Veterinary Protozoology

By

Zewdu S. (DVM, MSc, MTAH)

University of Gondar

College of Vet. Medicine and Animal Sciences

Learning Objectives

- **At the end of this session students will be able to:**
  - ✓ describe morphology, biology and feeding mechanisms of protozoa
  - ✓ identify the different types of vet. Important protozoa
  - ✓ explain factors that affect the occurrence of pathogenic protozoa
  - ✓ diagnose, treat and control vet. important protozoan infection

Introduction

- **Protozoology** is a branch of **biological** sciences that deals with the study of protozoans
- Protozoans are **unicellular**, **eukaryotic** **chemoheterotrophic** organisms, whereby different **activities** are carried out by different **organelles**: ER, mitochondria, Golgi bodies, lysosomes.
- They have much greater **complexity** than other unicellular life-forms, such as **bacteria**.

Introduction

- Like **multicellular** animals and plants, their **DNA** is mostly packaged into **chromosomes** within a nucleus.
- Discovered by **Anthony Van Leuwenhoek by 1674**
- More than **45** thousands species of protozoa were discovered; majorities are **free-living** while few are **parasitic**.
- Are **eukaryotic**, whereby their **genetic** information is stored in the chromosomes

Introduction

- **Nucleus** consists of the **nuclear membrane**, the **nucleoplasma** (fluid inside the nucleus) and **nucleolus**, which is a **chromatin** material
- **Cytoplasm** is extra nuclear part
- **Protozoans** obtain **their energy** by the **intake of organic material**, but no through **photosynthesis** in chloroplasts
- Do not possess a **rigid cellulose wall** exterior to the cell membrane

Introduction

- **Endostome** and **nucleolus** are collectively known as **chromatin** material
- Protozoans have **two types** of nucleus:
  - ✓ **Vesicular nucleus**: where the amount of **nucleoplasm** is greater than the **chromatin** material of nucleus. This is true for mastigophora (*Trypanosoma*) and sarcodina (*Amoeba*)
  - ✓ **Compact nucleus**: where the amount of nucleoplasm is very **small or absent**. This is true for ciliates (*Balantidium*).

Introduction

- Most protozoa have **two stages**
  - ✓ **Trophozoite** – the feeding and growing stage
  - ✓ Some protozoa will produce a **protective capsule** called a **cyst**.
    - A cyst allows the parasite to exist outside of the host and be the **infective stage** allowing the parasite to get to another host.

Protozoa: Locomotion

- **Protozoans** can move by means of:
  - ✓ **Gliding**: this is a feature of *Toxoplasma*, *Sarcocystis* and *Eimeria* (?).
  - ✓ **Pseudopodia**: it is the feature of ***Entamoeba***. It is **temporary** means of locomotion. **Pseudopodia** is a part of the body of protozoa, where accomplished by the **projection of cytoplasm** towards one side. It possess **phagocytic** capacity used for trapping **food molecules** via a vacuole.

Protozoa: Locomotion

- Protozoans can move by means of:
  - ✓ **Cilia**: are fine, minute, hair-like structures extending to the external part of the body. **Cilia** are organs used to take **feed**, **move** and as **organ of tactile** (sensory structure). E.g., *Balantidium* species
  - ✓ **Flagellum**: is a kind of whip like structure that arises from the **kinetoplast** (blepharoplast or basal body/granule): *Trypanosoma*, *Leishmania*, *Giardia* and *Trichomonas*

Some important characteristics of major protozoan groups

	Locomotion by	Intracellular stages?
Ciliates	Cilia	No
Amoebae	Pseudopodia	No
Flagellates	Flagellae	Some species
Apicomplexa	Gliding	Yes

Protozoa: Nutrition

- There are **three** types of protozoans based on their mode of nutrition:
  - ✓ **Halophytic** protozoans: synthesize carbohydrates by the help of **chlorophyll**. They are not important as causes of disease.
  - ✓ **Holozoic** protozoans: utilize food from other living plant or they may ingest tissue cells of their hosts by the help of **cytostomes** and therefore are parasitic. Example: *Balantidium* coli and *Entamoeba* species
  - ✓ **Saprozoic** protozoans: absorb food particles directly by a kind of **diffusion** into the body whereas holozoic protozoa ingest by the help of cytostomes

Types of feeding or ingestion

- Protozoa feed mainly on **particulate** material.
- There are **four** feeding mechanisms in protozoans:
  - ✓ **Pinocytosis:** is a mechanism by which protozoans take food in **liquid** form. The cell membrane **indents** and **folds** slowly over, thereby entrapping a small quantity of food and drawing it into the cell.
  - ✓ **Phagocytosis** (endocytosis): is a mechanism by which protozoans take **food in solid** form.

Types of feeding or ingestion

- There are four feeding ----------:
  - ✓ **Cytostomal** feeding: involves feeding through the **cytostome**. Cytostome is also refereed as **micro-pore** or **micropyle**. It is a feature of ciliates, where they take food particles by the action of cilia towards the base of a funnel-like structure: **cytostome**.
  - ✓ **Diffusion:** There are a number of biochemical processes in different feeding systems.

Reproduction

- Protozoa reproduce **sexually** and **asexually**
  - ✓ **Asexually**: Fission (mitosis), Budding, Schizogony
  - ✓ **Sexually**: Conjugation, Syngamy (Gamete formation)
  - ✓ **Definitive host:** harbors the **sexually** reproducing stage of parasite.
  - ✓ **Intermediate host:** harbors **asexually** reproducing portion of the parasite's life cycle.

Reproduction

- **Asexual reproduction:**
  - ✓ **Binary** fission: is the commonest form of reproduction, where the **parent cells** give rise to **two daughter cells**.
  - ✓ **Schizogony** (merogony): a **trophozoite** grows to a large size while the **nucleus** divides many times unlike binary fission where the nucleus is divided only into two parts. The end product of schizogony is **numerous daughter cells**. The parent cell in the process of schizogony is called **Schizonts**, while the resulting daughter cells are called **Merozoites**

Reproduction

- **Asexual reproduction**:
  - ✓ **Budding**: it is an asexual reproduction where the **cytoplasm** and the **nucleus** divide **unequally** so that **small offspring** bud off from a parent cell (example: *Babesia*). A bit of cytoplasm or a larger fragment of the parent cell will result in **daughter cell**.
  - ✓ **Sporogony**: It is a **multiple** fission of an encysted **zygote** or **oocyst** or **spores**, resulting in the formation of **sporozoites**. As a result of this process, mature ('sporulated') oocysts contain two or more infective organisms ('**sporozoites**'), often arranged in bundles within separate enclosing walls ('**sporocysts**').

Reproduction

- **Sexual reproduction:**
  - ✓ **Conjugation**: It is a **sexual** means where **two organisms** come together and they **exchange nuclear materials** and then they separate and **nuclear recognition** takes place. Then the daughter cells will form **exconjugant**.
  - ✓ **Syngamy**: is a form of **sexual** reproduction where two gametes come together to form a **zygote.**
    - The male gamete is called **microgamete**, which is formed from **microgametocyte.**

Reproduction

- The female gamete is known as **macrogamete**, which is derived from **macrogametocyte**.
- The process of transformation from gameteocytes to **gametes** is called **Gametogony.**

Host-parasite interaction

- **Hosts** rarely gain any benefit from the presence of parasites and are often harmed by them.
- **Defence** mechanisms have therefore evolved which, if totally effective, would have **extinguished parasitism** as a lifestyle.
- But the continued existence of an abundance of parasites indicates that **successful counter-strategies** have arisen through **natural selection**. These in turn have driven the development of **further protective measures.**

Host-parasite interaction

- Hosts have evolved many **behavioral** and **other strategies** to reduce the **risk of succumbing to parasitism**.
- The most powerful form of defence, however, is the **immune system.**
- This comprises of **chemical** and **cellular** weapons used to combat **invasive organisms**.
- **Immune reactions** may **completely** or **partially** disable the attacker or they may **alleviate** the **clinical** consequences of infection.

Host-parasite interaction

- Ideally, **immunity** should protect against **reinfection** after the invading parasites have been eliminated. This is called '**sterile immunity**'. It can last for a lifetime but often wanes with time.
- Sometimes; however, such **protection** persists only as long as a few parasites survive to continually boost the **immune processes**. This is known as '**premunity=none-sterilized immunity**'.

Host-parasite interaction

- In some cases, **parasite evasion** has gained an evolutionary advantage that renders **host immunity** relatively **ineffective**, so the **host remains vulnerable** despite being repeatedly exposed to infection. E.g.: *Trypanosoma* infection
- Some immune reactions directed at a parasite can produce collateral damage to host tissues. **Hypersensitivity** and **allergy** are well-known examples.
- Immunity can be: **innate immunity** & **acquired immunity**

Protozoan evasion of immunity

- The survival of parasitic species is dependent on being able to **escape the immune responses** of its host.
- There are several evasion strategies:
  - ✓ **Sequestration**: parasites hide themselves from the immune effects by:
    - adopting inaccessible predilection site (cells, organs: CNS, GIT)
    - generating a protective capsule or **cyst wall**

Protozoan evasion of immunity

- **Strategies**……

  - ✓ **Masking or changing surface antigens: by**
    - incorporating **host molecules** onto the surface of the parasite;
    - generating **parasite antigens** which mimic host molecules;
    - **antigen variance** – periodic changes of surface antigens
  - ✓ **Disturbance of immunological effector mechanisms**: by
    - surface shedding to remove adhering immune cells or Ab bound to parasite antigen

Protozoan evasion of immunity

- ✓ **Disturbance of immunological effector mechanisms**: by
  - **enzymatic digestion** of antibodies (Ab)
  - **inhibition of oxidative** products synthesized by leukocytes
  - reducing **MHC-expression on the surface of infected cells**: inhibiting antigen presentation to the immune system

Protozoan evasion of immunity

- ✓ **Modulation of the host immune response: by**
  - **induction of multiple clones** of T- and B-cells that produce nonspecific chemicals or Abs (polyclonal activation): reduce sufficient production of specific antibodies against the specific parasite
  - induction of **immune complexes** in the blood and **cleavage** of antibody/ complement factors, both of which result in **severe immune suppression**.

Host-parasite interaction disturbance

- The host–parasite relationship can be perturbed in two ways:
  - ✓ **Increased host susceptibility: if animals are:**
    - Stressed, debilitated or immunocompromised
    - **exposed to parasites** with which they have not coevolved
    - **selectively bred** for production traits at the expense of natural ability to resist infection (innate or acquired)
    - **inbreeding**

Host-parasite interaction disturbance

- **Increased parasite numbers:** host-seeking (**infective**) life-cycle stages may increase, for example, if:
  - ✓ host stocking density is increased, thereby increasing parasite exposure;
  - ✓ parasitized animals are introduced into a previously clean area (e.g. through livestock movements, global trade etc.), thereby infecting susceptible local livestock, potential wild-life reservoirs or vectors;
  - ✓ short-term weather patterns or longer-term trends such as global warming

Host-parasite interaction disturbance

- there is a **surge in the population of intermediate** hosts or vectors, or an **increase** in the number infected or their accessibility;
- the parasite population becomes **resistant** to anti-parasitic drugs.

- In general, **host defenses** and **parasite immune evasion** are both contributory factors to make **host-parasite** relationship **stable**: the total **elimination** of a parasite from the host population can have **unintended consequences**

Pathogenic effects of protozoans

- ✓ Protozoans can damage the tissues or adversely influence bodily functions of infected animals by:
  - **traumatic outcomes** and **mechanical defects**
  - parasite-induced **cellular** and **pathophysiological** changes
- ✓ Intracellular parasites not only use **their host cell** as a food source but may also **reprogram its genomic expression** to meet their **physiological** requirements.

