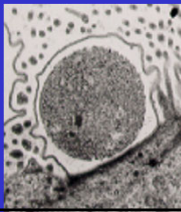
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Immunobiology

Characterization

Antibodies

- titres up to 1:400,000
- isotypes IgG₁, IgG₂, IgM, IgA
- reactivity against antigens from:
 - sporozoites
 - merozoites
 - gametocytes
- intracellular activity?



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Immunobiology

Immuno-therapy


Prophylaxis (administered before infection)

- partial protection in animals
- reduce severity of infection

Therapy (administered after infection)

- patent period reduced
- oocyst production reduced
- clinical resolution/ symptomatic improvement

Undergoing clinical trials (FDA, TGA)
 Problem with lactose intolerance (lactose reduce)



Drink milk!

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