Veterinary Parasitology (Protozoology and Arthropods) II (VETM-2082)

By

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Outline of the Lecture

- **Introduction to Protozoology**
  - Definition
  - Classfification
  - Structure
  - Reproduction
  - Nutrition

- **Trypanosomosis**
  - African animal trypanosomosis
  - Mechanically transmittied trypanosomosis
  - African human trypanosomosis (Sleeping Sickness)
  - American human trypanosomosis (Chagas Disease)

Cont..

- Leishmaniasis
- Trichomoniasis

- Malaria – Plasmodium
- 6 groups- will be assigned for presentation
  1. Group I - aetiology
  2. Group II - pathogensesis
  3. Group III - transmission
  4. Group IV - epidemiology
  5. Group V - diagnosis
  6. Group VI- control

Introduction

Definition

- **Protozoology is** the study of protozoa affecting animals and human beings.
- The science had begin in the latter half of the **17th** century when Antonie van Leeuwenhoek of the Netherlands first observed protozoans by the microscope.
- **Protozoans** are unicellular eukaryotic microorganisms.
- **They lie between prokaryotic and eukaryotic organisms, sharing any of the characteristics of each make their classification complex (Collier et al.,1998).**

- The word Protozoa meaning 'first animals' was coined by Goldfuss in **1818.**

Cont… Prokaryotes and Eukaryotes

Similarities

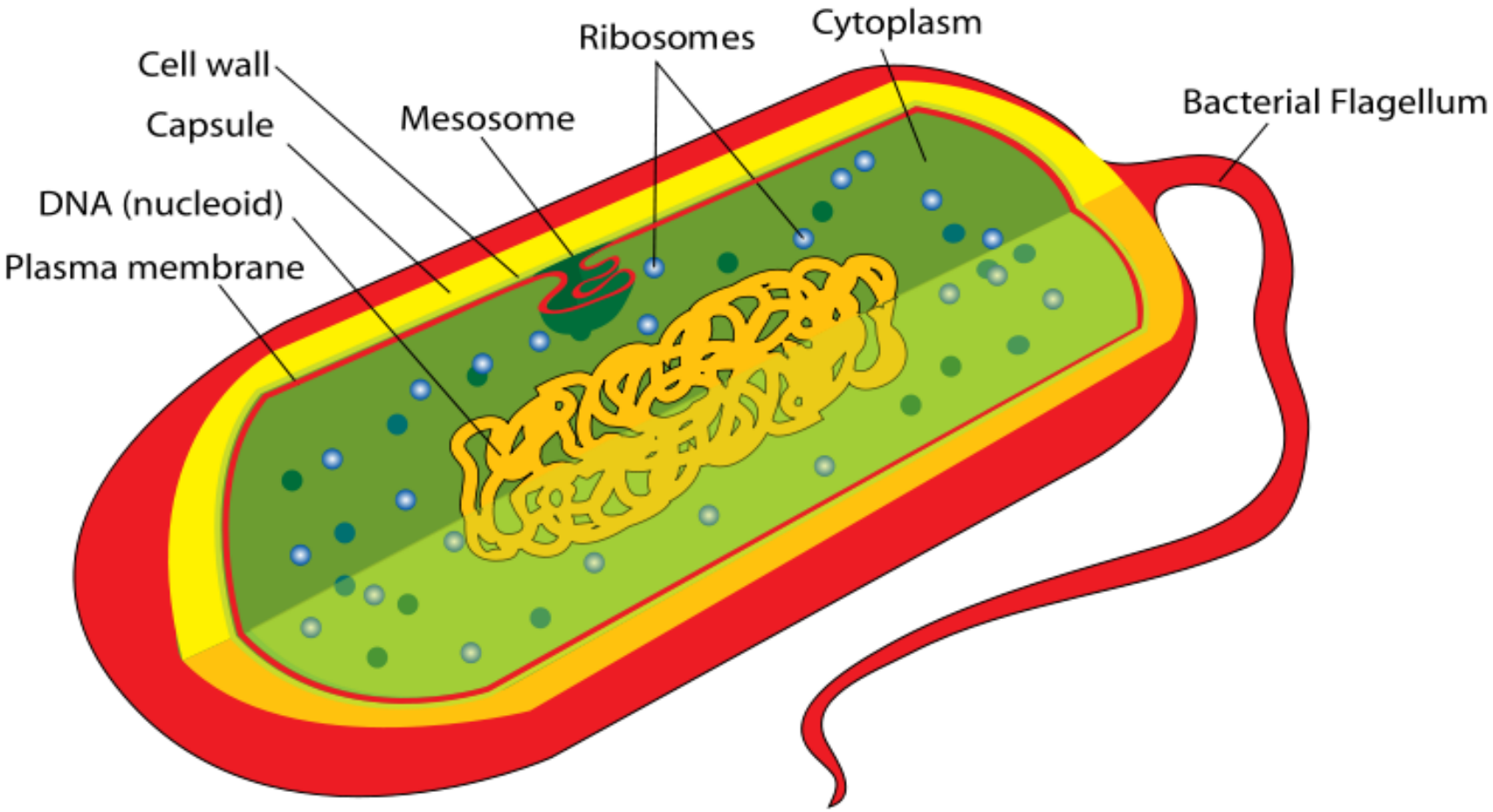
1. They both have DNA as their genetic material
2. They are both membrane bound
3. They both have ribosomes
4. They have similar basic metabolism
5. They are both amazingly diverse in form