Mechanisms of damage by protozoans

- ✓ Cell damage/necrosis by intracellular parasites: coccidiosis, ameobiasis, giardiasis, balantidiosis
- ✓ Malabsorption: villous atrophy: coccidiosis
- ✓ Anaemia: haemolysis: babesiosis
- ✓ Immunological damage: leishmaniasis
- ✓ Neurological damage: sarcocystosis
- ✓ abortion: toxoplasmosis

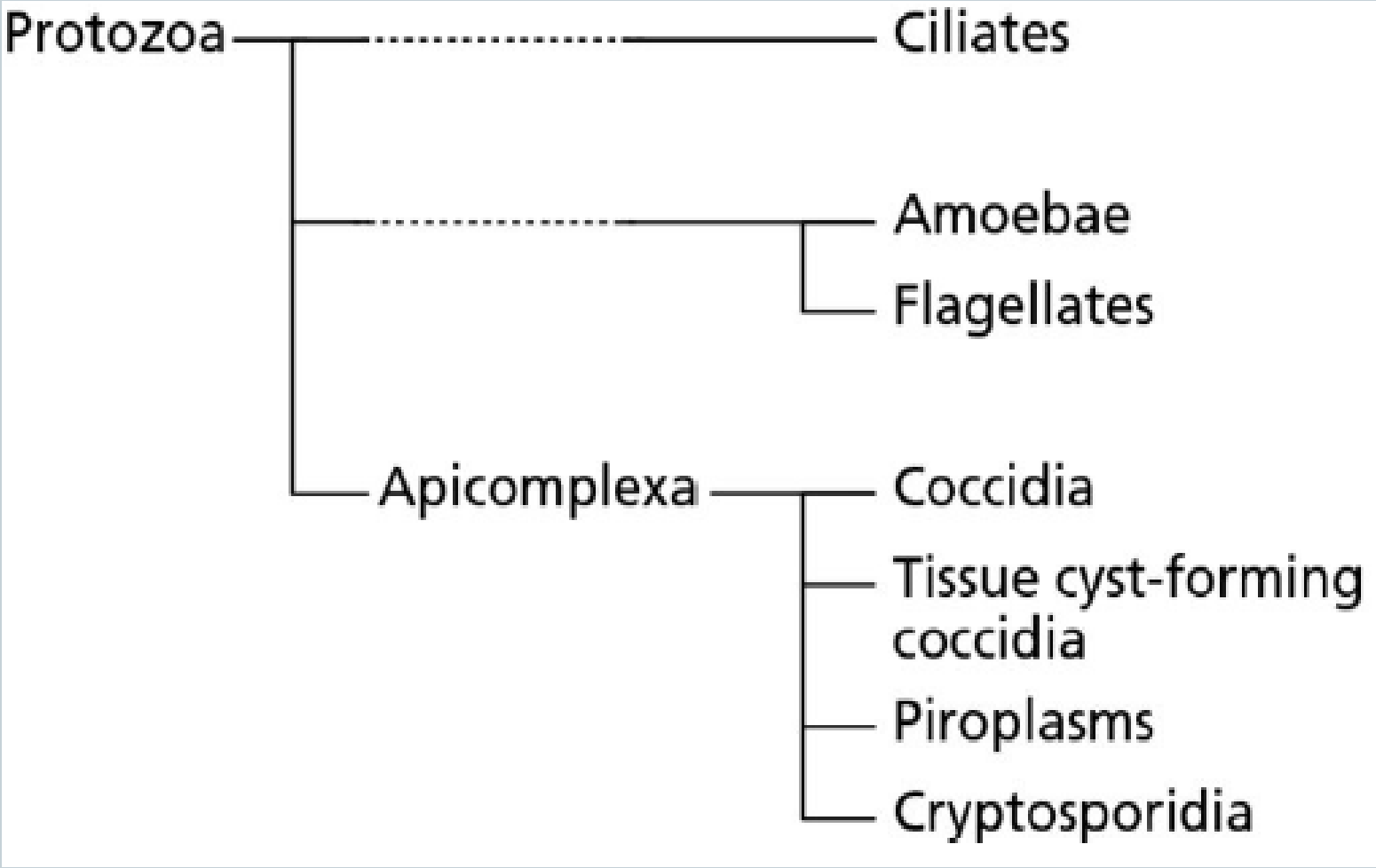
Importance of protozoan diseases

- Protozoan diseases have various impacts
  - ✓ **animal welfare**: cause pain, discomfort or stress to the host
  - ✓ **agricultural** impact:
    - **losses** due to death and diseases, subclinical disease is of significance as it prevents farm animals from attaining their full genetic potential.
    - constant use of bodily **resources**, imposed by the need to maintain the **immunological** battle against parasite and to repair the physiological and structural damage they cause→

Importance of protozoan diseases

- → can lead to reduced **weight-gain** or an increased **food conversion ratio**, or to a **reduction** in animal power, meat, milk or fiber (e.g. wool) yield and quality. This obviously affects agricultural production and economics. In turn affects the human population wellness
- **Veterinary public health:** many protozoans of animals are transmissible to humans and capable of causing disease.

Classification of Protozoans



Intestinal Protozoans

- ***Cryptosporidium***:
  - ✓ is an **enteric protozoa**, which is responsible for **clinical diseases** in animals and human beings
  - ✓ was first described by **Tyzzer in 1907** after isolating it from the **gastric glands of mice.**
  - ✓ its **infection** is associated with outbreaks of **diarrhoea** in young animals and immunocompromized man
  - ✓ unlike other coccidia, it does not enter into the cells of the host.

General description of *Cryptosporidium*

- The parasite is a **minute, colourless** and **transparent** protozoan parasite
- It is **spherical or ovoid** parasites that adhere to the **microvilli of enterocytes**, particularly in the **ileum**.
- Currently, it differentiated in to **13 various species** based on **their genetic profile** and the species of the host from which they were originally isolated. But only **2 species**: *Cryptosporidium parvum* and *C. andersoni* are involved in causing **clinical diseases**.

General description of *Cryptosporidium*

- *C. parvum*: measures 5x4.5 µm and infects **small intestines** of wide range of mammals including humans.
- *C. andersoni*: infects the **gastric glands of** laboratory rodents and several mammalian species and measures about 7.5x5.6 µm.
- *Cryptosporidium* species: **lack host specificity** so that **cross-infection** can occur between domestic animals, rodents and man.

General description of *Cryptosporidium*

- It develops just under the **surface membrane of the host cell or within its brush boarder** rather than in the **cell proper**: the parasite is not intracellular.
- Its species exhibit **three important differences** from other enteric coccidia:
  - ✓ **Excreted oocysts** are directly infective to new hosts,
  - ✓ They are **not host specific** so that infection can spread between hosts
  - ✓ They are **unaffected** by most existing anticoccidial drugs

Life-cycle

- **Direct**; the life cycle of the *Cryptosporidium* is basically similar to other **intestinal coccidia** although **sporulation** takes place within the host.
  - ✓ Infective, thick sporulated oocysts with **four sporozoites** are discharged in the feces and serve to disseminate the infection.
  - ✓ The oocysts remain **viable** for months unless exposed to **extremes of temperature** (below **0** degree centigrade, above **65** degree centigrade) & **desiccation**.

Life-cycle

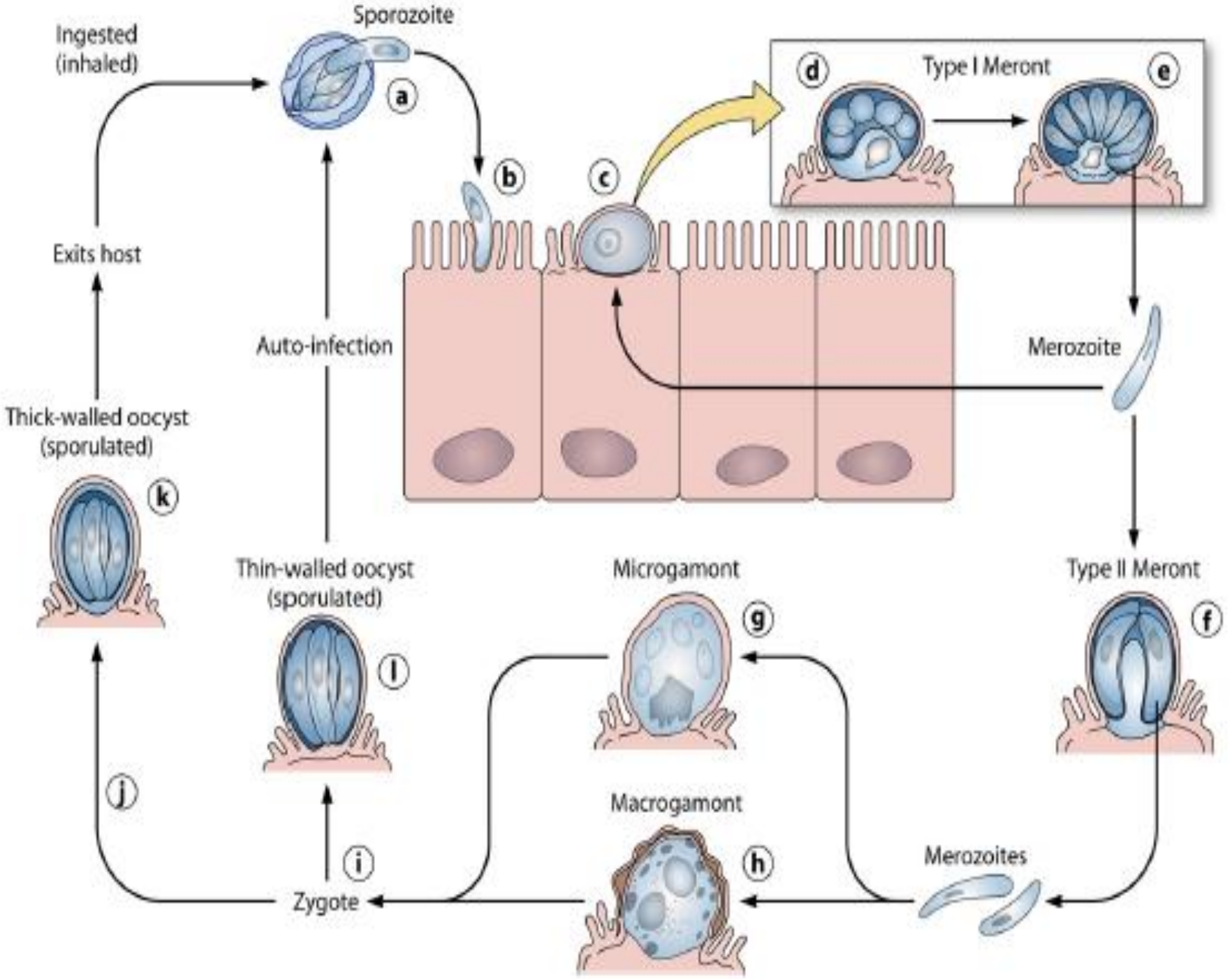
- Unlike ***Eimeria*** and ***Isospora*** species, which are intracellular, *Cryptosporidium* species are **intramembraneous** and resides within the **brush boarder** of the intestinal epithelial cells.
- Following ingestion by suitable host, the **thick walled oocysts** breakout to release the **four sporozoites** that invade **microvillus border of gastric glands (the enterocytes) or lower half of the small intestine**

Life-cycle

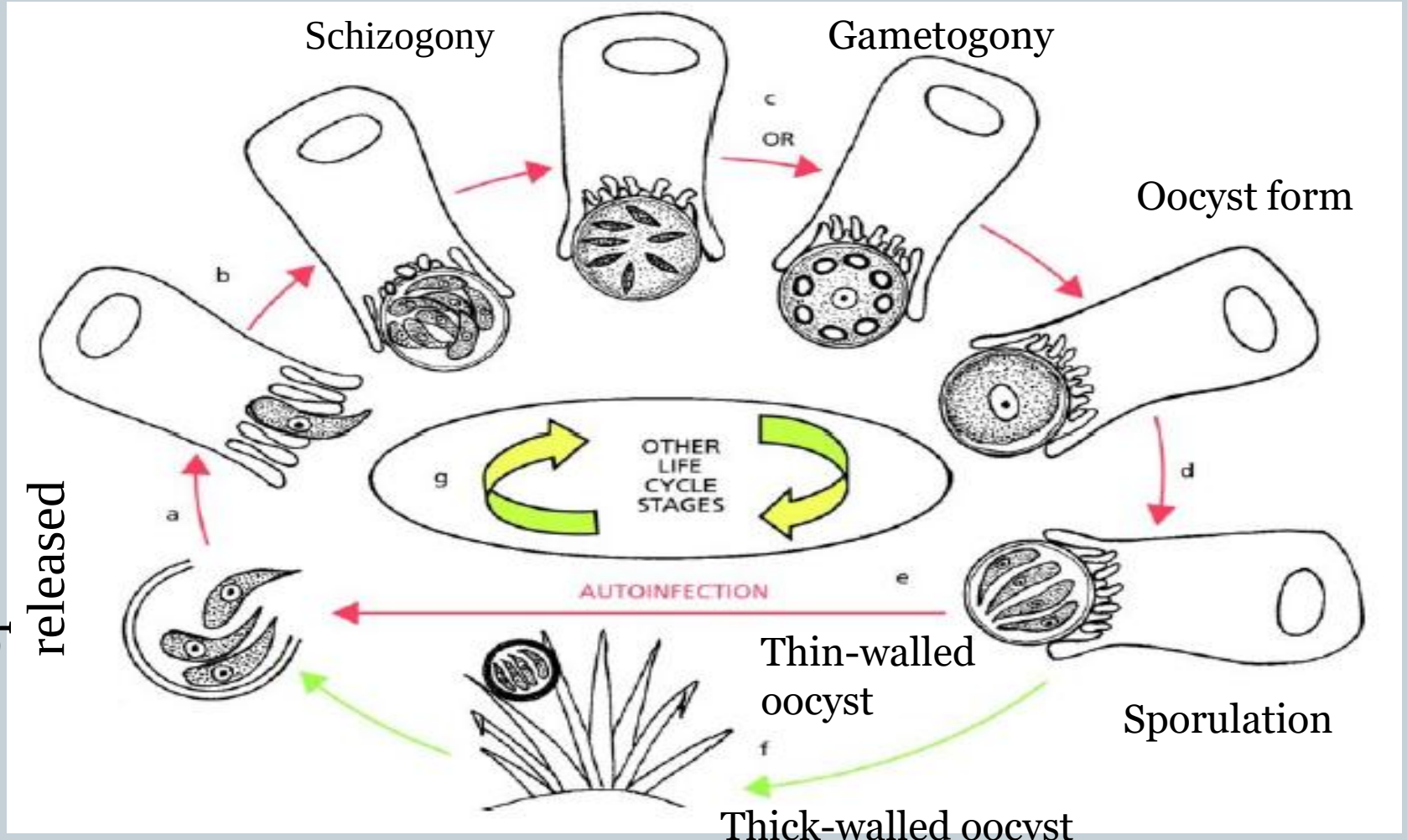
- In the **parasitophorous vacuoles** (just beneath the host cell membrane) of the microvillus boarder, the **organism (trophozoite)** undergoes **two** or **three** meront generations (**Schizogony, gametogony and sporogony generation**) and oocyst production within 72 hours; oocysts sporulate before leaving the cells
- **Two types** of oocysts are produced: thin-walled that breaks and re-infects the host, and thick-walled that passes in the faeces.

