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**Veterinary Protozoology
lecture note**

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INTRODUCTION

- **Protozoa** are unicellular organism that are eukaryotic (their genetic information is stored in chromosomes contained in a nuclear envelope) in which various activities taking place in the organelles of the cell.
- They were first discovered by Antony Van Leeuwenhoek in the 18th century and around 45,000 species have been described.

Protozoa ...

- As other eukaryotic cells, they have a **nucleus**, an **endoplasmic reticulum**, **mitochondria** and a **Golgi body** and **lysosomes**.
- In addition, because they lead independent existence they possess a variety of other sub-cellular structures or organelles with distinct organizational features and functions.

Protozoa ...

- The majorities of protozoan species are free living and are found in almost every habitat on land and in water.
- As parasites, many protozoa are responsible for some of the most important diseases of animals and humans.
- *Malaria* is most important disease of man.
- *Trypanosomes* interdicted vast African grazing lands for livestock.
- *Ameba* and *coccidia* cause dysentery in man and domestic animals, respectively.

Protozoa ...

- Generally, protozoan parasites kill, debilitate & mutilate more animal in the world than any other group of disease causing organisms.
- *But other protozoa* that swarm in the rumen and reticulum of ruminants and cecum and colon of equine can digest cellulose which intern be utilized by the host.

Structure

- **Protozoa** are single-celled **eukaryotes** and therefore most have a typical complement of organelles *surrounded by a plasma membrane.*
- with simple appearance but have developed complexity through specialized organelles that aid in *attachment, locomotion, feeding and cell entry.*
 - ✓ Examples glycosomes - contain glycolytic enzymes; kinetoplasts - contain extrachromosomal DNA (modified mitochondria) etc.

Protozoa...

- They usually *have two nuclei* including *micro* and *macronucleus*.
- The first is important for *reproduction* whereas the later *regulates the cytoplasm functions* (metabolism, respiration, locomotion and other activities).
- *Protozoans* do not have a rigid cell wall.

- **Locomotion**

- Protozoa move by means of

- ✓ *pseudopodia*,

- ✓ *flagella* or

- ✓ *cilia*

- In those without locomotary structure, *gliding* is the common way of movement. E.g. seen in *Babesia*, *Toxoplasma*, *Sarcocysts* and others.

- **Flagella:** it is a whip-like filamentous structure which arises from a basal granule in the cytoplasm. Some protozoa like Trypanosoma species have a single flagella and other are multi-flagellated. Eg. Trichomonas
- **Cilia:** they are short hair- like structures each arising from a basal granule. They are structures of locomotion in the ciliates, but also aid in the ingestion of food or as tactile structures. They are similar to flagella but smaller and many in number arranged in rows over the body of the protozoan.

- ***Pseudopodia***: it is a temporary locomotary structure formed as the result of prolongation of the cytoplasm.
- Movement occurs as the rest of the cytoplasm flows into this prolongation.
- The pseudopodium also possesses a phagocytic capacity and can function as a cup which closes, enveloping particulate food in a vacuole.

Nutrition

- Nutrition in parasitic protozoa may be *holozoic* in which food material are ingested using pseudopodia, *phagocytosis* or *pinocytosis*, or through a cytosome and passes to food vacuole for digestion by lysosomes.
- The *saprozoic* protozoa absorb nutrients through the body wall, these being utilized directly by the organisms.

Reproduction

- Reproduction in the protozoan may be
 - ✓ Asexual
 - ✓ Sexual or
 - ✓ A combination of both

asexual reproductions

- ⇒ Binary fission (the commonest form),
- ⇒ Schizogony (multiple fissions) or
- ⇒ Budding in some protozoan parasites.

- **Binary fission** -two daughter cells result from a 'parent' cell, division being along the longitudinal or transverse axis.
- **Schizogony** - the nucleus divides several times before the cytoplasm does.
 - The dividing form is called *schizont* and the daughter forms are called *merozoites*.
- **Budding** - is an asexual reproduction in which *two or more daughter* forms are produced by the 'parent' cell

sexual reproductions

- **Conjugation:** it is a form of sexual reproduction in which two organisms pair and exchange nuclear material followed by separation and nuclear reorganization.
- **Syngamy:** it is a form of sexual reproduction in which two gametes fuse to form a zygote.

Protozoa ...

- **Host range**

- All animals are susceptible. Some protozoan parasites have highly specific host ranges (e.g. *Eimeria*).
- Others are less discriminate and will infect many hosts e.g. *Giardia* & *Cryptosporidium*.

- **Site of Infection**

- Most organs & tissues e.g. **intestine, muscle, brain, liver & blood** in which some live free within the intestine or blood.
- while others are intracellular

Protozoa ...

- **Life Cycle**

- Reproduction can be *asexual*, *sexual* or a *combination* of both.

- **Asexual** e.g. budding, binary fission or schizogony (multiple fission)
- **Sexual reproduction** involves fusion of identical gametes (isogametes) or gametes that differ in size (anisogametes).

Protozoa ...

- Some have a cyst stage (**infective** or **resting** stage) with a resistant covering that protects from environmental factors.
 - Some protozoa also encyst within the hosts' tissue (e.g. *Toxoplasma*).
 - Life cycles may be simple occurring within a single host (e.g. *Isospora*) while others are complex and involve multiple hosts (intermediate and paratenic)
 - Some infect hosts **directly** while **others rely on a vector** (e.g. insects) for successful transmission.

Protozoa

Classification:

- phylum protozoa consist of **four** sub-phylums including:

1. Sarcomastigophora: locomotion by pseudopodia,

2. Sporozoa: locomotion by gliding,

3. Ciliphora: locomotion by cilia,

1. Subphylum Sarcomastigophora

- Class **Sarcodina**: organisms here are characterized by amoeboid movement by pseudopodia. Eg. Amoeba
- Class **Mastigophora**: here the presence of ***one or more flagella*** is characteristic of the class.

2. Subphylum Sporozoa

a. Class **Coccidia**: are characterized by having both sexual and asexual reproduction and commonly localize on epithelia cells. The class includes the genera: Eimeria, Isospora, Cryptosporidium, Toxoplasma, Sarcocystis and others.

b. Class **Piroplasma**: these are parasites of blood cells involving ticks as vectors in which the protozoa reproduce by sexual reproduction. Eg. Babesia, Theileria

c. Class **Haemosporidia**: they are parasites of the blood cells involving blood-sucking arthropods as vectors in which sexual reproduction take place

3. Subphylum Ciliophora

- These groups of protozoa move by means of cilia.
- Eg. Balantidium.

Simplified classification

1. Flagellates: all possess flagella at some life stage

– e.g. *Giardia*, *Histomonas*, *Trichomonas*, *Trypanosoma*,
Leishmania, etc....

2. Ciliates: all possess cilia at some life stage e.g. *Balantidium*

3. Amoebae: all use pseudopodia for locomotion at some life stage
e.g. *Entamoeba*, *Naegleria*

4. Apicomplexa: includes the coccidians and hemosporidians (the most important group of human and veterinary protozoan parasites).