Cont… Prokaryotes and Eukaryotes

Differences

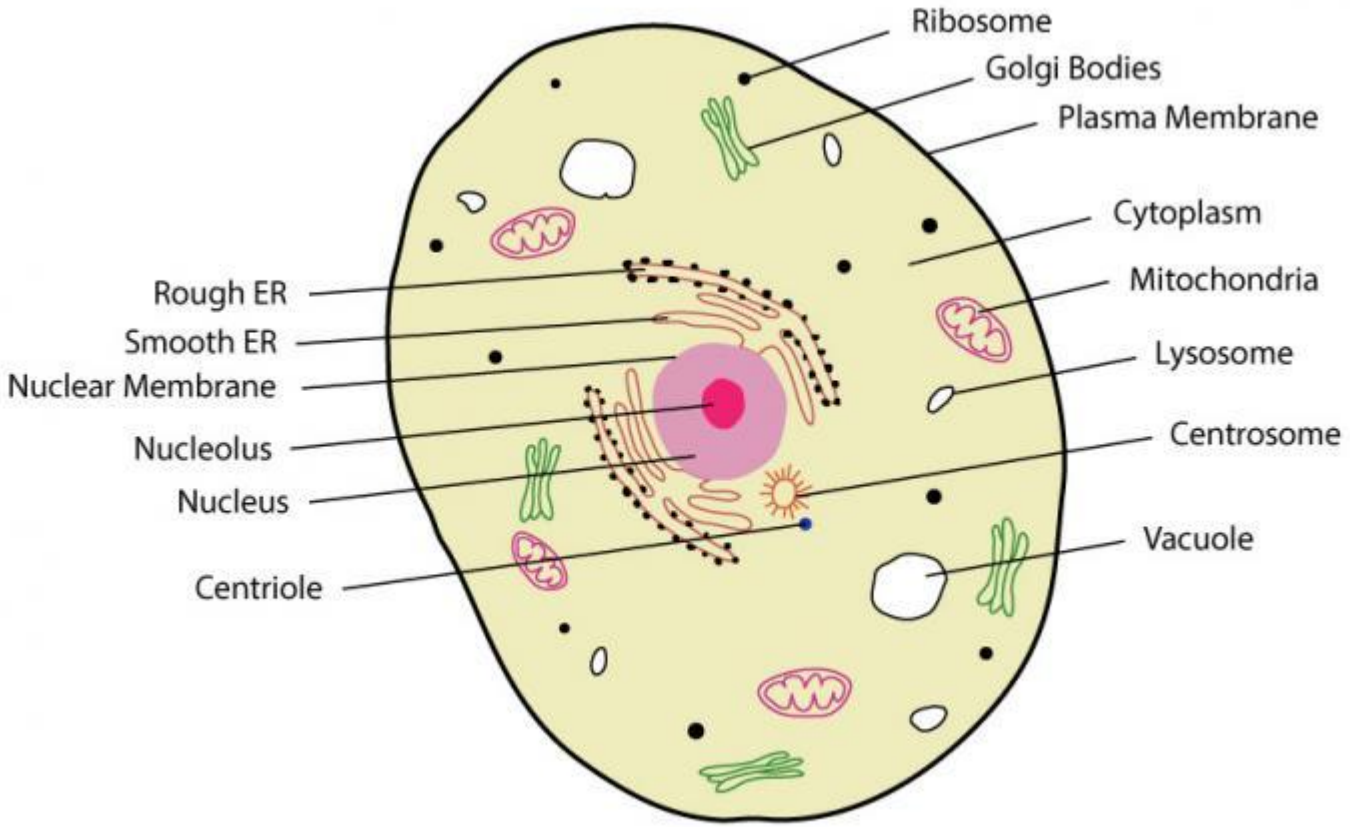
1. Eukaryotes have a nucleus, while prokaryotes do not.
2. Eukaryotes have membrane-bound organelles, while prokaryotes not.
3. Eukaryotic cells are, on average, ten times the size of prokaryotic cells.
4. The DNA of eukaryotes is much more complex and therefore much more extensive than the DNA of prokaryotes.
5. Prokaryotes have a cell wall composed of peptidoglycan, a single large polymer of amino acids and sugar.
6. The DNA of prokaryotes floats freely around the cell; the DNA of eukaryotes is held within its nucleus and associated with histones (proteins).
7. Eukaryotes undergo mitosis; prokaryotes divide by binary fission.