Life-cycle

- ✓ In general:
  - About **20% of the oocysts** produced have thin walls and break in the GIT thus releasing their sporozoites, which reinvade host cells (**causing autoinfection**).
  - The other **80%** are **thick walled oocysts** that pass out with the faeces.
- ✓ Oocysts are extremely resistant to environmental conditions and most man-made chemicals.
- ✓ Prepatent period can be as short as 3 days.


Ingested
(inhaled)
Sporozoite
a
b
c
d
Type I Meront
e
Exits host
Auto-infection
Merozoite
Thick-walled oocyst
(sporulated)
k
Thin-walled oocyst
(sporulated)
l
Microgamont
g
Type II Meront
f
j
i
Macrogamont
h
Merozoites
Zygote

Life-cycle


Schizogony
Gametogony
c
OR
Oocyst form
b
sporozoites released
OTHER LIFE CYCLE STAGES
g
d
a
AUTOINFECTION
e
Thin-walled oocyst
Sporulation
f
Thick-walled oocyst

Epidemiology, Source of infection & transmission

- *Cryptosporidium* affects primarily **neonatal calves, lambs, kids and piglets;** mainly animals b/n 1st and 3rd week of life.
- **Older animals** generally develop **poor infection.**
- Oocysts can **sporulate** within the host cell and are infective when passed in faeces
- Oocysts are **resistant** to most chemicals including chlorine based compounds and can survive for months in **moist and cool conditions**

Epidemiology, Source of infection & transmission

- Since the parasite can **cross host species barriers**, infections in domestic animals, wildlife and companion animals must be regarded as possible reservoirs of infection for humans.
- **Humans and dairy effluents** are probably the most important sources of environment and surface water contamination
- Transmission: **ingestion of oocysts excreted in the faces of infected humans or animals**: faeco-oral route through contaminated feed and water from the environment.

Risk factors

- **Host range**: variety of animals act as hosts to *Cryptosporidium* species.
- **Immune status of the host**: immunocompromized animals are more susceptible to clinical disease.
- **Host factor**: young animals are more susceptible and become sources of infection without clinical signs for others.
- **Parasite factor**: not dependent on environmental factors for sporulation or maturation.

Risk factors

- **Nutritional status of the host and intercurrent infections**: intercurrent infections or nutritional or mineral deficiencies could exacerbate or increase the likelihood of the disease.
- **Environmental resistant oocysts**: oocysts are resistant to direct sunlight and chemicals.
- **Environmental factors**: moist and cool conditions, adverse weather conditions, overcrowding, stress of early weaning, transport and marketing, together with **low levels of hygiene**, will increase the risk of clinical infections.

Pathogenesis

- Transmission among people or animals is by **fecal contamination**; infection may occur in any age of the animal, but disease is usually limited to the very young (≤3 weeks old) or the immunocompromized animals.
- Pathogenesis of *Cryptosporidium* infection is **not completely clear**; but the parasite causes **varying degrees of tissue reaction (villous atrophy)** suggest that the digestion and absorption of food may be impaired→ resulting in **diarrhea**.

Pathogenesis

- There is also evidence of **hyperplastic crypt epithelium** which along with **damaged villous epithelium** and **atrophic villi** indicates that the lesions develop as a result of accelerated **destruction of epithelial cells**.
- The parasite may decrease **disaccharidase** activity → resulting in **reduced breakdown of sugars** → resulting in **bacterial overgrowth**, **formation of volatile fatty acids**, and **changes in osmotic pressure**→ these changes then cause the characteristic **severe, watery diarrhea**.

Pathogenesis

- **Other causes of diarrhea** related with an important **secretory process of the parasite**, with the inhibition of the **absorption of sodium** and the **high production of prostaglandins** in the intestinal mucosa, and an **increase in the permeability of this mucosa**→ resulting from the increase of the **interferon level**→ results in **severe watery diarrhea.**

Pathogenesis

- **Cryptosporidiosis** is a **self-limiting disease**, lasting 1–3 weeks in healthy animals; **lesions** present are **mild to moderate villous atrophy**, changes in the **surface epithelium** and **shortening of microvilli**; **severity of disease** is exacerbated in the presence of **other pathogens** (e.g., rotavirus in calves).
- *C. andersoni* (possibly *C. muris*): infects the **abomasum**; clinically mild with possible adverse effects on **weight gain** and **milk production**; generally found in older cattle.

Pathogenesis

- *C. baileyi* and *C. meleagrids* may affect **bursa of Fabricius (BF)**, **cloaca or respiratory tract** of birds
  - ✓ **infection of BF** may diminish **humoral immune response to vaccines.**
  - ✓ severity of disease is exacerbated in the presence of other pathogens (e.g., **infectious bronchitis virus**)

Clinical findings

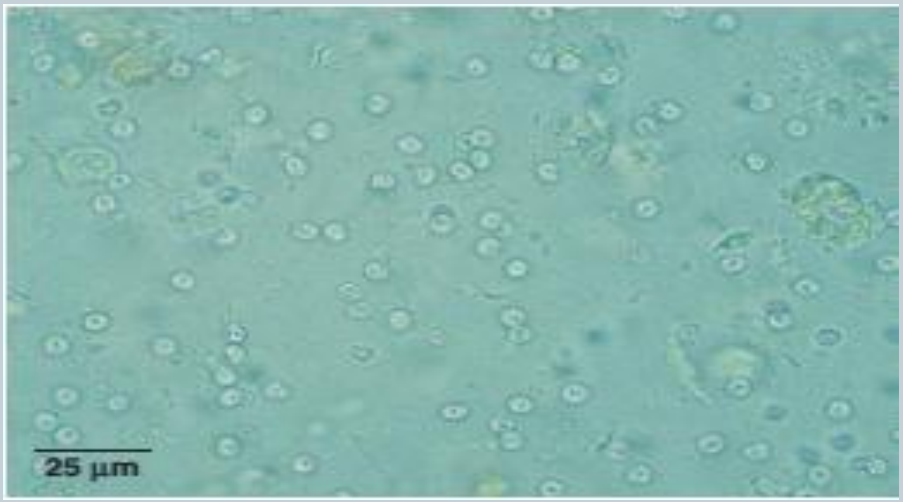
- Depression and inappetite/anorexia
- Foul smelling profuse diarrhea and dehydration→ requires treatment with **oral rehydration solutions**
- Loss of weight and poor growth rates
- Death→ due to **loss of electrolytes** and **fluids** through diarrhea.

Immunity

- The immune response of the host against the parasite is characterized by:
  - An increased number of **T CD4+ and CD8+lymphocytes**
  - By the production of **cytokines (interlukin-12[IL-12])** and **interferon-γ [IFN-γ])**
- Resistant of older animals is linked to **their specific immune status** rather than to their age.

Diagnosis

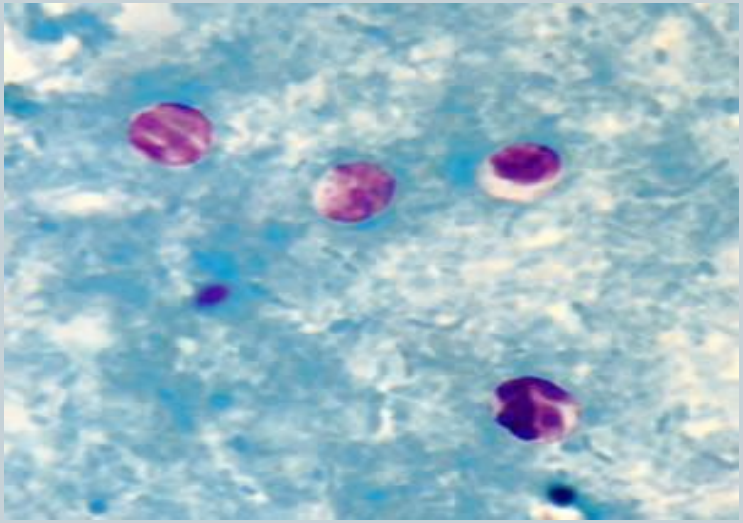
- **Faecal examination:**
  - ✓ Faecal flotation using **saturated sugar** (**sheather's**) solution (454gm/355ml of H2O) to detect sporulated oocysts in the faeces.



***Cryptosporidium* species in a sugar flotation preparation**

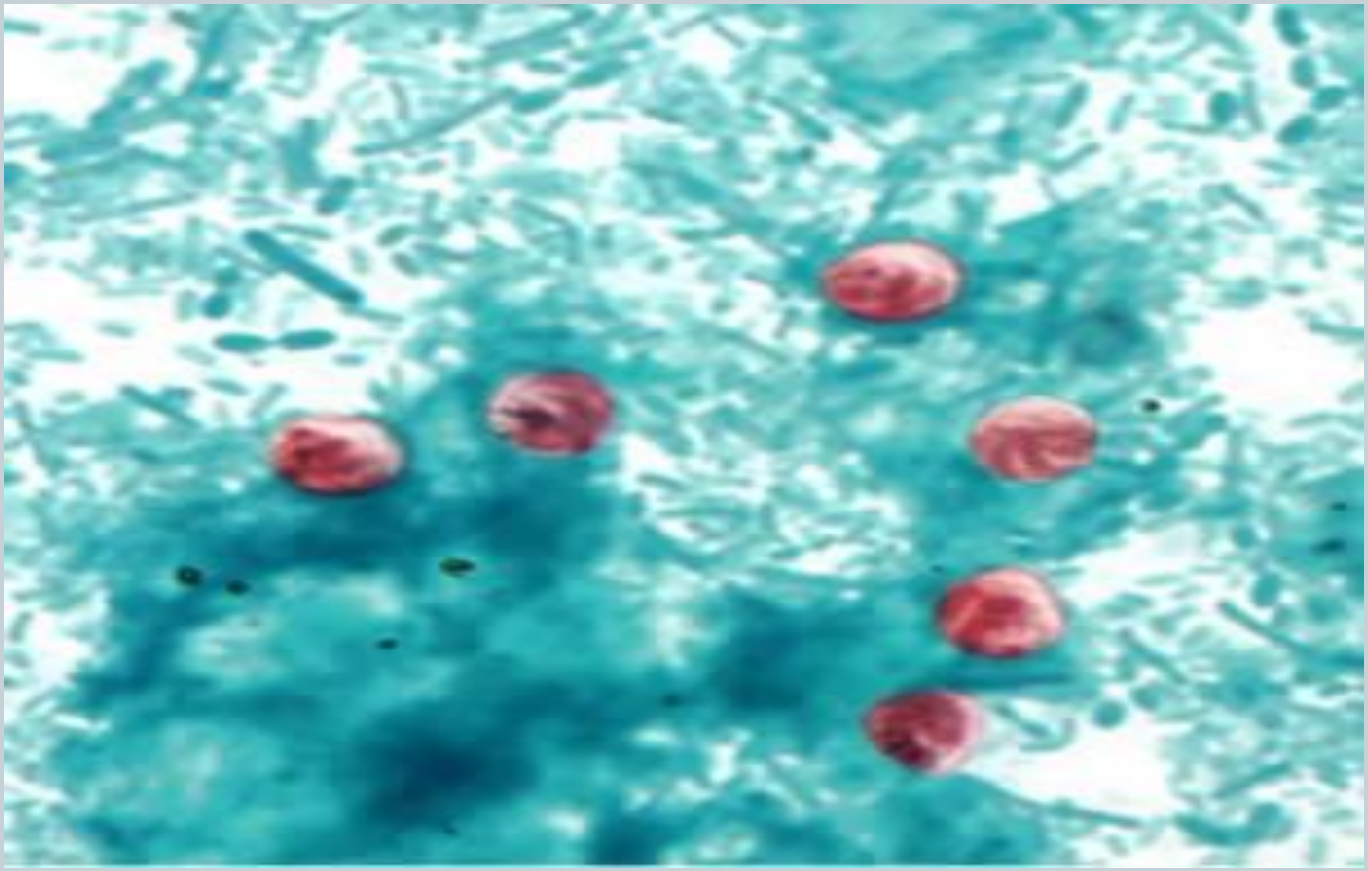
Diagnosis

- Faecal examination:
  - ✓ **Modified acid fast staining** of faecal smear (Ziehl-Nielsen staining technique)→ gives bright red granules or pink colour of oocysts with sporozoites



Oocysts of *Cryptosporidium species* (Ziehl-Nielsen stain)

Diagnosis



***Cryptosporidium* sp. oocysts stained with safranin**

Diagnosis

- **ELISA test→** detects parasite antigen in feces; can be expensive.
- **Immunofluorescence→** better sensitivity and specificity than stained smears; however, requires immunofluorescent microscope.
- PCR or DNA-based techniques for molecular identification
- Oocysts are tiny, subspherical, 3–7 μm in diameter.
- **Histological examination** of the intestinal tissue where it may reveal atrophy of the villi

Treatment, control & prevention

- There are no many **effective drugs**; few ones like Spiramycin, Halofuginone, and Paromomycin have found to be effective in preventing clinical signs and mortality and decreasing oocysts output in calves, kids, and cats.
- These drugs cause a **partial reduction in faecal oocyst elimination**
- Since the disease is **self-limiting**, **supportive therapy** such as rehydration and maintenance of energy is usually sufficient

Treatment, control & prevention

- Control is difficult; but can be prevented using
  - ✓ Good management and hygienic practices
  - ✓ Avoiding overcrowding/stocking and handling of diarrheic animals
  - ✓ Separate susceptible animals from dams
  - ✓ Desiccation of oocysts in sunlight or disinfect with formol-saline and ammonia

Public health significance

- Cryptosporidiosis is considered to be **zoonotic** and can produce transient **watery diarrhea** in humans (mainly in immunocompromized persons)
- The disease is pronounced in **young children** and the elderly, immunocompromized person (mainly in HIV/AIDS patients)
- **Acute** phase from 3 to 7 days
- **Chronic** wasting syndromes can persist for weeks to a few months

Status of Cryptosporidiosis in Ethiopia

- Few reports are available:
  - ✓ 6.7% in calves in Debre-Zeit dairy farms (Wudu, 2004)
  - ✓ 17.6% in calves in Debre-Zeit and Addis Abeba dairy farms (Abebe *et al*., 2008)
  - ✓ 7.3% in human and 7.8% in cattle in North-Shewa Zone, Ethiopia (Wegayehu *et al*., 2013).
  - ✓ 27.8% in calves, 22.2% in lambs and 12.2% in kids at Haremaya, eastern Ethiopia (Regassa *et al*., 2013).
  - ✓ 0.52% in sheep and goats in and around Debre-Zeit (Dinka *et al*., 2006).