– e.g. *Eimeria*, *Isospora*, *Cryptosporidium*, *Sarcocystis*, *Toxoplasma*,
Neospora, *Babesia*, *Theileria*, *Plasmodium* (Malaria).

Classification based on their location in relation to cells

- Protozoan parasites basically can be classified into *extracellular* and *intracellular* parasites.
- **Example of *intracellular protozoa***
 - Intracellular blood protozoa
 - Erythrocytes - *Babesia*, *Theileria*, *malaria*
 - Lymphocytes – *Theileria*
- Intracellular gut:
 - Coccidian including *Eimeria*, *Cryptosporidium*, etc
- **Example of *extracellular protozoa***
 - Haemoflagellates like *Trypanosoma*, *Leishmania spp* (transmitted by biting flies)

Trypanosomes and Trypanosomosis

- are unicellular, flagellated organisms
- live in the bloodstream or tissue fluids of their vertebrate hosts including human.
- They are under subphylum Sarcomastigophora and class Mastigophora.
- With one exception(*T. equiperdum*), all have an arthropod vector *Trypanosomes* are blood parasites (haemoparasites)

- occur in the blood and tissue fluid and are known as haemoflagellates
- as they progress actively by the movement of the thread-like filament called flagellum

Morphological features

- They have leaf like or sometimes rounded body containing a vesicular nucleus.
- They have a single flagellum connected to the cell of the organism with an undulating membrane.

- The following main morphological features are used for differentiation of species of trypanosomes.
- Position of kinetoplast:
 - terminal, sub-terminal or marginal.
- Size of kinetoplast:
 - large, medium or small.
- Free flagellum: present or absent.
- Undulating membrane: distinct or not.
- Posterior end: blunt or pointed.

Classification

- trypanosomes of veterinary and medical importance are sub-divided into **two** *based on the mode of development in the insect vectors.*

Stercoraria: multiplication and transformation occurs in the gut and the infective forms migrate to the rectum and passed with the feces, this is a posterior station development.

Salivaria: here multiplication occurs in the digestive tract and proboscis so that the new infection is transmitted when feeding; the process is known as *anterior station development*.

☛ The group here includes:

1. Duttonella (*T. vivax*, *T. uniforme*)

2. Nannomonas (*T. congolense*, *T. simiae*)

3. Pycnomonas (*T. suis*)

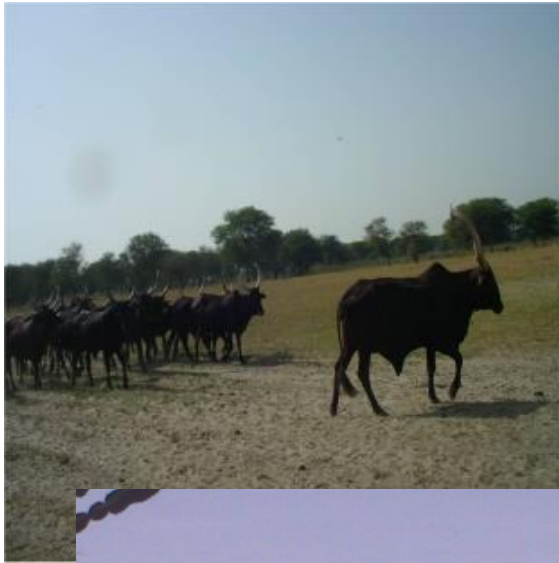
4. Trypanozoon (*T. brucei*, *T. rhodensiense*, *T. gambiense*, *T. evansi*, *T. equinum*, *T. equiperdum*)

Distribution of trypanosomes in Ethiopia

- ⇒ *T. congolense*, *T. vivax*, *T. brucei* ... Tsetse infested areas of Ethiopia.
- ⇒ *T. evansi*--- camel production area of Ethiopia
- ⇒ *T. equiperdum* --- Arsi and Bale highlands
- ⇒ *T. brucei rhodensiense*--- Gambella

Trypanosomosis

- ☛ Trypanosomosis/ Trypanosomiasis: is a disease caused by trypanosomes.
- ☛ Trypanosomoses are group of diseases of *man* and *animals* caused by parasitic trypanosomes.
- ☛ The disease results from the interaction of the
 - Trypanosomes (Pathogenic),
 - Vectors (biting flies, tsetse flies) and
 - Animals (camels, cattle, wild animals, etc.)



Tsetse transmitted Trypanosomoses

Etiology: *T. congolense*, *T. vivax* and *T. brucei* etc.

Occurrence

- Some 40 African countries including Ethiopia are affected
- 10 million km² of land in Sub-Saharan Africa (one third of the continent) is infested with tsetse flies
- *T. vivax* has also established itself outside the tsetse infested areas

Species affected

- Cattle (50 million head at risk)
- All species of domestic animals are susceptible to infection with one or more species of the trypanosomes, but trypanosome infections are economically of greatest importance in cattle
- The ingested trypanosomes undergo a cycle of development between 8 and 35 days before infective Metacyclic trypanosomes are produced.
- Once infected, a fly is usually capable of transmitting trypanosomes for the rest of its life.

Clinical features

- The clinical signs are reflections of pathogenesis
- Chancre
- Lymphadenopathy
- Anemia
- Tissue damage
- Cattle exposed to infection by tsetse flies develop the disease after a prepatent period of 8 to 10 days (*T. vivax*), 12-16 days (*T. congolense*) and 5-20 days (*T. brucei*). In endemic areas the disease is mostly in chronic form but for new animals it is acute.

Acute form: fever, parasitemia, enlargement of superficial lymph nodes, anemia.

Chronic form: intermittent fever, anemia, progressive loss of condition, prominent lymph nodes.

- Infected animal are dull, they have a staring coat, lose weight and are easily exhausted, lagging behind the herd.
- As anemia becomes more severe, cattle invariably die of congestive heart failure which results from anemia, myocardial damage and increased permeability.

Pathogenicity may depend on

Species of trypanosome

- *T. congolense* and *T. vivax* are parasites of the blood plasma and produce tissue injury primarily by the anemia.
- *T. brucei* is more of tissue parasites, infecting the intracellular fluids of connective tissue of various organs and extracellular fluids of the body cavities.

Stages of the disease

- **In the acute stage** of the disease postmortem findings may show extensive small hemorrhage on mucous and serous surfaces, emphysema in the lungs and mild gastro-enteritis. The spleen and lymphatic glands are grossly enlarged. The red bone marrow is present throughout the shaft of long bones.
- **In the chronic stage**, the carcass may be anemic and emaciated. The spleen is small and atrophied but the lymphatic glands have the same appearance as in the acute stage. Atrophy of body fat especially around the heart and kidneys. Hypertrophy of the heart, enlarged liver and chronic venous congestion may be visible. The red bone marrow disappears from the long bones but not from the ribs and vertebrae.

Mechanically transmitted Trypanosomes

Surra is a disease affecting horses, camels, buffalo, goats, sheep and pigs caused by *T. evansi*. In Ethiopia Surra is the disease of camels.