Prokaryote cell diagram

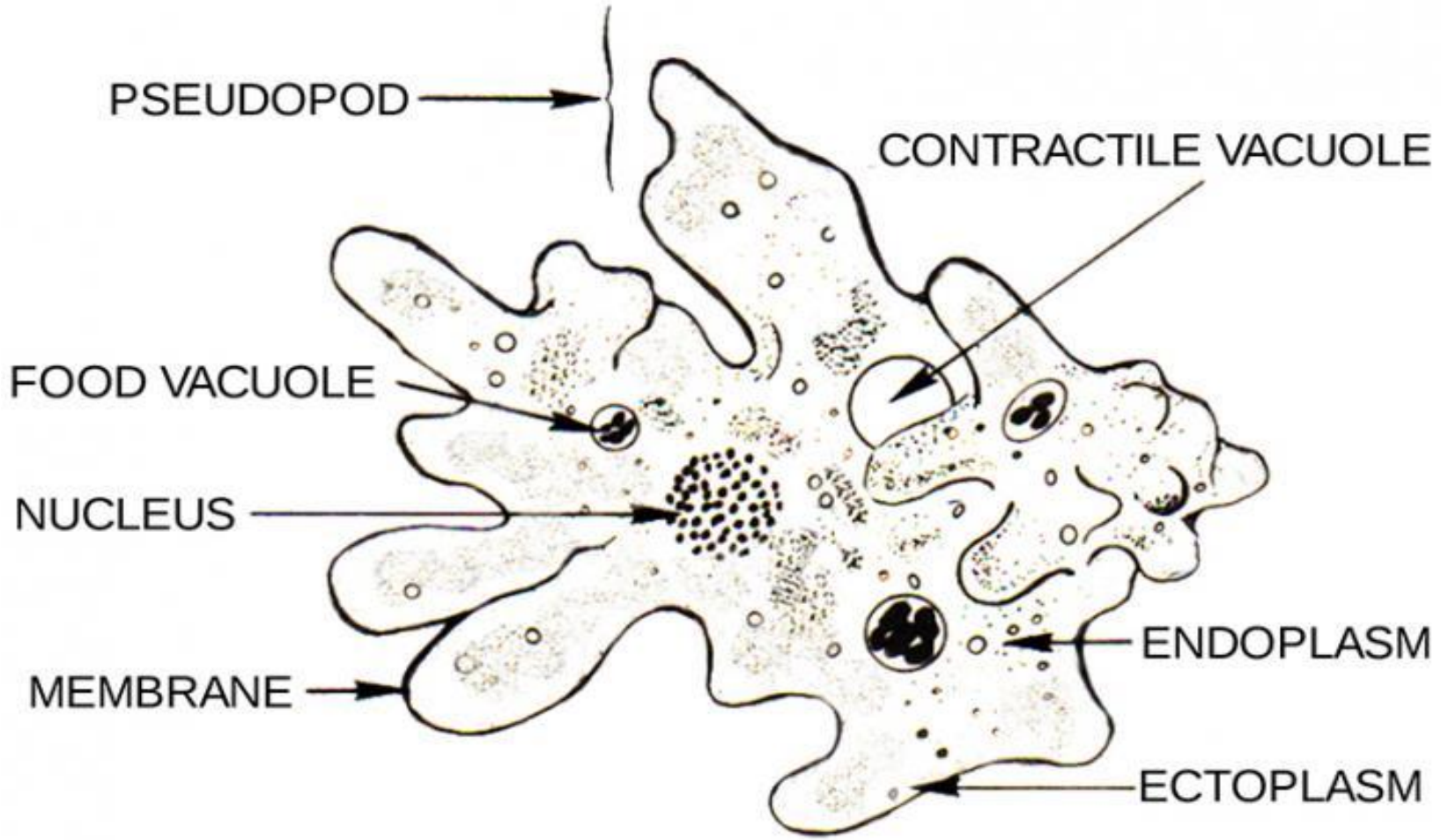


Eukaryotic cell diagram

- Have defined nucleus along with other membrane bound cell organelles.
- All these cell organelles are held in their position by cytoplasm which is protected by plasma membrane.
- And the plasma membrane is further protected by the cell wall.