Coccidiosis

- General overview:
  - ✓ It is a **contagious parasitic enteritis** of predominantly young animals caused by infection with *Eimeria* and *Isospora* species
  - ✓ The parasite, coccidian , attaches to the **epithelia** lining in the intestine
  - ✓ It is characterized by **dysentery**, **anemia**, inferior growth rates and production

Coccidiosis

- General overview:
  - ✓ It affects a wide variety of animals
  - ✓ Serious disease in sheep, cattle, goats, pigs, poultry and rabbits
    - In dogs, cats and horses it is less diagnosed but it can cause **illness**
  - ✓ Host-specific

Coccidiosis

- The disease is transmitted **orally**
- It is directly related to **contamination** of feeding and watering trough, calving, kidding and lambing areas.
- It is most **common** in young animals like lambs, calves and kids (3-6 wks), chicken and piglets
- It has a **seasonal** effect being more common in the **wet** months
- The infection can occur from **residual contamination** of the environment or from parasites being shed by dams

Etiology

- Genus *Eimeria* and *Isospora* belong to the family Eimeriidae
- *Eimeria* species are highly **host-specific** & are mainly **intracellular** parasites of the intestinal epithelium. In horses and rabbits it can also infect **kidney** and **liver**, respectively
- Various species of *Eimeria* exist
- *Isospora* canis and *Isospora* felis predominantly affects dog and cat, respectively

Eimeria species affecting poultry

- ✓ *Eimeria tennella* (the most pathogenic)
- ✓ *Eimeria necatrix* (the second most pathogenic)
- ✓ *Eimeria brunetti (highly pathogenic)*
- ✓ *Eimeria maxima (moderately pathogenic)*
- ✓ *Eimeria acervulina (moderately pathogenic)*
- ✓ *Eimeria mitis (??)* and *Eimeria praecox* (Non pathogenic ones)

Eimeria species affecting bovine

- ✓ *Eimeria zuernii* (the most pathogenic one)
- ✓ *Eimeria bovis* (equally pathogenic as *E. zuernii*)
- ✓ *Eimeria alabamensis*
- ✓ *Eimeria ellipsoidalis*
- ✓ *Eimeria auburnensis* etc.

Eimeria species affecting ovine

- *Eimeria parva*
- *Eimeria ovinoidalis (highly pathogenic)*
- *Eimeria crandallis (highly pathogenic)*
- *Eimeria pallida*
- *Eimeria bakuensis*
- *Eimeria intricata* (largest one) etc

Eimeria species affecting caprine

- *Eimeria arlongi* (highly pathogenic)
- *Eimeria ninakohlykimovae*
- *Eimeria faurei*
- *Eimeria christenseni*

Identification

- This can he made **microscopically**, either by examining the faeces for the presence of oocysts or by examination of **scrapings** or **histological** sections of affected tissues
- **Oocysts** may be identified based on:.
  - ✓ **Shape**: the most common shapes are spherical, ovoid or ellipsoidal
  - ✓ **Size**: the common size ranges between 15-50µm
  - ✓ The presence of **micropyle**: some species possess a small pore at one end, the micropyle
  - ✓ Time taken for **sporulation** can also used

Identification

- **Tissue stages**
  - ✓ mature **schizonts** may be identified histologically by:
    - location/site of infection
    - size and number of **merozoites** (a series of **crescent shaped** organism: 5-10µm) they contain

Life-cycle

- Infective stage is **sporulated oocyst**
- Sporulation of oocysts occur in the external environment under the presence of:
  - ✓ adequate moisture/humidity
  - ✓ optimum temperature (~ 27oC)
  - ✓ sufficient O2 supply

Life-cycle

- Life cycle is divided into 3 phases:
  - ✓ **sporulation**
  - ✓ infection and **schizogony**
  - ✓ **gametogony** and oocyst formation
- **Schizogony**: This initial phase of abundant asexual reproduction takes place in the host.
- **Gametogony**: prolific **sexual replication** follows, the products of which are egg-like '**oocysts**'.

Life-cycle

- **Sporogony:** A more modest phase of **asexual division** occurs within the oocyst after it has been shed into the environment.
- In general, the cycle as follows
  - ✓ young or susceptible animals ingest **sporulated oocyst** and the **sporozoites** in the oocyst are released due to **mechanical** force or by **CO2** and invaded epithelial cells of the gut: **trophozoites.**
  - ✓ Then, the parasites grow within the **host cell** and divides by **multiple fission** (‘**schizogony**’) to form a bunch of banana-shaped daughter organisms (‘**merozoites**’) called a ‘**schizont**’

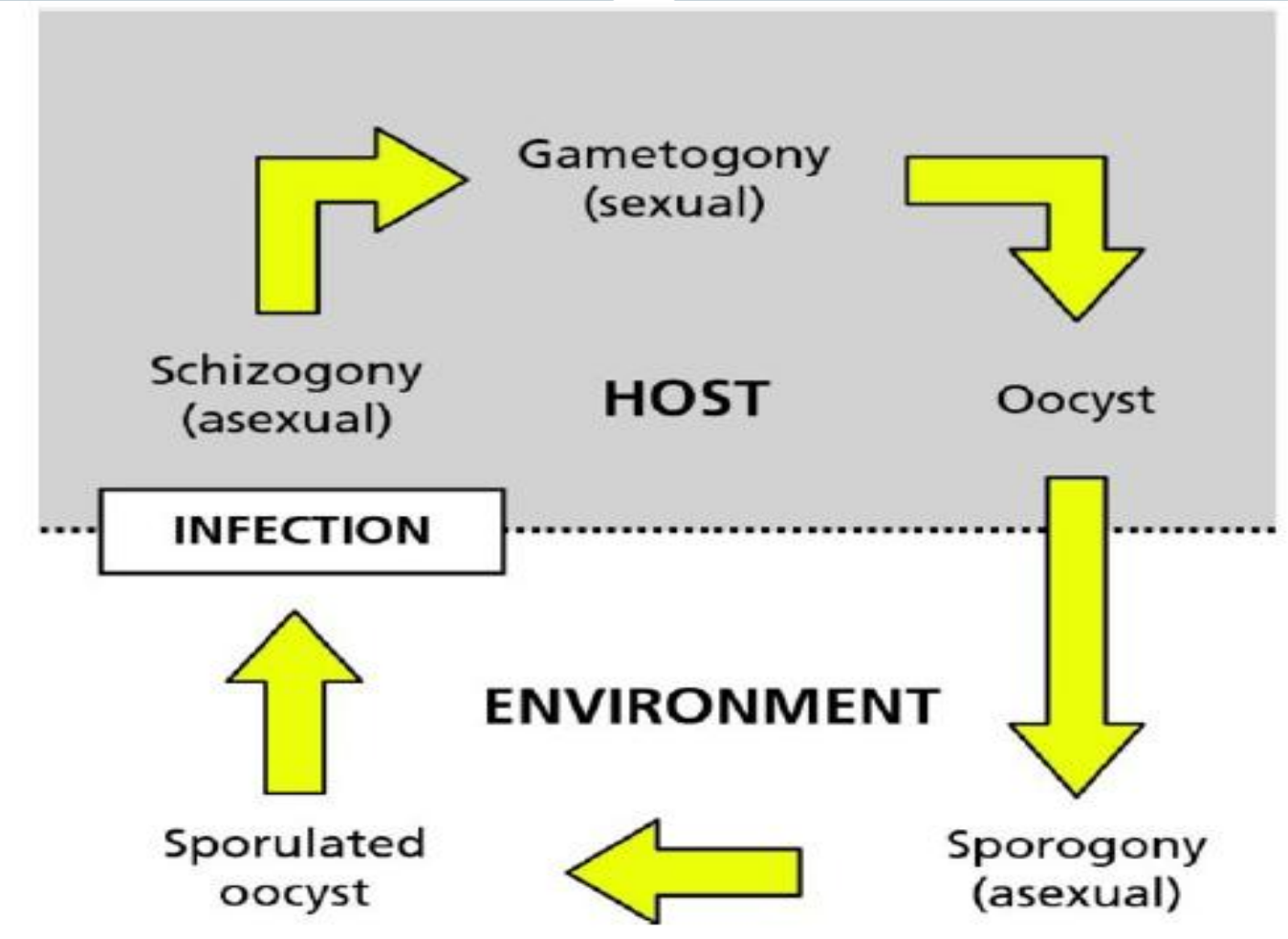
Life-cycle

- In general, the cycle as …….
  - ✓ The host cell bursts and merozoites disperse to invade further epithelial cells. Depending on **species** and **stage** of infection, therefore, the **merozoite** either develops into another schizont or progresses to the next phase of the life-cycle(gametogony).
  - ✓ A merozoite entering gametogony develops either into a single **female macrogametocyte**, which grows to occupy most of the volume of its host cell, or into a male **microgametocyte**, which divides to become a mass of small motile male gametes.

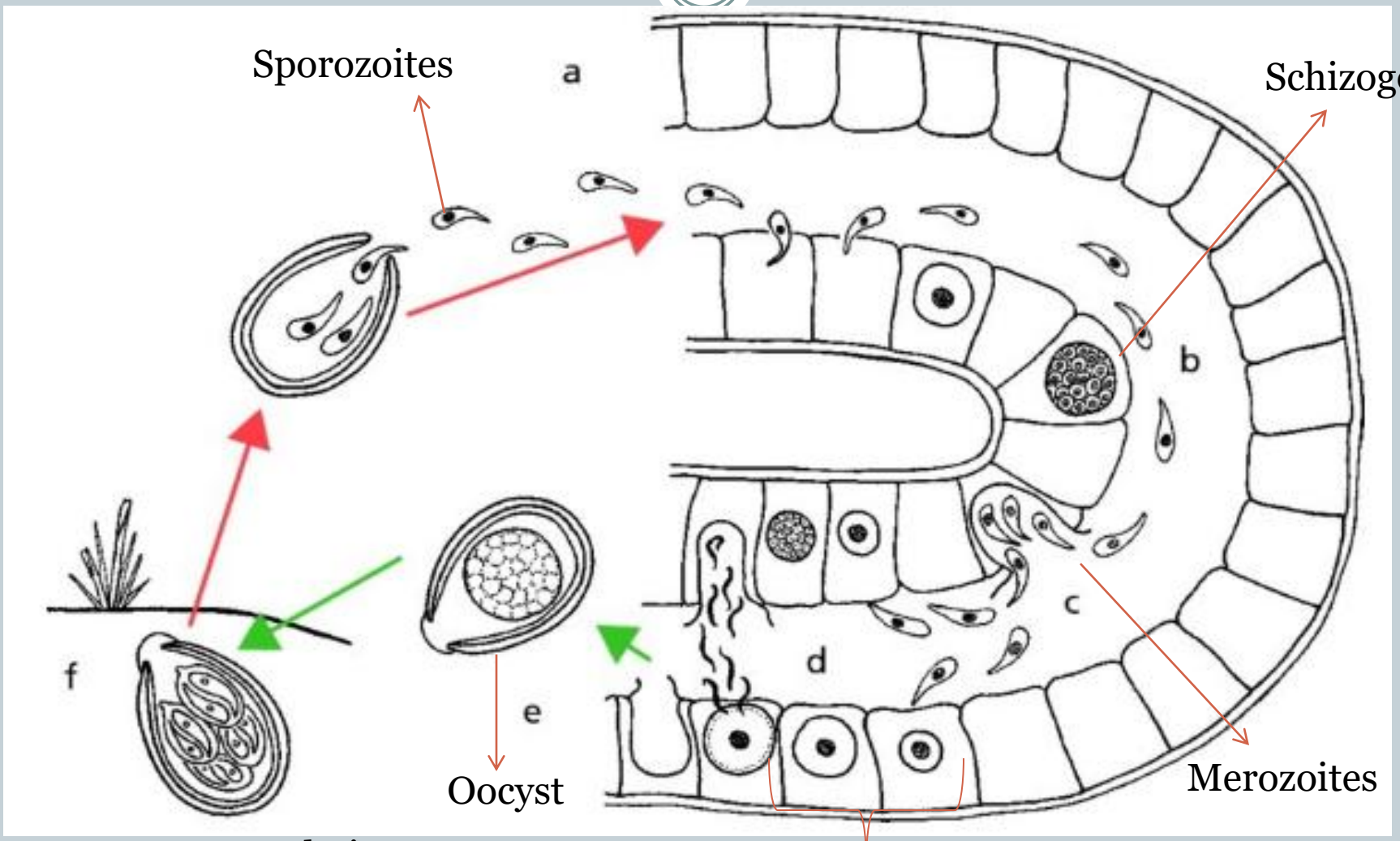
Life-cycle

- In general, the cycle as …….
  - ✓ The **macrogametes** (female sex cells) are fertilized by **microgametes** & results **zygote**
  - ✓ A protective wall forms around the zygote forming the oocyst.
  - ✓ The oocysts are then shed in feces which completes the life cycle
  - ✓ The oocyst is not **infective** until it has sporulated. This happens only after the oocyst has exited the host.
  - ✓ **Sporogony** is temperature dependent and will occur only if there is sufficient **humidity** and **oxygen**.