T. evansi. It is morphologically indistinguishable from the slender form of *T. brucei*. It can not develop in tsetse flies.

- **Occurrence:** the disease occurs in Afar, Ogaden and Borena.
- It has a marked seasonal incidence in association with wet climatic condition which favors the development of large biting fly populations.

Transmission: Mechanically by the bite of haematophagus flies (tabanus, stomoxys, haematopota etc.)

Clinical features

- Acute form: intermittent fever, parasitemia, subcutaneous edema, progressive anemia, dullness, lethargy....
- Chronic form: anemia, progressive emaciation (loss of hump), weakness.

Pathology

- Post mortem lesions are not well defined.
- Necrosis of the skin may occur on the thorax and abdomen.
- The carcass is anemic and ascites and hydrothorax may be present.
- Enlarged lymph nodes are visible.

Dourine

- It is a venereal infection of equids caused by *T. equiperdum* and characterized by a slow progressive course, genital edema, dermal plaques and terminal paralysis. It is the only trypanosome of veterinary importance that does not require an arthropod vector.

Etiology: *T. equiperdum* morphologically similar to *T. evansi*.

Occurrence: highland Ethiopia, (Arsi and Bale highlands).

—Species affected: Horses, Donkeys and Mules

Transmission

- It takes place at coitus
- Epidemics originate from the introduction of infected “carrier” breeding animals into a susceptible population.

Clinical features

- Inoculation period may extend from 1 to 20 weeks,
- Mucoid vaginal and urethral discharge,
- Low recurrent fever,
- Edema of genitalia,
- Ulceration of the genital mucosa may leave depigmentation scars,
- The animal loses condition, becomes weak and lame in one or both hind limbs, muscular atrophy of the gluteal region,
- Central nervous system disturbance, ataxia, paralysis
- the trypanosomes which destroy red cells directly by lysis (HAEMOLYSIS).
- Trypanosomosis is also associated with **immunodepression**, i.e. the host's immune system becomes less efficient to deal with infections.

Pathogenesis of trypanosomosis in general

- When the tsetse fly injects infective metacyclic trypanosomes into the skin of the host, there is a phase of local inflammation and a swelling (Chancere).
- The metatrypanosomes divide and multiply in the chancre and give rise to the typical blood forms which invade the lymphatics and lymph nodes, and then the blood stream.

- One of the main symptoms of the disease is **anemia** (decrease of haemoglobin in the blood).
- trypanosomes which destroy red cells directly by lysis (haemolysis).
- Trypanosomosis is also associated with **immunodepression**, i.e. the host's immune system becomes less efficient to deal with infections.
- **Edemas** (subcutaneous swellings caused by accumulation of tissue fluid)
- In chronic trypanosomosis the animal loses condition, there is **wasting**.

Diagnosis

- Diagnostic procedures vary not only according to the tools available, but often even more to what one wishes to know.

Laboratory methods

1. Direct methods

- **Wet blood films** (fresh blood preparation)
- A simple technique to examine fresh blood between a coverslip and a slide with the microscope,
- using medium magnification (objective of 40x or even less, and eye-pieces of 10x).
- Trypanosomes are seen either directly, moving between the blood cells, or indirectly, as they cause the blood cells to move.

Advantages:

- Simple and cheap.
- If trypanosomes are found, the disease is diagnosed on the spot.

Disadvantages:

- Unless the animals are brought to the veterinary centre, or the blood (with an anticoagulant) as the parasites lose their mobility after a limited time.
- Limited sensitivity, the detection limit is usually around 10^4 trypanosomes per ml of blood.
- The species of trypanosome cannot be identified.

- Fresh preparation of lymph

- The lymph is usually collected from a swollen prescapular lymph node, and examined between slide and coverslip, like blood.

- Thick blood film

- A drop of blood is applied on a clean slide and spread out with the corner of another slide, or with a match or a needle, to produce a circular area to a thickness such that, when dry, it just allows to see the hands of a watch or small print through the film.

Advantages:

- Simple and cheap.
- A field microscope is not needed, as the blood films are taken back to the centre for processing and examination at ease.
- It is sometimes (but mostly not) possible to identify the trypanosome species seen.

Disadvantages:

- An immediate diagnosis of trypanosomosis on the spot is not possible.
- The sensitivity of the method remains limited.

Thin blood smears

- These are made as in the case of blood smears to detect other blood parasites. They are fixed by methanol and stained with Giemsa stain, Diff quick stain

Advantages

- Specific diagnosis of trypanosomes is possible.

Disadvantages:

- The sensitivity is extremely low

Thin smears of lymph

- Lymph aspirated from a prescapular lymph node

Concentration methods

a. Buffy coat examination

- *This is also called the Woo method.*

Advantages:

- The sensitivity is higher than that of the direct methods
- The special centrifuge can be run on a portable generator, so that diagnosis in the field is possible, if one has also a field microscope.
- The PCV value is obtained at the same time.
- The special centrifuge can be run on a portable generator, so that diagnosis in the field is possible, if one has also a field microscope.
- The PCV value is obtained at the same time.

Disadvantages:

- Special equipment is needed.
- No specific identification is possible, although the type of motility may give some indication.

b. Darkground/phase contrast buffy coat technique

c. Use of experimental animals

2. Indirect methods;

▪ Serological tests - **Antibody-detecting tests, Antigen-detecting tests (Ag-ELISA)**

▪ Molecular tests

a) DNA-probes (nucleic acid probes)

The sample to be examined is heated to separate the two strands of DNA (this is also called denaturing of DNA), and these are fixed to a membrane, so that they cannot recombine again on cooling.

b) The polymerase chain reaction (PCR)

This is another molecular method of detecting parasite DNA. It is based on the use of an enzyme,

Tsetse Fly (*Glossina species*)

- Tsetse flies are yellow-brownish insects, 6- 13.5 mm long.
- They transmit Nagana between domestic animals and sleeping sickness in humans.
- wings while resting is characteristic: one wing covers the other like a pair of scissors.
- Once a tsetse fly has become infected with trypanosomes, it remains infected for the rest of its life and may infect any animal on which it feeds.

- The most important vector of bovine trypanosomosis is the *G. morsitans* group. It transmits *T. vivax*, *T. congolense* and *T. b. brucei* which cause Nagana. *Trypanosoma b. gambiense* and *T. b. rhodesiense*, which cause human sleeping sickness, are mostly transmitted by flies of the *G. palpalis* group, but also by species of the *G. fusca* group.

Control of Trypanosomosis

- Control of vector-borne diseases, (virtually all forms of trypanosomosis are in this category, *except dourine*), can be based on:
 1. Control of the causal agent
 2. Control of the vector
 3. Use of innate resistance of the host

Type of drugs

Curative drugs

- *Curative drugs* are meant to cure individual infected animals, not to protect the whole herd or group for a longer period.

Prophylactic drugs

- These are used where the risk is so high that the health of the herds cannot be maintained by individual application of curative compounds.

Names of commonly used drugs

To the user of drugs, ***two*** kinds of names are important:

1. The *generic name* which is based on a shortened form of the chemical formula of the active principle and constitutes an internationally used official name.