Unicellular cell diagram



Cont…

- Haekel (1876) used the term ‘Protista’ and his ideas were translated into a system of classification that divided the Animal kingdom into single celled organisms (Protozoa) and multicellular organisms (Metazoa).

- This has remained almost unchanged in zoological books until the present time and its basic concepts are clearly and unambiguously set out by Craig (1926).

- There are > 200, 000 named species of protozoa of which nearly 10, 000 are parasitic in invertebrates and in almost every species of vertebrate (Collier et al., 1998) ???

Cont…

Classification

- Three terms are currently widely used:
  - Protozoa
  - Protoctista
  - Protista

- However, parasitologists tend to be very conservative and the term **Protozoa** is now almost universally used.

- With the creation of **5** kingdoms, status of Protozoa was raised to that of a kingdom, which formerly was a Phylum.
- Thus, the subordinate groups automatically became Phyla.

Classification Methods

1. Isoenzyme Profiles

- The first most widely used technique for distinguishing between apparently identical parasites.

- The technique involves using a number of characteristic enzymes to type different populations of parasite isolates in parallel and with previously characterized ones.

- Isoenzymes have been used to distinguish the various species of Leishmania, subspecies of African trypanosomes etc...

- It is widely used in the typing and phylogenetic classification of Cryptosporidium.

Cont…

2. DNA and RNA Technology

- Is used for diagnosis of parasitic infections as well as for resolving taxonomic and phylogenetic problems.

- The first attempt to produce a comprehensive phylogenetic tree of the protozoa with special reference to the parasitic forms, using data derived from a small subunit of ribosomal RNA (srRNA) was done in (1989).
- Both DNA and RNA can be used to determine evolutionary distance as nucleotide sequences tend to diverge over time and do evolve at a more regular rate than do morphological characters.
- The development of the Polymerase Chain Reaction (**PCR**) has revolutionised the use of DNA techniques in Parasitology (Guo and Johnson,1995).