General overview of *Eimeria* life-cycle



Life-cycle


Sporozoites
a
Schizogony
b
c
d
e
f
Oocyst
Merozoites
Sporulation
Gametogony

Pathogenesis

- Coccidia of domestic animals pass through **all stages** of their life-cycle in the alimentary mucosa.
- *Eimeria* species vary considerably in their pathogenicity.
- The **pathogenic** effects of *Eimeria* species depends up on:
  - ✓ the **species** of the parasite
  - ✓ **infective** dose (amount of sporulated oocysts ingested)
  - ✓ **age** of the host
  - ✓ **immune** and **nutritional** status of the host
- The damage they cause depends on the **size** and **position** of each life-cycle stage.

Pathogenesis

- On the basis of the above issues, the *Eimeria* species are divided into **two major clinical** categories:
  - ✓ **The malabsorption group**: This includes species in which all life-cycle stages are developed superficially along the alimentary tract. Infection induces **villous atrophy** and **mucoid enteritis**, but little **haemorrhage**. The resulting **digestive** and **absorptive** abnormalities lead to **impaired food utilisation** and **reduced weight-gain**, with **diarrhoea** in severe cases.

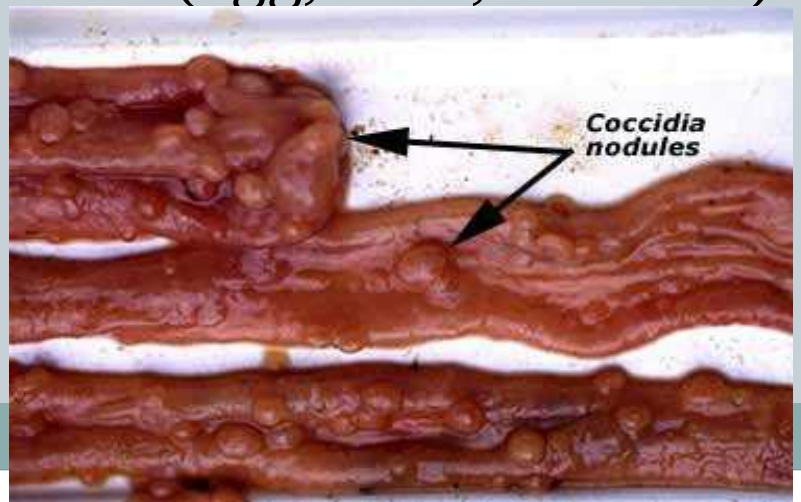
    Example: *E. acervullina* in chickens

Pathogenesis

- **The haemorrhagic group**: This embraces species which have a particularly **large lifecycle stage** (often the **second generation schizont**) situated in a subepithelial position at the base of an intestinal crypt. Example: *E. tenella* in chicken
- When **parasitized** host cells rupture by **cytolysis** to release the merozoites within, **deep erosions** are formed and **crypt stem cells** are destroyed.
- Thus, there is **villous atrophy**, **marked haemorrhage** into the gut leading to blood stained faeces. The outcome is often **severe** disease or death.

Clinical manifestations

- Blood diarrhoea: due to haemorrhage and malabsorption
- Dehydration, fever and severe straining
- Loss of condition, wool/feathery breaking
- Emaciation/ weight loss
- Reduced production (egg, milk, meat etc)
- Death



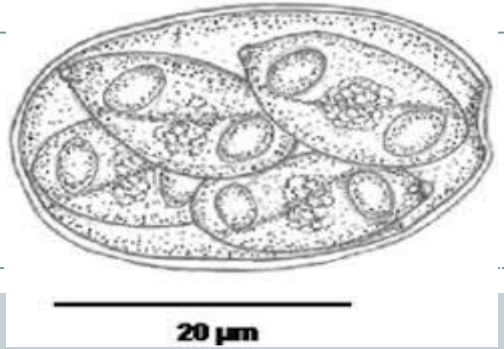
Immunity

- Coccidial infections are **self-limiting** and asexual reproduction does not continue indefinitely
- **Resistance** is **species specific** i.e., previous infection with one species of *Eimeria* will not preclude subsequent infection with other species.
- **Cellular immune** response (Th1 cells) plays a major role.

Epidemiology and mode of infection

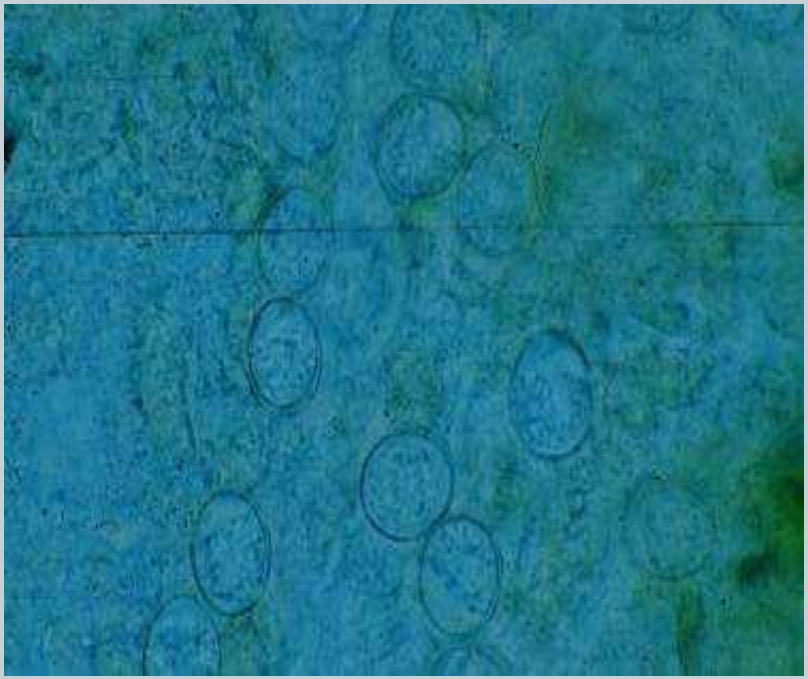
- **Production system** (intensive production system involving deep litter poultry houses, cattle yards: offer optimal conditions of temperature and humidity for oocyst sporulation)
- **Stress** (overcrowding): risk of heavy infections is increased
- **Age** of the host and arrested development at schizogony stage
- Poor hygiene condition
- Resistance of oocyst to external environment and different disinfectants
- Faecal oral transmission through contaminated feed and water

Diagnosis



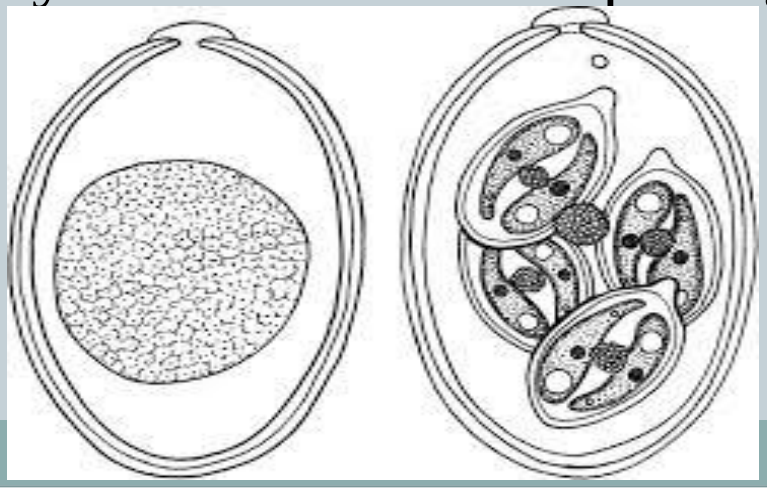
- Based on:
  - ✓ History of the disease (stress, management, high exposure)
  - ✓ Demonstration of the oocysts in faecal sample, intestinal scrapings
  - ✓ species identification based on **morphological** characteristics of the oocysts (**size** and **shape**), **sporulation** time, **prepatent** period, **predilection** site within the intestinal epithelium.
  - ✓ Postmortem examination: best approach for definitive diagnosis

Diagnosis



Diagnosis

- Diagnosis by **faecal** examination may lead erroneous results since in some instances, the major pathology is pronounced before **oocysts** are shed in the faeces.
- The presence of large numbers of oocysts may not necessarily indicate a serious pathogenic condition.



Treatment

- Life cycles of the protozoa are **self-limiting** and their life ends spontaneously, unless re-infection occurs
- **Timely medication** may slow or **inhibit** the life cycle of the oocysts, which can **shorten** the length of the infection, **alleviate** symptoms and can lessen the likelihood of re-infection and death
- Anticoccidial (coccidiacidal) drugs like Amprolium and Sulfur (Corid), Chloretracycline, and Sulphadimidine

Treatment

- Infected animals should be moved and treated separately to prevent infection of other animals and make sure they are being treated effectively.
- **Fluid** therapy with electrolytes and injections of antibiotics to control secondary bacterial infections.

Prevention

- Good feeding practices and good management (i.e. sanitation)
- Make sure neonatal receives colostrum
- Young susceptible animals should be kept in clean and dry areas
- Stress or overcrowding should be minimized
- Feeding and watering devices should be kept clean and clear from fecal contamination
- Use of vaccine: x-ray attenuated sporulated oocysts

Prevention

- Animals can be fed a ration containing **Coccidiostat** which slow down the shedding of coccidia into the environment
  - ✓ Monensin (Rumensin)
  - ✓ Lasalocid (Bovatec)
  - ✓ Decoquinate (Deccox)
- Prophylactic treatment for healthy animals in the group should be assessed
- Oocysts can be killed by heat, direct sunlight and drying

Status of coccidiosis in Ethiopia

- Few reports are available:
  - ✓ 68% prevalence in calves in Addis Ababa & Debre-Zeit dairy farms (Rahmeto 2008)
  - ✓ 22.9% in sheep in Addis-Zemen, Ethiopia (Abinet & Zewdu, 2016)
  - ✓ 25.8% in chickens in Central Ethiopia (Ashenafi *et al*., 2004).
  - ✓ 80.7% in Rhode Island Red and 61.3% in local breeds of chicken in Tiyo district, Arsi zone, Ethiopia (Getachew *et al*., 2008).

Giardiosis

- A protozoal disease that cause an **enteritis** and **chronic diarrhoea** in humans and also in wild and domesticated mammals (dogs, calves, cats).
- *Giardia* mainly affects the **upper small intestine** of hosts.
- Infection may be **inapparent** or can cause **severe enteritis**
- In animals, the parasites can provoke **acute**, **intermittent** or **chronic** diarrhoea of varying severity; however, many infections are asymptomatic

Giardia

- Several ***Giardia*** species have been recognized , including ***Giardia duodenalis*** ***(G. lamblia)*** in domestic and wild mammals (including humans) and ***G. muris*** in rodents; based on molecular analyses, more species may exist.
- Common cause of **chronic** diarrhea in man and infection has also reported in wild and domestic animals
- **Transmission:** among hosts occurs by **faecal-oral route**; **cysts** excreted in faces are immediately infective.