—For example a well-known trypanocidal drug is **isometamidium chloride**.

2. The *trade name (or brand name)*, used by a manufacturer for its commercial preparation. In addition to the active principle, commercial formulations

Commercial trypanocides

Generic name	Trade name	Dosage rate	Route
Suramin	Naganol	10 mg/kg	I.V.
Diminazene aceturate	Berenil, Ganaseg, Trypazen, Veriben	3.5-7 mg/kg	I.M.
Homidium Bromide	Ethidium bromide	1 mg/kg (1 ml/25 kg)	I.M.
Homidium chloride	Ethidium C, Novidium	1 mg/kg	I.M.
Quinapyramine methyl sulphate	Antrycide, Trypacide, Noroquin, Quintrycide	5 mg/kg	S.C.
Melcy	Cymelarsan	0.25-0.5 mg/kg	I.M./ S.C.
Isometamidium Chloride	Samorin, Trypamidium	0.25-0.5 mg/kg 1.0 mg/kg	I.M.

favorable circumstances for the development of resistance

1. Under high challenge and widespread use of a curative drug with an appreciable persistence in the system.
2. During the course of a prophylactic regime with intervals which are too long, which have been incorrectly assessed. The plasma drug levels drop into the subcurative range before each re-inoculation.
3. In all cases of serious underdosing.
4. Where a prophylactic cover is interrupted while the challenge remains high.

Resistance to drugs

- ➡ **Individual resistance** can be dealt with by the use of an alternative drug to which the parasite remains susceptible. Once the resistance is widely disseminated (area resistance) it will be necessary to use in that area, for at least a year, a drug to which the trypanosomes remain sensitive.
- ➡ The phenomenon of **cross resistance** is of utmost importance. *Cross resistance is resistance to a drug which has arisen as a result of previous exposure of the trypanosome strain to a different drug.* Cross resistant drugs are often members of the same chemical family. The resistance which appears to the drug used is known as **primary resistance**, that which appears to a different compound is called **secondary resistance**.

Cross resistance between trypanocidal drugs

	<u>QP</u>	<u>HM,DA</u>	<u>PB</u>	<u>IM</u>	<u>DA</u>	<u>HM</u>	<u>PB</u>	<u>I</u>
QP	+	+	+	+	+	±	-	-
HM	+	+	+	+	-	+	+	-
PB	+	+	+	+	-	+	+	+
IM	+	+	+	+	-	+	+	-
DA	+	-	-	-	+	-	-	-

QP = Quinapyramine; HM = Homidium; PB = Pyrrithidium bromide; IM = Isometamidium; DA = Diminazene aceturate

+ = resistant; - = not resistant; ± = some strains resistant.

Vector control

Past methods based on *ecological* control e.g. Bush clearing.

- Another ecological method was to starve tsetse flies by depriving them of the hosts they feed on.
- *The most effective ecological control method is to avoid contact with tsetse flies.*

Use of insecticides

- Tsetse flies are highly susceptible to the action of insecticides, and many different products, starting with DDT and dieldrin up to the more recently introduced and less harmful pyrethroids, have been used over the past 50 years to control and eradicate tsetse.

Sterile male technique

- It has been known for a long time that male tsetse flies which have been rendered sterile by gamma irradiation or by certain chemical compounds.
- The males to be sterilized have to be mass reared in the laboratory.

Use of traps or screens

- Another approach is the use of traps or insecticide-impregnated targets (screens).

Innate resistance to trypanosomosis

- It is well-known that genetically determined innate resistance to many diseases occurs in animal populations which have been subject to natural selection by exposure to disease pressure over many generations.
- The central Sudan (Nuba Mountains) and even western Ethiopia. *N'Dama* cattle (which originate from Guinea) have rather long horns, while breeds with short horns comprise for example the Baoulé (Burkina Faso and northern Ivory Coast) and the *Muturu* (Nigeria). They are “dwarf” cattle (although a N'Dama cow can weigh as much as 200 kg, similar to the size of many of the smaller zebu breeds).

Integrated control

- we should use all available methods that can be applied in each particular situation and are cost-effective. Combining different control methods against a parasitic disease is called *integrated disease control* or *integrated disease management*

The sterile insect technique

- The *Sterile Insect Technique* (**SIT**) involves the rearing of large numbers of insect, separation of the males, **irradiation** of these **flies** with carefully controlled doses of **gamma radiation** to make them **sterile** and systematic releases of sterile insects among the indigenous target population.

Babesiosis

Synonyms: *Piroplasmosis, Texas fever, redwater, tick fever*

- *Babesiosis* is caused by intraerythrocytic protozoan parasites of the genus *Babesia* that infects a wide variety of vertebrate hosts, including domestic and wild animals, as well as man.
- The disease is transmitted by ticks.

- The tick species mostly incriminated as vector are *Boophilus spp.*
- In Ethiopia *B. decoloratus* is a common vector
- Other tick vectors include *Rhipicephalus spp.*, *Haemaphysalis spp.*

Distribution: worldwide in distribution.

- **Host:** As indicated above all domestic animals can be infected.

Morphology

- Pear- shaped but also round or elongated.
- In side RBCs, they are present singly or as pairs arranged at a characteristic angle with their narrow ends opposed.
- The two organisms inside the RBC form an acute angle. In stained smear it has a blue cytoplasm and a red chromatin granule at one pole.
- Generally two forms are recognized:
- Small babesia ===== which have a size of 1-2.5 μm .
- Large babesia ===== which are 2.5 – 5 μm .

Around **71** species yet are discovered out of which
around **18 spp** are found in domestic animals.

<u>Host</u>	<u>Large babesia spp</u>	<u>Small babesia spp</u>
• Cattle	<i>B. bigemina</i>	<i>B. bovis, B. divergens,</i> <i>B. major</i>
• Dog's	<i>B. canis</i>	<i>B. gibsoni</i>
• Horses	<i>B. cabali</i>	<i>B. equi</i>
• Sheep and goats	<i>B. motasi</i>	<i>B. ovis</i>
• Pigs	<i>B. trautmanni</i>	

Life cycle and transmission

A. Final hosts

- Asexual multiplication in RBC by *binary fission* so that the resulting merozoites burst the cell (erythrocytes) – the released merozoites invade new RBCs and the cycle continues.

B. Intermediate hosts

- Ticks including *Boophilus*, *Haemaphysalis* and *Rhipicephalus* are IHs.
- There are two types of transmission: *transovarian* and *transstadial* (stage to stage).

Transovarian transmission

- The organism divides asexually, by binary fission within the red cell.
- Eventually, the host cell ruptures and the organisms are liberated to penetrate new red cells.
- sexual phase occurs in the tick gut followed by schizogony which results in the production of elongated, motile, club-shaped bodies, called vermicules.
- These migrate to the tissues of the tick, especially the ovary, and undergo further multiplication to produce more vermicules.
- The entire process takes around seven days.

Trnasstadial tranmission

- When stage-to-stage transmission occurs, vermicules again reach the salivary glands of the next stage of the tick when feeding commences, and mature to become infective forms.
- some species of Babesia may be transmitted through the ovary for two or more generations of female ticks; this is known as **vertical transmission**.