Cont…

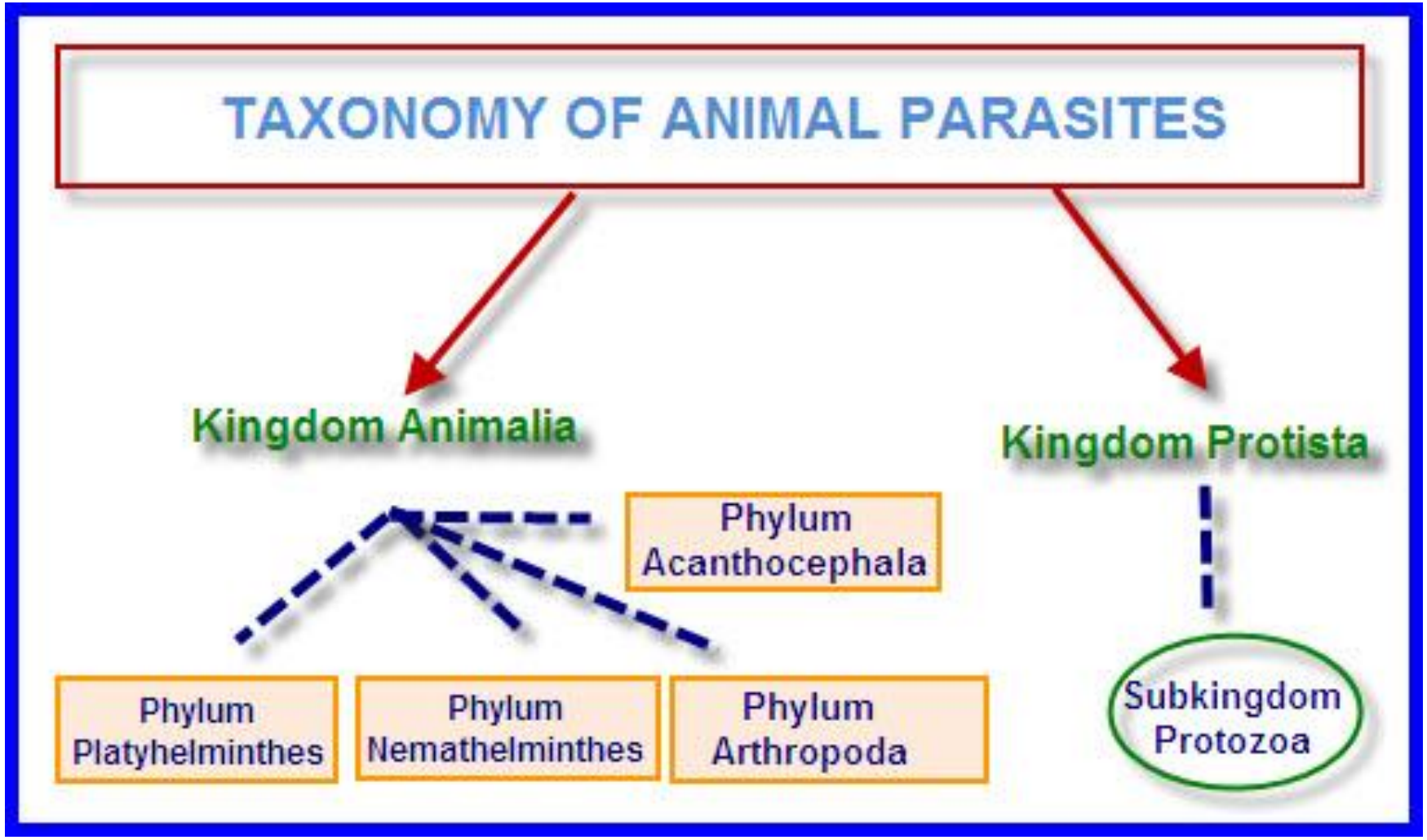
3. Molecular Karyotyping

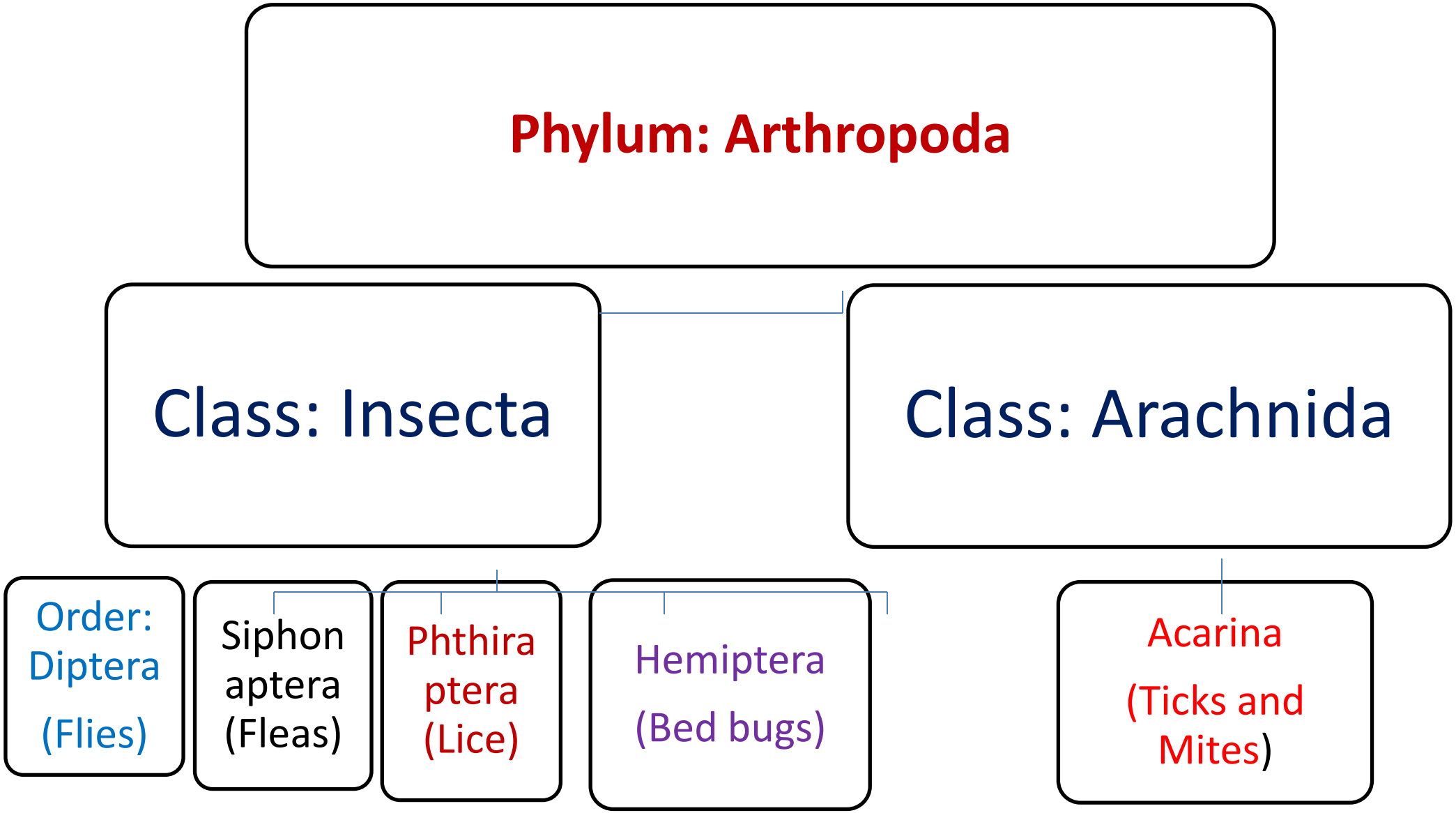
- Which involves measuring size differences between chromosomes (Dujardin, 1995)