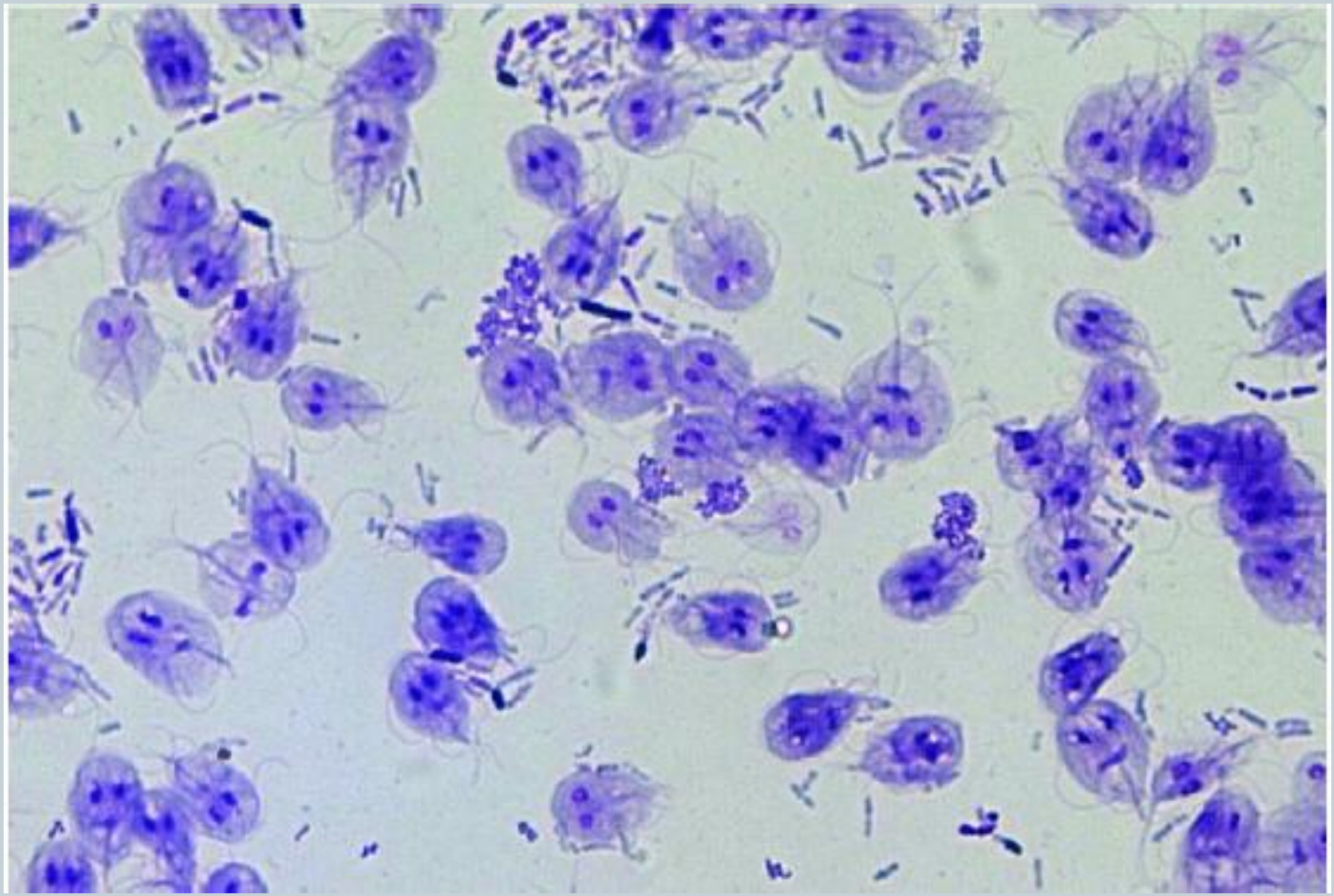
Identification

- The organism (**trophozoite**) has a **pyriform to ellipsoidal**, bilaterally symmetrical (size: 9–21 × 5–15 μm) shape.
- It also possesses **eight flagellae** (in four pairs), **six** of which emerge as **free flagella** at intervals around the body.
- It is unique in having a large **disc (adhesive disc)** on the flat ventral surface of the body which facilitates **attachment to epithelial cells** of the intestinal mucosa.

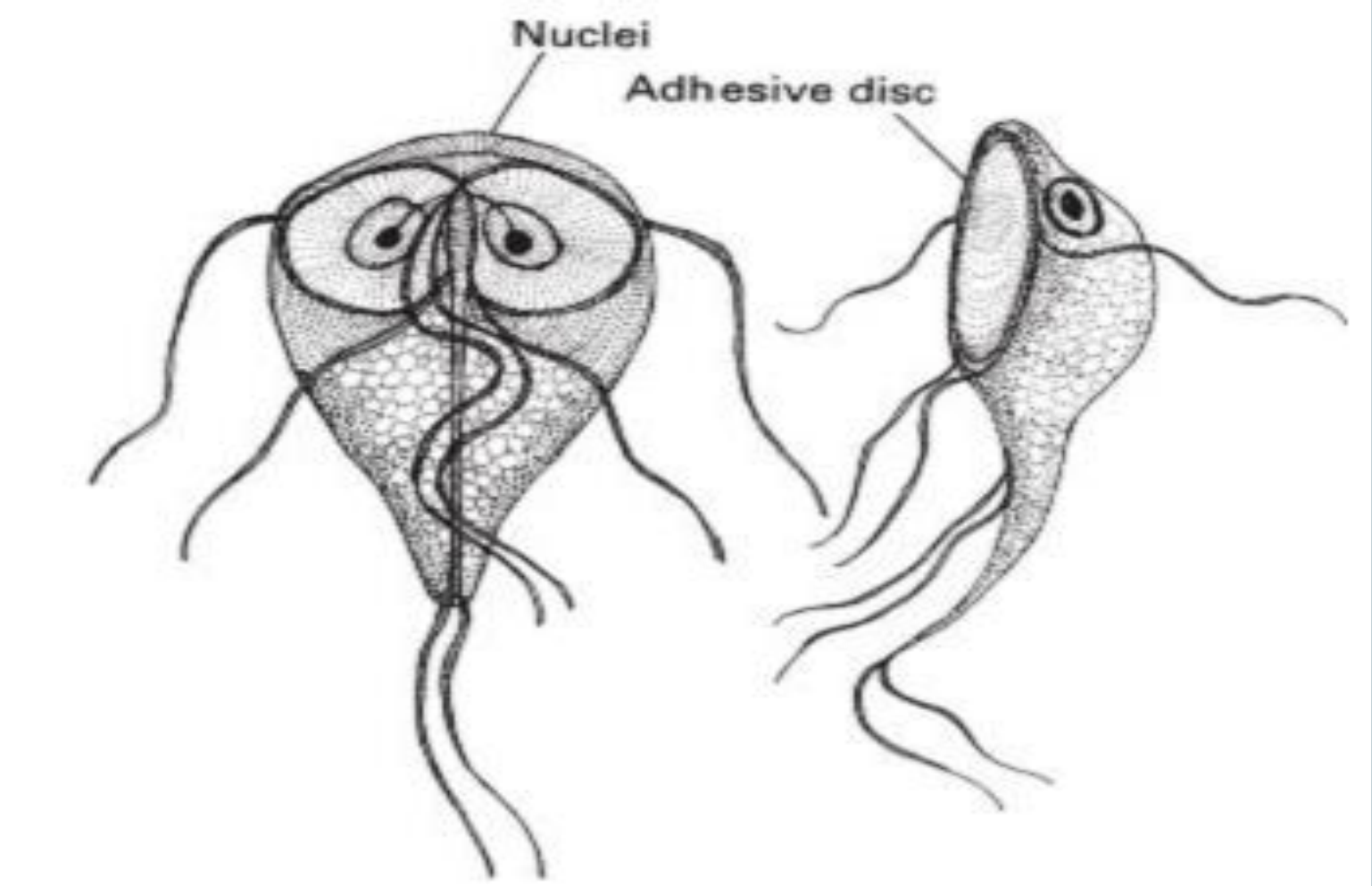
Identification

- It has **two nuclei**, each with a **large endosome** that makes the organism look like a **tennis racket with eyes** when viewed bottom side up under the compound microscope.
- The organism is passed as **multinucleated cysts** in which the flagella are visible and occasionally as **trophozoites** in feces (detection of these is the basis for laboratory diagnosis).
- **Cysts:** are **ovoid** (8–13× 7–10 μm) and contain **four nuclei**.

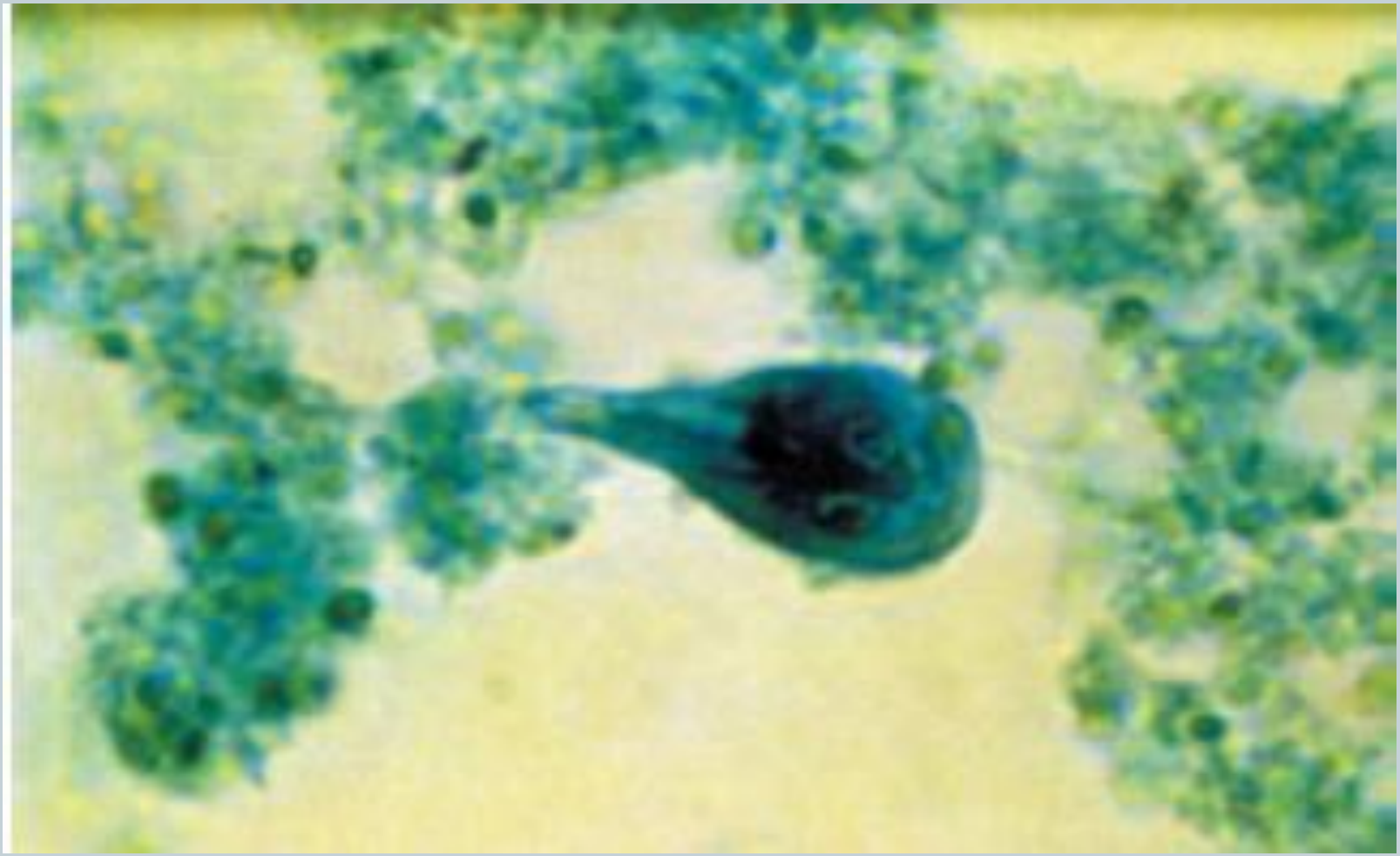
Giardia trophozoites growing in a culture medium



Nuclei, flagella and adhesive disc of *Gardia* species



Giardia intestinalis or *duodenalis* trophozoite



Life-cycle

- Transmitted by the **fecal-oral route**, primarily through feed and/or water contaminated with cysts; **cysts** are passed with the feces; each cyst contains **two trophozoites**
- Following ingestion, **trophozoites** leave the cyst, attach to the **brush border of the jejunum**, and multiply through **binary fission**; subsequently, each **trophozoite** forms a cyst; asexual reproduction occurs resulting in **two trophozoites** within the cyst.

Life-cycle

- **Trophozoites** can also be passed with the faeces, particularly during **acute infections**, resulting in transmission of the parasite; however, cysts are **more resistant** to external environmental conditions and are the stage most often responsible for **continued transmission**.
- PPP: 5-16 days

Life-cycle

- Infections are more common in **young animals**; they are most important **source of environmental contamination**; adults, especially dams, can be sources for their offspring; a **peripaturient rise** in cyst excretion has been shown to occur in sheep.