Pathogenesis

- The rapidly dividing parasites in the red cells produce rapid **destruction of the erythrocytes** with accompanying ***hemoglobinaemia, hemoglobinuria*** and ***fever***.
- This may be so acute as to cause death within a few days, when the ***packed red cell volume falls below 20%***.
- **RBC destruction** result in the release of pharmacologically active substance (kinin) which results in increased vascular permeability leading to edema.
- At necropsy, the carcass is pale and **jaundiced (yellow discoloration)**.

Clinical manifestations

- Incubation period is around 2 weeks, high fever, anemia, jaundice, haemoglobinuria, emaciation, anorexia and restlessness, profuse diarrhea followed by constipation, labored breathing, abortion in pregnant animals, and death due to anaemic anoxia.

Diagnosis

1. Common clinical manifestations are suggestive.
2. Confirmation is by examination of blood to demonstrate the organisms. Negative result of blood smear examination does not indicate negative diagnosis, several smears should be examined.
3. Serological examination: CFT, ELISA, IFA, IHA
 - Detection of Ag is better than Ab to confirm or know the current status. This is because Abs could also be from previous exposure.
3. PCR

Treatment

1. Diminazine aceturate (Berenil): 2-3.5 mg/kg, IM
2. Quinoronium Sulphate (Pirevan): 1 -2 mg/kg, SC
3. Imidocarb: 1.2- 2.4 mg/kg, SC. This has therapeutic and prophylactic use.
4. Amicarbalid: 5-10 mg/kg, IM. This is used mainly for therapeutic use.

Control

- Treatment of infected animal.
- Regular spraying or dipping with acaricides. Enzootic stability should be maintained.
- Introduction of tick resistant cattle breed.
- attenuated and non-attenuated babesia strains (*B. canis*) cell culture babesia vaccine (not much commercialized)

Theileriosis

- *Theileriosis* are a group of tick borne diseases caused by *Theileria* spp.
- The most important species are *T. parva* and *T. annulata*, which cause widespread death in cattle in tropical and subtropical areas.
- The parasites, are tick transmitted, undergo repeated *schizogony* in the lymphocytes, ultimately releasing small *merozoites* which invade the **red** cells.

- Both *Theileria* and *Babesia* are members of the suborder Piroplasmorina. While *Babesia* is primarily parasites of **RBC**, *Theileria* use, successively, WBC and RBC for completion of their life cycle in mammalian hosts.
- The infective *sporozoite* stage of the parasite is transmitted in the saliva of infected ticks as they feed. Sporozoites **invade lymphocytes** (monocytes in the case of *T. annulata*), within a few days, develop to *schizonts*.
- In the most pathogenic species of *Theileria*, development of schizonts causes the host WBC to divide; at each cell division, the parasite also divides.
- There is **no transovarial transmission** as occurs in *Babesia*.

Transmission

- *T. parva* sporozoites are injected into the host by infected ticks, *Rhipicephalus appendiculatus*, during feeding.
- Based on clinical and epidemiologic parameters, 3 subtypes of *T parva* are recognized, but these are probably not true subspecies. *T parva parva* , transmitted mainly between cattle, and *T parva lawrencei* , transmitted mainly from buffalo to cattle, are both highly pathogenic and can cause high levels of mortality, whereas *T parva bovis* , transmitted between cattle, is less pathogenic.

Pathogenesis, Clinical Findings, and Diagnosis

- An occult phase of 5-10 days follows before infected lymphocytes can be detected in Giemsa-stained smears of cells aspirated from the local draining lymph node.
- Subsequently, the number of parasitized cells increases rapidly throughout the lymphoid system, and from about day 14 onwards, cells undergoing merogony are observed.
- This is associated with widespread lymphocytolysis, marked lymphoid depletion, and leukopenia. Piroplasms in RBC infected by the resultant merozoites assume various forms, but typically they are small and rod-shaped or oval.
- Lymph node swelling becomes pronounced and generalized.

- Clinical signs vary according to the level of challenge and range from inapparent or mild to severe and fatal.
- Typically, fever occurs 7-10 days after parasites are introduced by feeding ticks, continues throughout the course of infection, and may be $>42^{\circ}\text{C}$.
- Lymphoblasts in *Giemsa*-stained lymph node biopsy smears contain multinuclear *schizonts*.
- Anorexia develops and the animal rapidly loses condition;
- Lacrimation and nasal discharge may occur.
- Terminally, dyspnea is common
- Just before death, a sharp fall in body temperature is usual, and pulmonary exudate pours from the nostrils.
- Death usually occurs 18-24 days after infection.

Treatment and Control

- Prospects for survival of cattle with clinical East Coast fever or *T. annulata* infection were enhanced by the development of the theilericidal compound *parvaquone* and, subsequently, its derivative *buparvaquone*.
- Treatment with these compounds is highly effective when applied in the early stages of clinical disease but is less effective in the advanced stages in which there is extensive destruction of lymphoid and hematopoietic tissues.

Trichomonas

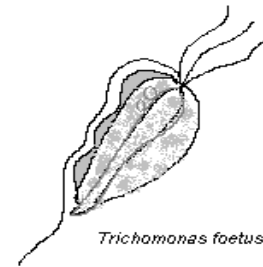
Site: digestive tract mainly and reproductive tract

Host: domestic animals and man

- Important species in this genus is *Trichomonas foetus*, a venereal transmitted multiflagellated organism of the reproductive tract of cattle.
- In bulls the infection is inapparent, but in pregnant cows it produces early fetal death which is usually first recognized as an infertility problem.

- *Trichomonas* that occur in the digestive tract of animals are commensals,
- only one species, *T. gallinae*, is clearly pathogenic in the esophagus and crop of pigeons.

Morphology



- pyriform, rounded anteriorly and pointed posteriorly (pear-shaped)
- with single nucleus 3 anterior flagella and 1 posterior flagella.
- In fresh preparations, the organism is motile and progresses by rolling jerky movements, the flickering flagella and the movements of the undulating membrane being readily seen. Occasionally, rounded immobile forms are observed.

Life cycle

- Bulls, once infected, remain infective permanently.
- The organisms inhabit the preputial cavity and transmission to cows occurs during coitus.
- From the vagina, the trichomonads reach the uterus.
- Intermittently organisms are flushed into the vagina, often two or three days before oestrus.
- Infection is usually followed by early abortion, the organisms being found in the amniotic and allantoic fluid.
- Subsequently cows appear to 'self cure' and, in most cases, appear to develop a sterile immunity.

Pathogenesis

- In cows, abortion before the fourth month of pregnancy is the commonest sequel and this is normally followed by recovery.
- developing fetal membranes are retained leading to purulent endometritis, a persistent uterine discharge and anoestrus;
- corpus luteum is retained and the cervical seal remains closed, when a massive pyometra develops which, visually appear pregnant.
- In bulls, a preputial discharge associated with small nodules on the preputial and penile membranes may develop shortly after infection.