4. Traditional Classification of Parasitic Protozoa

- This classification is simply outlined focusing on the central problem of protozoa classification, the need to meet up with the protozoologists requirement.
- It is based on 1980 Classification published by The Society of Protozoologists and that of Lee et al. (1985).

Classification of Parasites




Phylum: Arthropoda
Class: Insecta
Class: Arachnida
Order: Diptera (Flies)
Siphon aptera (Fleas)
Phthira ptera (Lice)
Hemiptera (Bed bugs)
Acarina (Ticks and Mites)

Traditional Classification

Kingdom: Protozoa

- **Phylum:** Sarcomastigophora
  - **Subphylum:** Mastigophora: e.g. Giardia, Trichomonas, Trypanosoma, Leishmania,
  - **Subphylum:** Sarcodina: e.g. Entamoeba, Endolimax, Acanthamoeba, Balamuthia

Cont..

- **Phylum:** Apicomplexa (the sporozoans)
  - **Class:** Sporozoea
    - **Subclass:** Coccidia
      - **Order:** Eucoccidiida
        - **Suborder:** Eimeriina e.g. Isospora, Sarcocystis, Toxoplasma, Cryptosporidium, Cyclospora
        - **Suborder:** Haemosporina e.g. Plasmodium
    - **Subclass:** Piroplasmea e.g. Babesia

- **Phylum:** Microspora e.g. Enterocytozoon, Encephalitozoon
- **Phylum:** Ciliophora (the ciliates) e.g. Balantidium

Cont…

1. Mastigophora

- Locomotion is effected by one or more whip-like, thin structures called flagella

- Examples: Free - living forms like Euglena viridis, and parasitic forms like *Trichomonas vaginalis, Trypanosoma gambiense, Giardia lamblia*

2. Sarcodina

- Motility is due to the streaming of ectoplasm, producing protoplasmic projections called **pseudopodia** (false feet)
- Examples: Free-living form like Amoeba proteus and parasitic form like Entamoeba histolytica

Cont…

3. Ciliophora

- Locomotion is carried out by means of short hair-like projections called cilia

- Examples: Free-living forms like Paramecium and parasitic form like Balantidium

4. Sporzoa

- Unlike the above three classess of protozoa, members of the class sporozoa do not have locomotory organelles

- Examples: Plasmodium, Eimeria, Toxoplasma etc.

Cont…

Sub phylum	**Sarcomastigrophara** Locomotion by pseudopodia and/or flagella	***Sporozoa*** *(By gliding the cycle, largely intracellular both sexual and asexual phases occur)*	**Ciliophora** By cilia Balantidium	**Microspora** Intracellular parasites multiplying by asexually

Cont…

Class	Sarcodina	Mastigophora	*Coccidia*	*Piroplasmidia*	*Haemosporidia*
	Movement by pseudopodia	One or more flagella	Parasites of the epithelial cells Asexual and sexual reproduction takes place	Parasites of blood cells, have ticks as vectors in which sexual reproduction occurs	Parasites of blood cells, have blood sucking diptera as vectors in which sexual reproduction occurs

Cont…

Class	Sarcodina	Mastigophora	*Coccidia*	*Piroplasmidia*	*Haemosporidia*
	Entamoeba	Trypanosoma Leishmania Trichomonas Histomonas Giardia	Eimeria Isospora Cryptosporidium Toxoplasma Sarcocystis Besnoitia Neospora	Babesia, Theileria, Cytauxzoon	Plasmodium, Haemoproteus Leucocytozoan

Cont…

Structure

- **Protozoa are unicellular eukaryotes**
  - They do not have cell wall
  - The nucleus is enclosed in a membrane
  - The organelles of protozoa have functions similar to higher animals
  - The plasma membrane covers the projecting locomotor structures
  - The outer surface layer of some protozoa termed a pellicle
  - It is sufficiently rigid to maintain a distinctive shape
  - Some protozoa have a cytosome or cell “mouth” for ingesting
  - Some have a distinctive undulating membrane between the body wall and a flagellum
  - Other structures occur in parasitic protozoa: Golgi apparatus, mitochondria…
- **Electron microscopy is essential to visualize the details of protozoal structure**