Pathogenesis

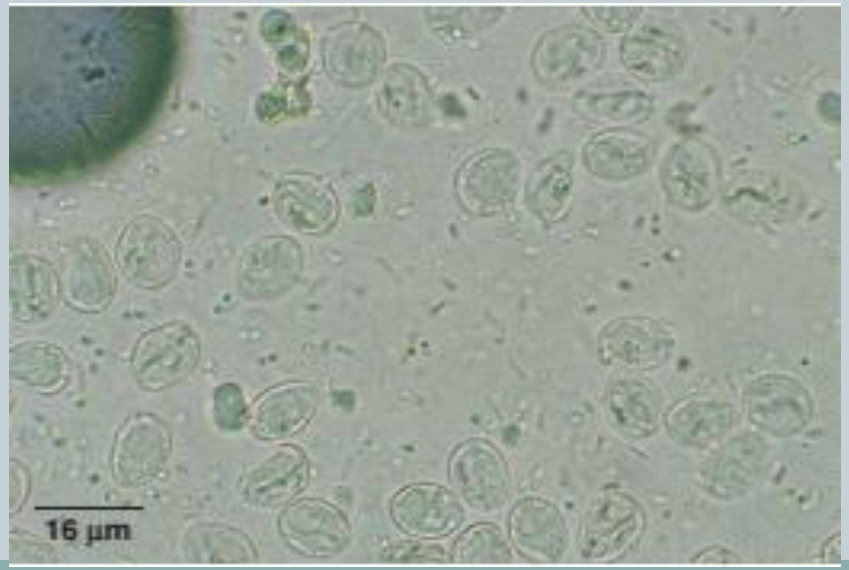
- Infections can cause **villous atrophy** and **crypt hyperplasia**→ results in a **decrease in the absorptive surface area** of the small intestine→ results in **hindrance of glucose, water, and sodium absorption.**
- Decreased activity of **disaccharidase** also occurs, which **impairs digestion**.

Clinical manifestations

- The severity of clinical outcome of *Giardia* infection varied up on the host involved; signs often include **chronic**, **pasty diarrhoea**, **weight loss**, **lethargy** and failure to **thrive**.
  - ✓ clinical signs in ruminants and horses are almost absent; when present: diarrhea, weight loss, impaired growth rate in youngs
  - ✓ in dogs and cats the clinical signs are apparent; when present: acute chronic or intermittent **foul smelling**, fatty diarrhea, vomiting, dehydration and anorexia.
  - ✓ **Dogs and cats** unlike ***Amoeba histolytica*** they are significant reservoirs for **human infection**

Diagnosis

- ***Giardia* cysts or trophozoites** can be detected in faeces by a number of methods:
  - ✓ **Faecal flotation**: centrifugal or simple flotation using **33% ZnSO4** solution or Sheather's solution: to detect the cysts of the parasite

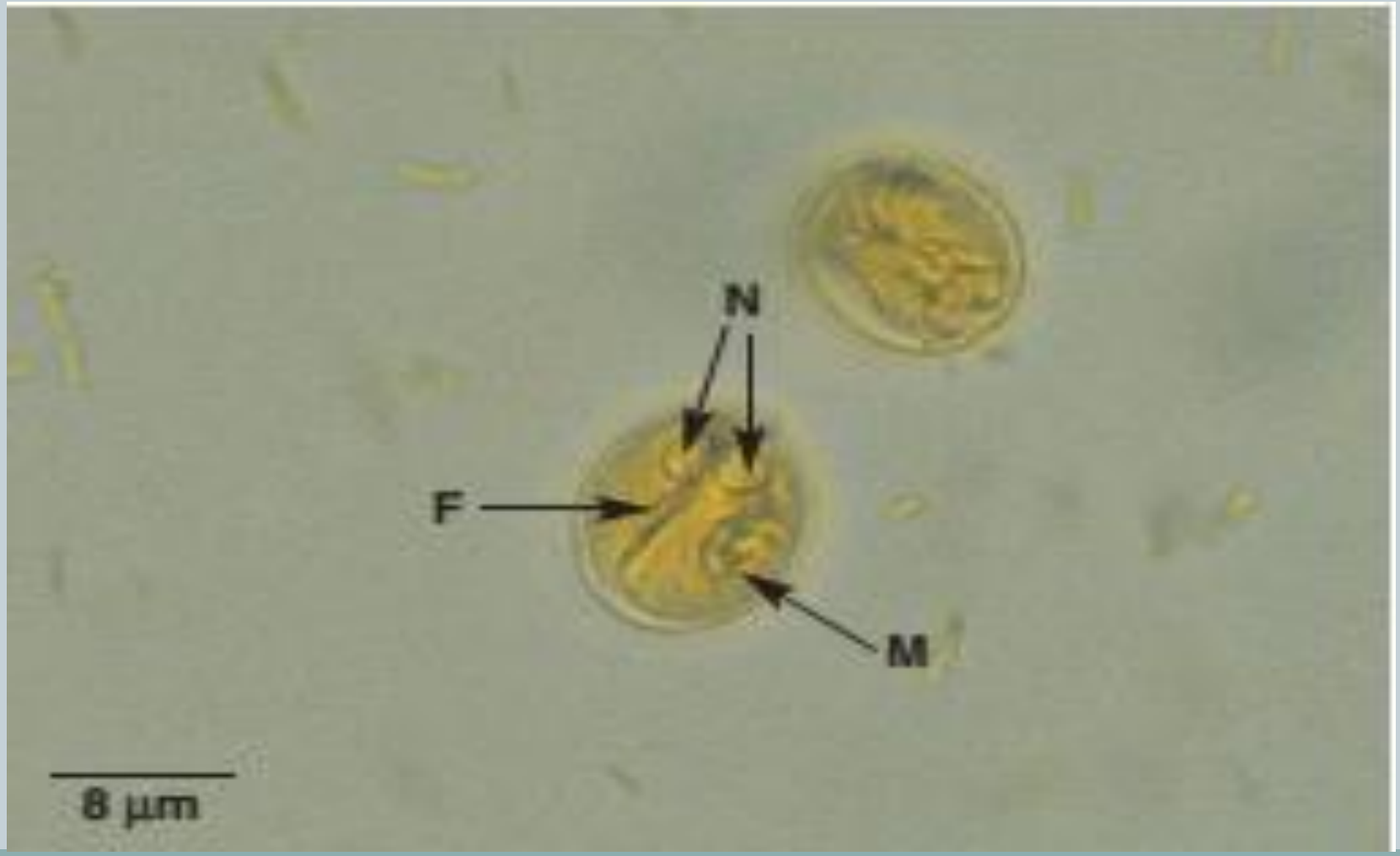


***Giardia* cysts recovered using 33% ZnSO4**

Diagnosis

- ✓ **Direct saline smear examination of fresh diarrheic faeces**
- ✓ Faecal concentration using **formalin-ethyl acetate**→best if performed within **20 minutes** of sample collection to detect **motile trophozoites** in unstained preparation
- ✓ **A drop of Lugol's solution of iodine** at the edge of the cover slip will kill and stain the **trophozoites** and **cysts**: and make them easier to identify.
- ✓ Use p**hase contrast** microscopy to identify the trophozoites and cysts.

Giardia cysts using Lugol's iodine stain



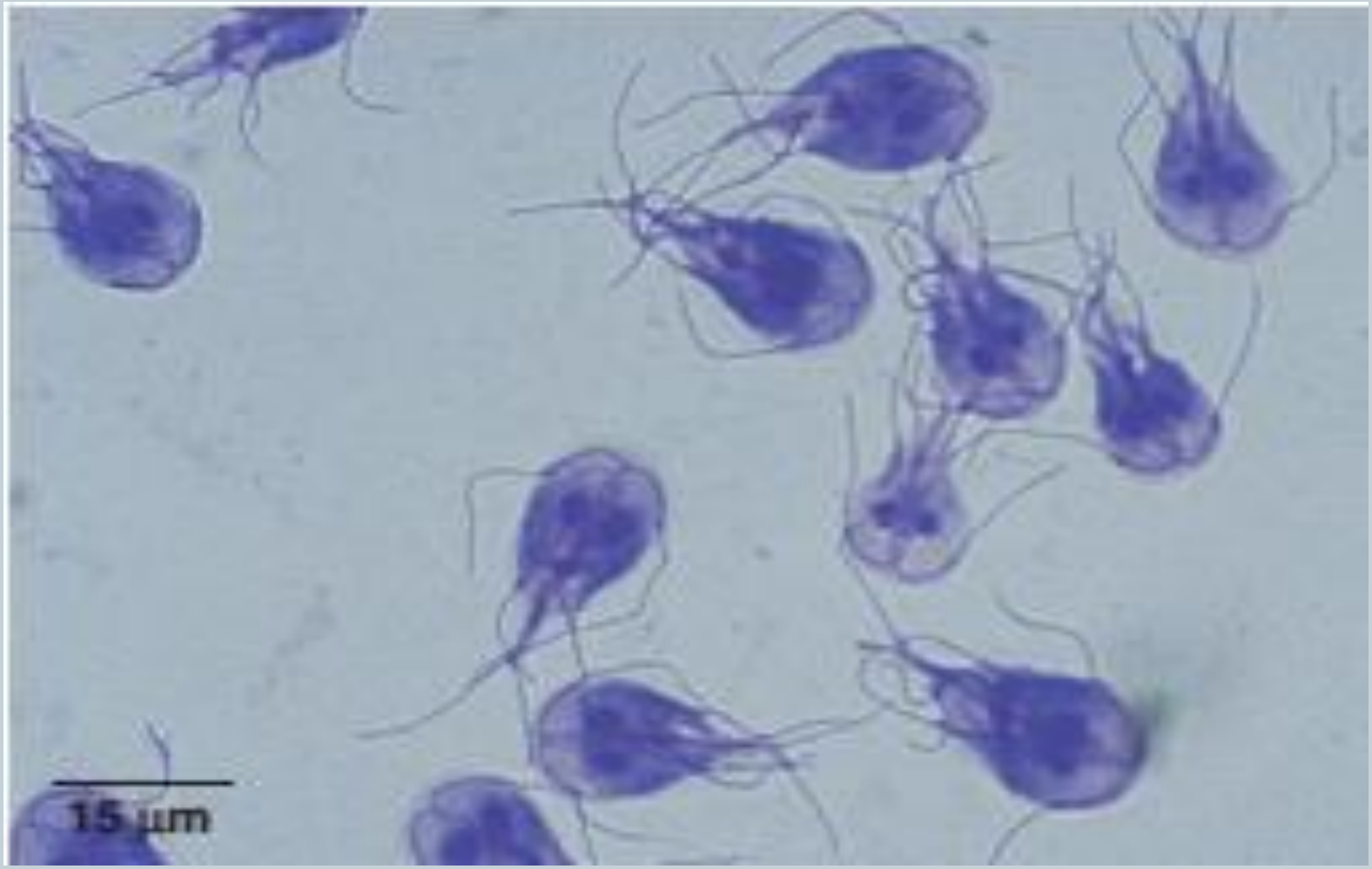
Diagnosis

- ✓ ELISA test→ detects parasite antigen in faeces
- ✓ Immunofluorescence→ detects cysts and/or trophozoites with fluorescent antibodies
- ✓ Duodenal aspiration—fluid is aspirated and the sediment examined for trophozoites

Trophozoites and cysts

- Trophozoites are **pear-shaped**, **bilaterally** symmetrical with a **concave adhesive disc** on the ventral surface, with two nuclei and four pairs of flagella
- **Cysts:** are **oval**, with 2–4 nuclei and other elements characteristic of the trophozoites that they contain.

Stained *Giardia* trophozoites in a direct smear



Treatment

- **Dog** and **cat**: Metronidazole; is the drug of choice followed by fenbendazole.
- **Calves**: **Calves**: fenbendazole or albendazole

Control

- Since the parasite **potential zoonotic** to human, treatment of household pets is warranted.
- **keeping areas dry**: because cysts survive best under cool, humid conditions.
- **Kennel disinfection** with proper disposal of faeces
  - ✓ use quaternary ammonium solutions

Control

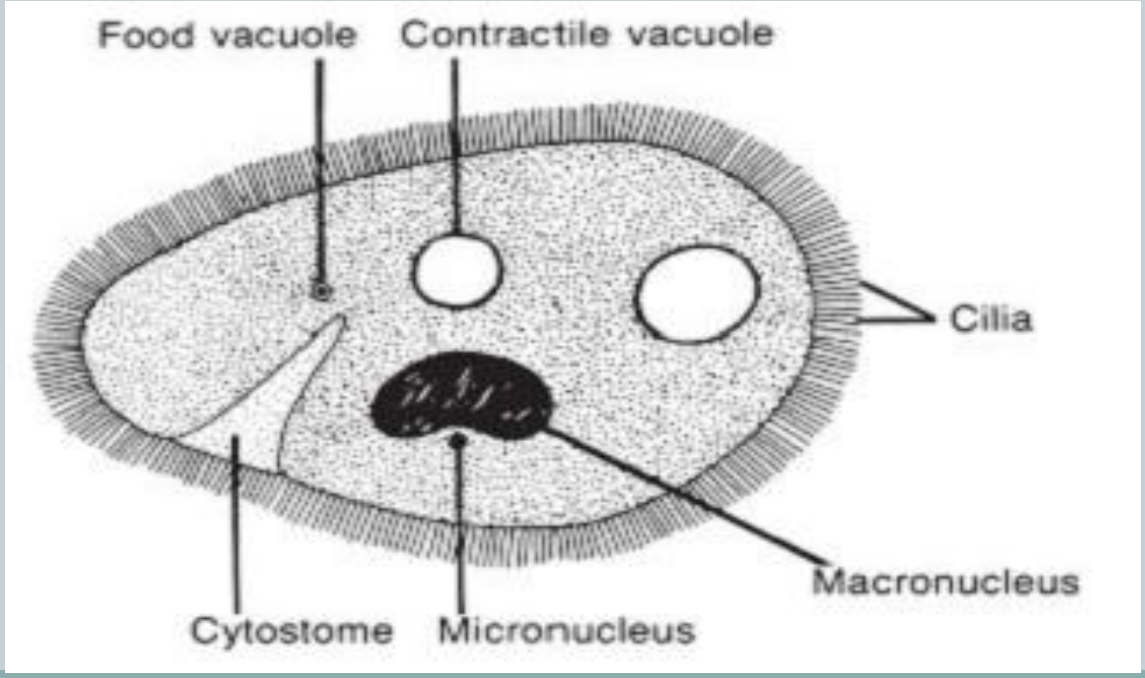
- In human beings, giardiosis can be controlled using the following methods:
  - ✓ Personal and sanitary hygiene
  - ✓ Improved sewage disposal
  - ✓ Preventing faecal contamination
  - ✓ Boiling or filtering of drinking water as chlorination doesn't kill the cysts
  - ✓ Cooking vegetables