Diagnosis

- History and clinical findings are very important
- samples could be taken from vaginal mucus or preputial washings examined using a warm-stage microscope for the presence of organisms.
- Concentration by centrifugation is possible.

Treatment and control

- Cows: only symptomatic treatment and sexual rest because the disease is self limiting.
- Bulls: very difficult to treat so culling by slaughtering is recommended.
- Dimetridazole orally or intravenously has been reported to be effective.
- Artificial insemination from non-infected donors is the only entirely satisfactory method of control.

SUB PHYLUM SPOROZOA

- Protozoa within the subphylum *Sporozoa* are characterized by occurring intracellularly and having an apical complex at some stage of their development.
- The trophozoites have no cilia or flagella.
- Reproduction involves both asexual (schizogony) and sexual (gametogony) phases.
- Following gametogony, a zygote is formed which divides to produce spores (sporogony).
- most important are *Coccidia* or *alimentary sporozoa* and the *Piroplasmidia* which are *blood sporozoa*.

Class coccidia

- The class coccidia residing in GI epithelium, liver, kidneys, blood cells, and other tissues of vertebrates
- They are typically intracellular with direct or indirect life cycle.
- Those of veterinary importance fall into *two* distinct family groups, the *Eimeriidae* and *Sarcocystidae*.

Some terminologies

- **Oocyst:** is a cyst that contains a zygote but may or may not contain sporocyst (depending on the stage of development).
- **Sporocyst:** is a cyst within an oocyst which may or may not contain sporozoite.
- **Sporozoite:** is the infective stage of coccidian parasite.
- **Gamete:** is sexual stage of protozoan parasite. It can be microgamete or macrogamete.
- **Zygote:** is a single cell stage which result from joining a male and female sexual stages.
- **Homozenous cycle:** is part of life cycle where there is the same host for the development of both sexual and asexual stages (it is a direct life cycle).
- **Heterozenous cycle:** here asexual stage of development takes place in one host and sexual stage in another and completely different host (indirect life cycle).

How do different coccidia?

1. The time required for the completion of the life cycle (longer in heterozenous cycle than homozenous one).
2. The number of asexual stages: some have a single asexual stage whereas others have two or more generations.
3. The predilection site: most are found in the GIT (some in proximal, some in the middle and some distally/in large intestine).
4. The type of host cell affected.
5. Size and shape of oocyst: some are very small and some are larger. Some are oval and others are circular in shape.

6. The pathogenicity : some are highly pathogenic producing high mortality and morbidity where as others can cause a very mild infection.
7. Sensitivity to anti-coccidial drugs: some are easily treated whereas in some there is a strong drug resistance.
8. The number of sporocysts and sprozoites within an oocyst: oocysts might contain four sporocysts containing two sporozoites each OR two sporocysts containing four sporozoites in each. There are some oocycts without sporocycts but having sporozoites.

Family Eimeriidae

- These are mainly intracellular parasites of the intestinal epithelium.
- Schizogony and gametogony occur within the host and sporulation, or maturation of the fertilized zygote, usually takes place outside the host.
- three genera, *Eimeria*, *Isospora* and *Cryptosporidium*, are of veterinary importance
- the term coccidiosis is usually reserved for infections caused by *Eimeria* and *Isospora species*.

Genus Eimeria

Hosts: Poultry, cattle, sheep, goats, pigs and horses.

Site: Epithelial cells of the intestine, kidney and liver.

- The **oocyst** has four **sporocysts** each containing two **sporozoites**, 8 being the total number of **sporozoites**.

Important Species

- *Eimeria tenella*, *E. necatrix*, *E. brunetti*, *E. maxima*,
E. mitis and *E. acervulina* – **chickens**
- *E. zuernii*, *E. bovis* and *E. alabamensis* - in **cattle**

Identification

- This can be made at *microscopic level* either by examining the *feces* for the presence of *oocysts*.
- or by examination of scrapings or histological sections of affected tissues.
- The oocysts may be identified according to *shape* and *size*.
- The most common shapes are *spherical, ovoid or ellipsoidal* and the size of the common species ranges from 15 to 50 μm . Oocysts have a retractile shell and some species possess a small pore at one end called the micropyle, which is often covered by a polar cap.

Tissue stages

- The mature ***schizonts*** may be identified histologically by their ***location, size*** and ***the number*** of merozoites they contain.

Life cycle

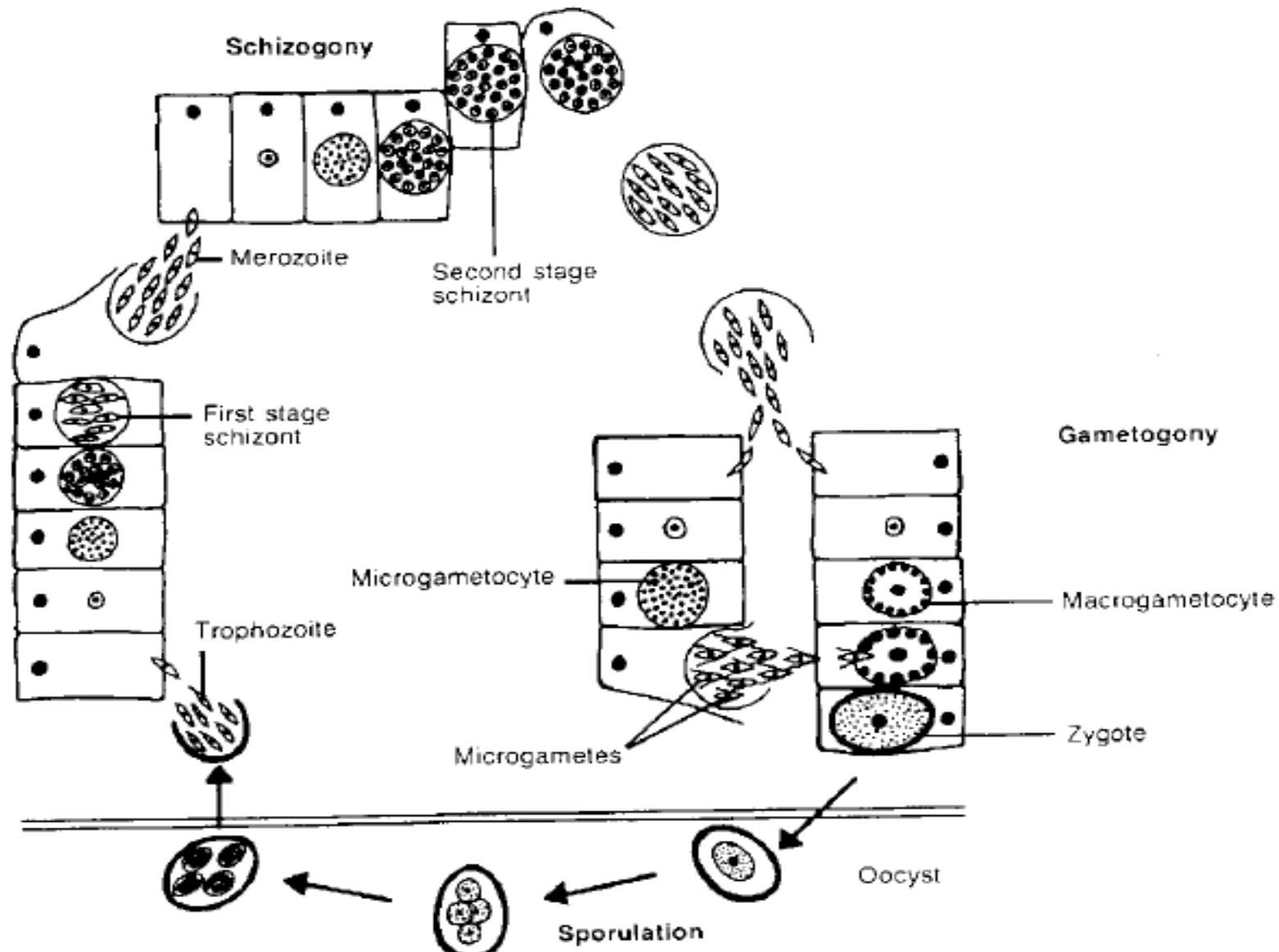
- The life cycle can be ***direct*** or ***indirect***.
- Most have a ***direct life cycle***.
- There are ***three major*** components.
 - ***Sporogony***: this takes place in the outside environment (multiplication in the early stage).
 - ***Merogony***: asexual reproduction within the host.
 - ***Gametogony***: sexual stage within the host.