The structure of the bloodstream forms of a trypanosome with Electron Microscopy

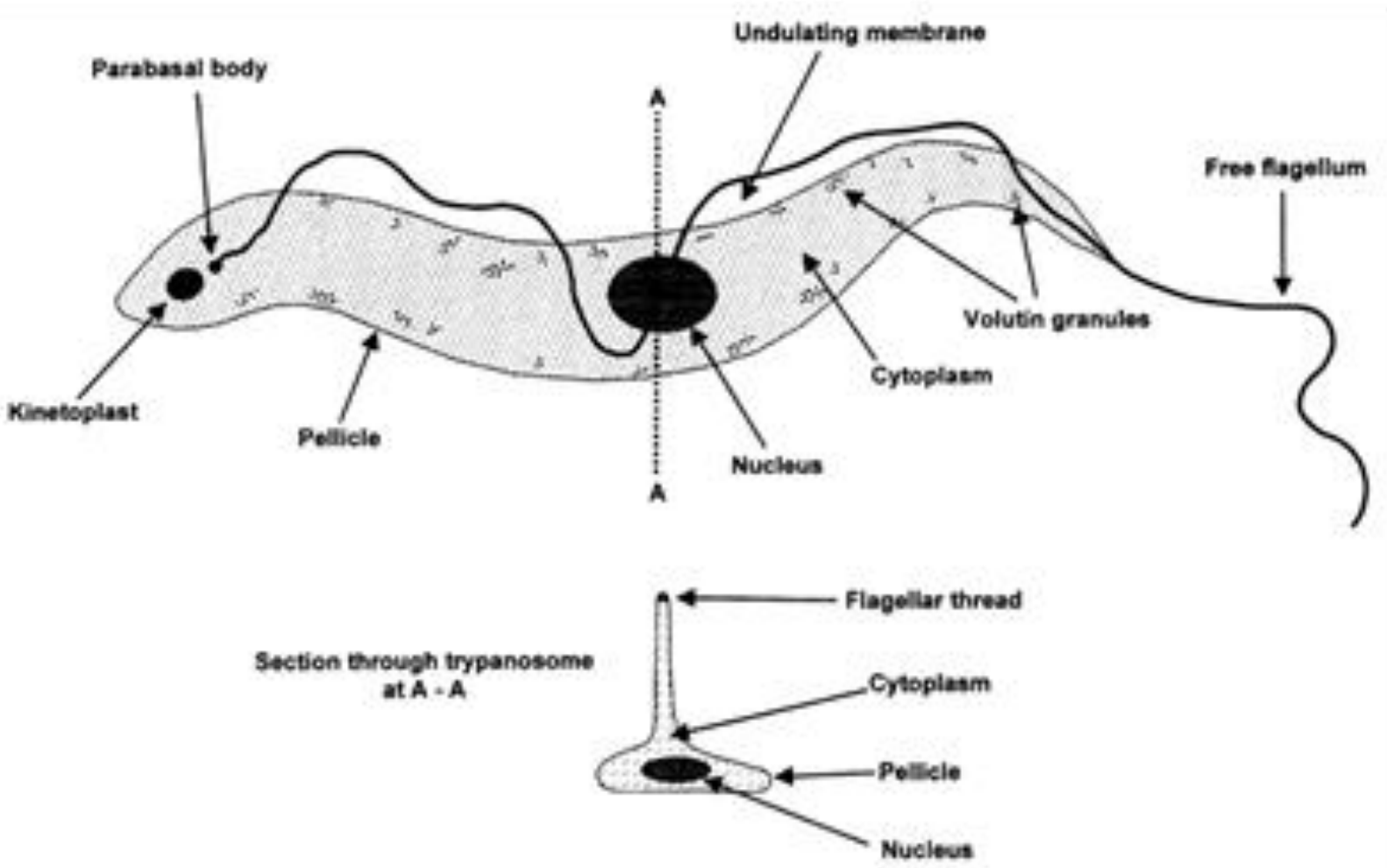
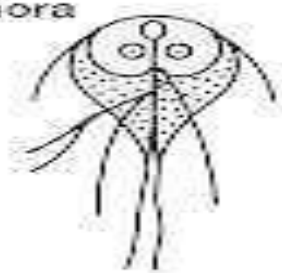
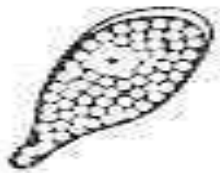
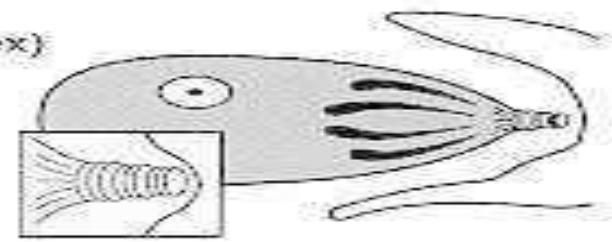
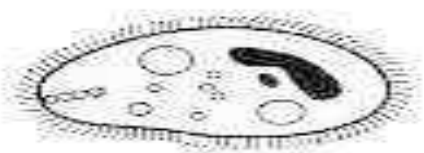