Balantidiosis

- Is an **enteric protozoal disease** affecting humans, domestic animals (pig, cattle, dogs) and other primates
- Caused by a **ciliate protozoa** known as *Balantidium coli*
- The parasite affects large intestine of infected host
- *B. coli* is the **only member** of the ciliate phylum known to be **pathogenic**

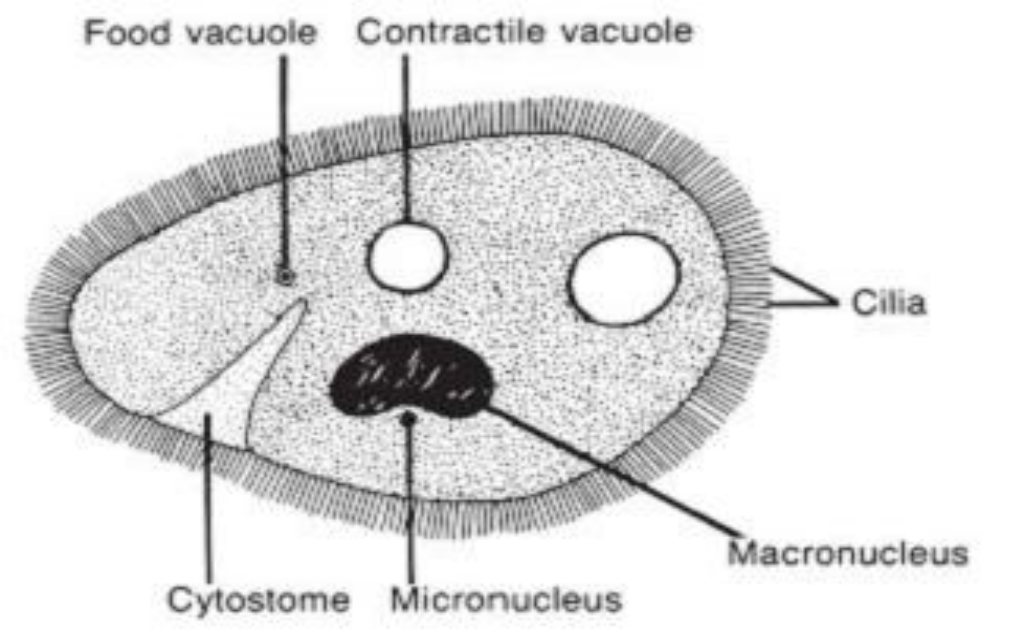
Balantidium coli

- It is **motile** by its cilia in fresh faecal sample
- It has **cytostome or mouth**: through this food vacuoles are passed to vacuoles in the cytoplasm



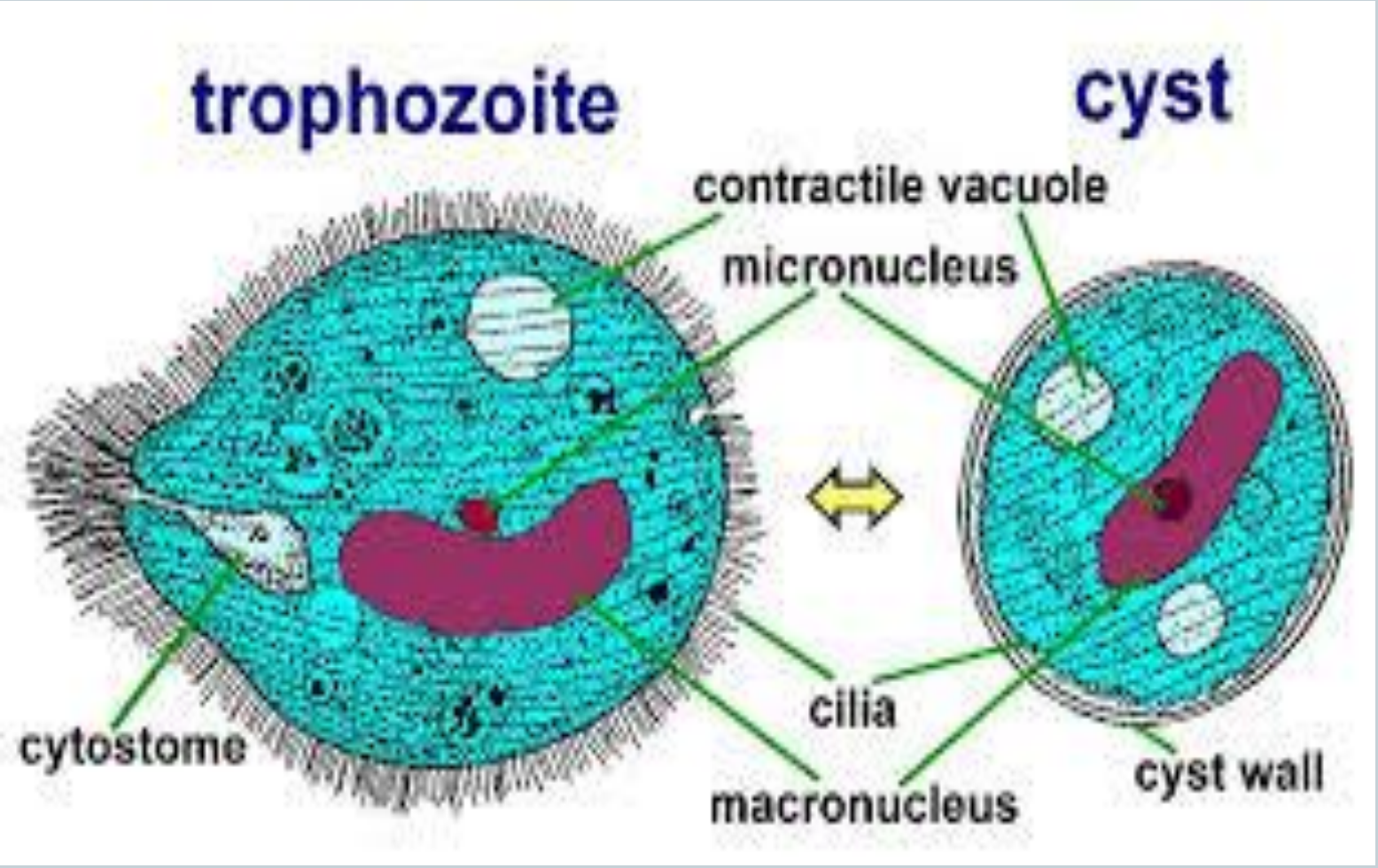
Balantidium coli

- It has **two nuclei** (macro and micro-nucleus) and **two contractile vacuoles**: that regulate osmotic pressure
- Cysts are spherical to ovoid, 40-60 µm in diameter



Trophozoite stage

Cyst


trophozoite
cyst
contractile vacuole
micronucleus
cytostome
cilia
macronucleus
cyst wall

Transmission and Source of Infection

- Transmission occurs by **ingestion of cysts or trophozoites**.
- Balantidiasis is a **zoonotic** disease and is acquired by humans via the **faecal-oral route from the normal host, the pig, dog, cattle,** where it is asymptomatic**.**
- **Contaminated water and food with cysts** is the common source of infection.

Role of *Balantidium coli* in disease

- *Balantidium coli* lives in the **caecum** and **colon** of humans, pigs, rats and other mammals.
- It is not readily **transmissible** from one species of host to another because it requires a **period of time** to adjust to the **symbiotic flora** of the new host.
- Once it has adapted to a host species, the protozoan can become a serious pathogen, especially in humans.

Role of *Balantidium coli* in disease

- Trophozoites multiply by **binary fission** and encyst due to the **dehydration** of faeces
- Infection occurs when the cysts are ingested, usually through contaminated food or water.

Life-cycle

- **Direct**.
  - ✓Transmitted through the fecal-oral route via **cysts** or **trophozoites**; cysts more resistant to external environment.

Epidemiology

- Balantidiasis in humans is common in the **Philippines**, but it can be found anywhere in the world , especially among those that are in **close contact with swine.**
- The disease poses a problem mostly in **developing countries**, where water sources may be contaminated with **swine or human faeces**.
- The disease is considered to be rare and occurs in less than 1% of the human population.

Epidemiology

- The **cysts** are **resistant to environmental conditions** and can survive for weeks in pig faeces.
- The pig is the **usual source of infection** for man and dogs.

Pathogenesis and clinical syndromes

- In pigs, it is generally **nonpathogenic**; the protozoa may invade the colonic mucosa if damaged by other pathogens.
- *Balantidium* infection in **immunocompetent individuals** is not unheard of, but it rarely causes a serious disease of the gastrointestinal tract.
- It can thrive in the gastrointestinal tract as long as there is a **balance** between the protozoan and the host without causing dysenteric symptoms

Pathogenesis and clinical syndromes

- Infection most likely occurs in people with **malnutrition** due to the **low stomach acidity** or people with **immune-compromised systems**.
- In acute disease, **explosive diarrhea** may occur as often as every twenty minutes.
- **Perforation of the colon** may also occur in acute infections which can lead to **life-threatening situations**.

Diagnosis

- **Faecal examination**
  - ✓ **Trophozoites** can be recovered from **direct smear** of feces or **cysts** on fecal flotation.
    - Trophozoites are oval, with funnel-shaped mouth, macronucleus and usually micronucleus, covered with cilia, 30–150× 25–120 μm.
    - Cysts are spherical, with double membrane, 40–60 μm.
- **Postmortem examination**: may find trophozoites in histological sections of colonic tissue

Treatment & control

- Generally do not treat pigs for infection; however, **tetracyclines** are effective in treatment.
- **Routine hygiene measures** to prevent ingestion of cysts or faeces to prevent human or animal infections.

Entamoeba or *Amoeba*

- *Entamoeba histolytica* is the most important species.
- Classified under the class of Sarcodina
- Cause of amoebic dysentery in humans
- Affects dogs and cats, and humans
- Transmission: occurs by faecal-oral-route
- Infected human is an important source of infection of dog and cat

Entamoeba or *Amoeba*

- ✓ Distribution: worldwide, but most common in the tropics
- ✓ Multiplies by binary fission and eventually encysts and is passed in faeces
- ✓ The **cysts form** is relatively resistant and is the infective stage for the next host.
- ✓ Trophozoites (growing and feeding stage) secrete proteolytic enzymes and produce characteristic flask shaped ulcers in the mucosa of the large intestine.

Entamoeba or *Amoeba*

- The erosion may allow the parasite to enter the blood stream when the most common sequel is the formation of amoebic abscesses in the liver.
- Natural infections without clinical signs can occasionally occur in dogs from human reservoir of active or carrier infections.
- Monkeys have their own strains of *E. histolytica* and these can be infective to humans.

Symptoms, diagnosis, treatment & control

- Clinical signs: chronic diarrhea, dysentery, weight loss, anorexia
- Dx: demonstration of motile trophozoites and cysts in the smear of faeces. Cysts contain four nuclei.
- Rx: Metronidazole and other drugs
- Control: Dogs are not significant reservoir of infection for man so that prophylaxis ultimately depends on personal hygiene and sanitary in human population.

Additional references

- Kahin, M. (2007):The Merck veterinary manual
- Hoy, Marajorie, A. (2003): Insect molecular genetics: Sf.765. H-6.
- Shapiro, Leland, S. (2004): Pathology and parasitology of veterinary technicians. Sf. 769. J-82.
- Levine, Norman,D. (1985):Veterinary protozoology. Sk.780. P-49.
- Ballwebuler, Lora, R. (2001): Veterinary protozoology.Sf.810. F-67.
- Hendrix, Charless. M. (1998): Diagnostic veterinary parasitology. Sf 810. H-46.
- Lamann Gregory, V.(1987): Veterinary parasitology. SF.810. L-36.
- Sloss, Margaret, W. (1994): Veterinary clinical parasitology. Sf810. S-58.
- Urguhort, G.M. (1996): Veterinary parasitology. Sf810. U-77.
- Tylor,M.A. (2007): Veterinary parasitology. Sf.810. T-425.
- Zajec, Anne.M.(2006): Veterinary clinical parasitology. Sf. 810.Z-35.
- Maximav, V.I.(1982): A series of parasitological studies of the helminthes arthropods and protozoa . Sf.810. M-39.
- Lefebvre. (2010):Infectious and parasitic disease of livestock one and two. Sf. 996, I. 54.

I THANK YOU VERY MUCH

FOR YOUR PATIENCE!!!