- The hosts get infected by the ingestion of *sporulated oocyst*.
- The *sporozoites* penetrate GIT mucosa and undergo a series of asexual multiplication (reproduction).
- Each cycle of asexual reproduction takes place within new viable epithelial cells and these stages are called *schizonts*.

Generally, the life cycle is divided into three phases:

- Sporulation,
- Infection and schizogony,
- Gametogony and oocyst formation

Life cycle of Eimeria species.



Genus *Isospora*

- The genus *Isospora* contains many species and like *Eimeria* parasitizes a wide range of hosts. This has an oocyst with two sporocysts each containing four sporozoites.
- The important species include
 - *I. suis* - pig,
 - *I. canis* and *I. ohioensis* - dog
 - *I. felis* and *I. rivolta* - cat.

❑ The life cycle of *Isospora* species differs from *Eimeria* in three respects.

- **First** the sporulated oocyst contains **two** sporocysts each with **four** sporozoites.
- **Secondly**, extra-intestinal stages occurring in the spleen, liver and lymph nodes of the pig may reinvade the intestinal mucosa and cause clinical signs.
- **Thirdly**, rodents may become infected by the ingestion of oocysts from the dog and cat, with asexual stages and act as reservoirs

Genus *Cryptosporidium*

- It poses *problem in human beings* and *in calves*.
- *In man it is an opportunistic infection* whereas in calves, is a major cause of diarrhea especially in exotic breeds.
- The surface cells of GIT called “brush border cells” are the predilection sites.
- There are different species affecting a number of different hosts (lack of host specificity).

Life cycle: it is not well understood but assumed to be direct involving feco-oral contamination.

Pathogenic significance

- **Diarrhea** is the major problem both in **man** and other animals.
- Excessive loss of electrolytes as the result of diarrhea in HIV infected patients is a feature of cryptosporidiosis.
- Being very small (4-5 μm) oocysts require staining

Family Sarcocystidae

- Two genera, *Toxoplasma* and *Sarcocystis* are of veterinary importance.
- Their life cycles are similar to *Eimeria* and *Isospora* except that the asexual and sexual stages occur in intermediate and final hosts respectively (heteroxenous).
- The **definitive hosts** are usually ***carnivores*** and **IHs** are ***herbivores animals***. Man could also be infected and serve as intermediate host (asexual stage takes place).
- The sexual stage takes place in the definitive hosts.

- they are normally non-pathogenic to their **FH**
- and their significance is due to the cystic tissue stages in the **IH** which include *ruminants, pigs, horses and man*.
- The tissue phase in the **IH** is obligatory, except in ***Toxoplasma*** where it is facultative.

Genus Sarcocystis

- The life cycle is exclusively ***heteroxenous*** so that all species have two hosts (**IH** and **Definitive Host**).
- **Final hosts:** Dogs, cats, wild carnivores and man
- **Intermediate hosts:** Ruminants, pigs and horses.
- **Site in final host:** Small intestine.
- **Site in intermediate host:** Schizonts in endothelial cells of blood vessels; large cysts containing bradyzoites in muscles.
- **Distribution:** Worldwide.

Species:

- The previously complex nomenclature for the large number of *Sarcocystis* spp. has largely been discarded by many workers in favor of a new system based on their biology. The new names generally incorporate those of the **intermediate** and **final hosts** in that order.
- Although unacceptable to systematizes, this practice has the virtue of simplicity.

- At present the most important species recognized with the dog as a final host are
 - *Sarcocystis bovicanis* (Synonym *S. cruzi*)
 - *S. ovicanis* (syn. *S. tenella*)
 - *S. capricanis*
 - *S. porcicanis* (syn. *S. miescheriana*)
 - *S. equicanis* (syn. *S. bertrami*)
- Those with the **cat** as the **Final host** include:
 - *S. bovifelis* (syn. *S. hirsuta*)
 - *S. ovifelis* (syn. *S. tenella*)
 - *S. Porcifelis*

Life cycle

- **Definitive hosts** (**dogs/cats**) release the **oocyst** within the feces---**sporulation** in the environment---ingestion of sporulated oocyst by the IH--**sporozoites** are released and penetrate intestinal mucosa, join circulation and transport to different body parts in which they become encysted and exist as a **cyst**.
- **Brain, kidney, liver** and **muscle** are the most frequently affected organs. The cyst remain there longer since they are well encapsulated.
- ***The cyst in the muscle can easily be seen during meat inspection.*** Finally, *the definitive host is infected by ingesting organs and tissues that contain the cyst (zoocyst)---penetrate intestinal mucosa---differentiate into macro and microgametes---sexual reproduction.*

Pathogenic significance

- Unless they reside in vital organs (*brain* and *lungs*), it is not a major problem in the **IHs**.
- except in man where it act as opportunistic infection in **HIV/AIDS**. In the definitive hosts, there are no clear clinical signs.

Treatment

- Anti-coccidial drugs for the final hosts.
- In the IHs, it is very difficult since they are well encapsulated.

Genus Toxoplasma

- The genus *Toxoplasma* has a single species, *T gondii*, which is *an intestinal intracellular coccidian of cats*.
- It can develop in any host cells except non-nucleated RBCs. The life cycle includes a facultative systemic phase which is an important cause of abortion in sheep and *may also cause a zoonosis*.
- Human infections are particularly serious if they occur during pregnancy and may result in abortion or congenitally acquired disorders which primarily affect the central nervous system.

Species: *Toxoplasma gondii*

Final hosts: all felids. The domestic cat is the most important.

Intermediate hosts: any mammal including man, or birds.

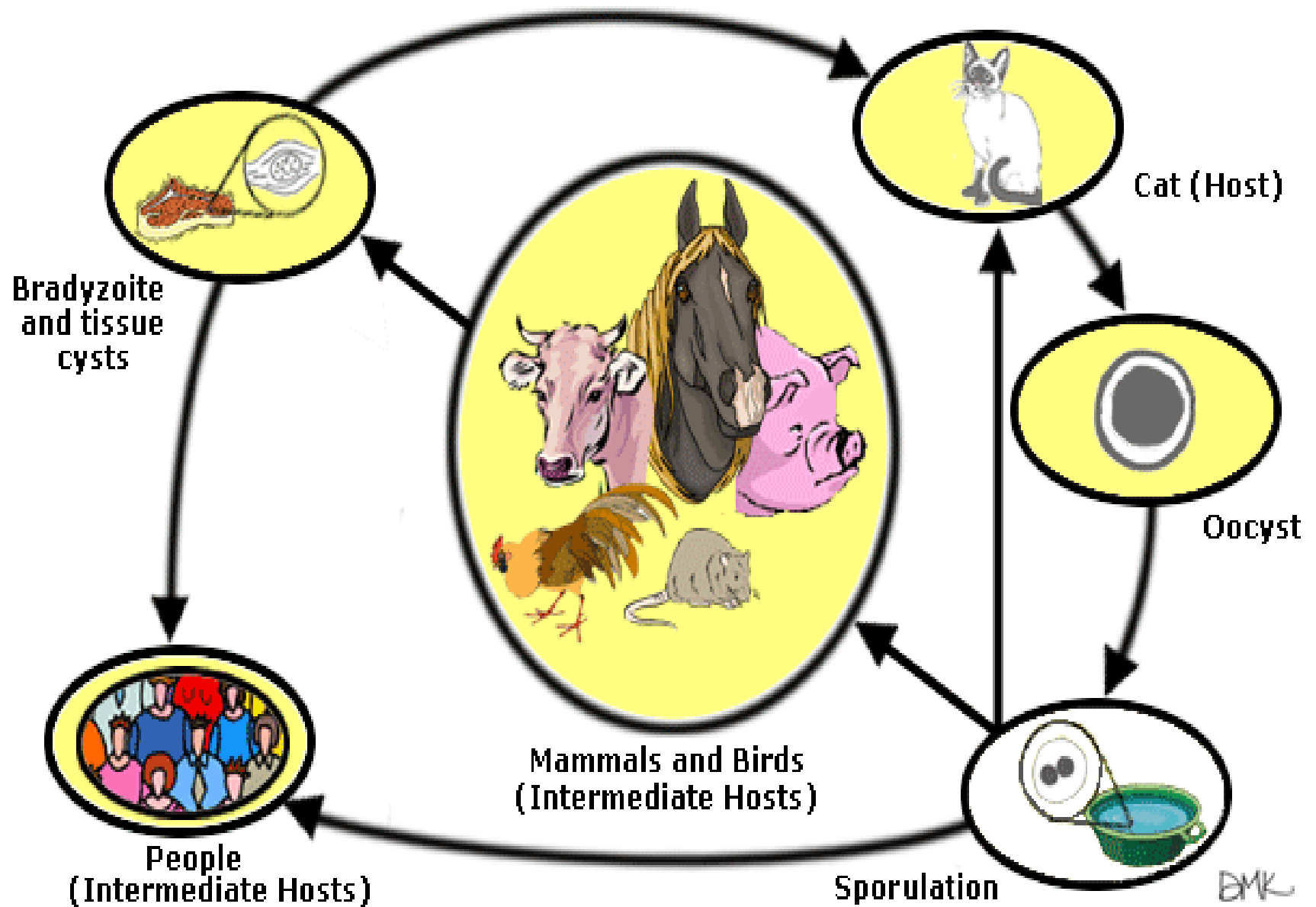
- Note that the final host, the cat, may also be an intermediate host and harbour extra-intestinal stages.

Life cycle

- The infected definitive hosts (domestic and wild cats) release oocysts---sporulation resulting in oocysts containing sporocysts—ingestion by IHs (human, rodents, herbivores, etc)---asexual stage in the IHs (brain, muscle, liver, etc...)---encapsulation in the sites above--- ingestion of the organ/tissue containing the cyst by the definitive hosts---sexual stages and the cycle continues.
- Following infection, the cyst wall is digested in the cat's stomach, and in the intestinal epithelium liberated bradyzoites initiate a cycle of schizogonous and gametogonous development terminating in the production of oocysts in 3-10 days. During this cycle in the intestinal mucosa, the organisms may invade the extra-intestinal organs where the development of tachyzoites and bradyzoites proceeds as in intermediate hosts.
- If pregnant animals are infected, the infection can pass to the progeny.

- **Tachyzoite:** Extra-intestinal stage of *Toxoplasma* species that are found in vacuoles of muscle, liver, brain, and lung found in intermediate and definitive hosts.
- Sporulated oocysts are ingested and the liberated sporozoites rapidly penetrate the intestinal wall and through the blood. It is the invasive and proliferative stage of *Toxoplasma*.

The life cycle of *Toxoplasma gondii*



Dogs:-

- The onset of illness is marked by fever with lassitude, anorexia and diarrhoea.
- Pneumonia and neurological manifestations are common.

Ruminants:-

- There are only a few reports of clinical toxoplasmosis associated with fever, dyspnoea, nervous signs and abortion.
- At postmortem, bradyzoites are demonstrable in the brain with focal necrosis in acute cases.
- The most important role of toxoplasmosis in ruminants is its association with abortion in ewes and perinatal mortality in lambs.
- If infection of the ewes occurs early in gestation (<55 days) there is death and exulsion of the small fetus.

Man

- Infection of man may be **acquired** or **congenital**.
- Acquired infections occur in two ways.

☛ **First**, from the ingestion of oocysts shed in the feces of cats.

This may be directly from hands contaminated, for example, during the cleaning of litter trays or, more likely, indirectly from the ingestion of vegetables or food contaminated by cat feces. Flies may also transfer oocysts on to food.

☛ **Secondly**, an important source of infection is the ingestion of undercooked meat containing *Toxoplasma* cysts.

Diagnosis

- Specific diagnosis is made by serological tests or by demonstration of the organisms in tissues of mice inoculated with suspected material.
- Two of the most commonly used tests measure antibody, the ***Sabin-Feldman dye test*** and the ***indirect immunofluorescent antibody test (IFA)***.
- The later is preferred since it does not require live organisms.

- More recently, an ***ELISA test*** has been developed which is capable detecting a recent infection by the estimation of IgM, as compared to IgG antibody.
- The most convincing diagnosis is obtained by inoculating Toxoplasma-free mice by the ***intrapерitoneal*** or ***intracerebral*** route with test material and the subsequent demonstration of ***tachyzoites*** or ***bradyzoites*** in smears of organs or serous cavities.
- Clinical, apparent infections present as low grade fever and malaise.
- Involvement of vital organs is rare although myocarditis and encephalitis have been recorded.