Table 77-1. Classification of Parasitic Protozoa and Associated Diseases

Phylum	Subphylum	Representative Genera	Major Diseases Produced in Human Beings	Chapter
Sarcomastigophora (with flagella, pseudopodia, or both) 	Mastigophora (flagella)	*Leishmania*	Visceral, cutaneous and mucocutaneous infection	82
		Trypanosoma	Sleeping sickness Chagas' disease	
		Giardia	Diarrhea	80
		Trichomonas	Vaginitis	
	Sarcodina (pseudopodia)	*Entamoeba*	Dysentery, liver abscess	79
		Dientamoeba	Colitis	
		Naegleria and *Acanthamoeba*	Central nervous system and corneal ulcers	81
		Babesia	Babesiosis	
Apicomplexa (apical complex) 		*Plasmodium*	Malaria	83
		Isospora	Diarrhea	80
		Sarcocystis	Diarrhea	
		Cryptosporidium	Diarrhea	
		Toxoplasma	Toxoplasmosis	84
Microspora		*Enterocytozoon*	Diarrhea	—
Ciliophora (with cilia) 		*Balantidium*	Dysentery	80
Unclassified	—	*Pneumocystis*	Pneumonia	85

Cont…

Reproduction

- Reproduction may be asexual as in the amoebas and flagellates or both asexual and sexual as in the Apicomplexa
- The most common type of asexual multiplication is binary fission
- Division is longitudinal in the flagellates and transverse in the ciliates

Cont…

- Endodyogeny is a form of asexual division seen in ***Toxoplasma*** and some related organisms:
  - In which no separate nuclear division occurs; the two daughters develop internally within the parent, without nuclear conjugation
- **Schizogony** a common form of asexual division in the Apicomplexa, the nucleus divides a number of times, and then the cytoplasm divides into smaller uninucleate merozoites

Cont…

- **The sexual cycle involves:**
- The production of gametes (gamogony)
- Fertilization to form the zygote
- Encystation of the zygote to form an oocyst
- The formation of infective sporozoites (sporogony) within the oocyst

- Some protozoa have complex life cycles requiring two different host species
- Others require only a single host to complete the life cycle
- A single infective protozoa entering a susceptible host has the potential to produce an immense population

Cont…

Nutrition

- They have heterotrophic mode of nutrition:
  - The free-living forms ingest particulates
  - The parasitic forms derive nutrients from the body fluids of their hosts

- Many protozoa have a permanent mouth, the cytosome or micropore, through which ingested food passes to become enclosed in food vacuoles

- Protozoa have metabolic pathways similar to those of higher animals and require the same types of organic and inorganic compounds

Cont…

- Competition for nutrients is not usually an important factor in pathogenesis because the amounts utilized by parasitic protozoa are relatively small

- Some parasites that inhabit the small intestine can significantly interfere with digestion and absorption and affect the nutritional status of the host (*Giardia* and *Cryptosporidium)*

- The destruction of the host's cells and tissues as a result of the parasites' metabolic activities increases the host's nutritional needs

- Finally, extracellular or intracellular parasites that destroy cells while feeding can lead to organ dysfunction and serious or life-threatening consequences

Significance of protozoal diseases

- Protozoal diseases occur on every continent and affect all domestic animals.
- In the field of vet. medicine, protozoan diseases are by far the most important.
- The FAO estimated in 1984 total annual losses due to ticks and tick-borne diseases is about 7 billion US$.
- Similarly, other protozoal diseases transmitted by vectors have also significant impact on the livelihood of peoples particularly in tropical world (Africa trypanosomosis costs more than 4.75 billion US$ annually).
- The public health impacts directly from zoonotic diseases and indirectly from low protein supply because of the diseases is becoming a serious challenge for current human population growth.

5%

- **Define the following terms:**
  1. Protozoa
  2. Hetrotrophic mode of nutrition
  3. Cilia
  4. Flagella

- **Compare and contrast the following phrases:**
  1. Longitudinal/Transverse binnary fission
  2. Cyclical/Non-cyclical transmission in protzooal diseases

Trypanosomosis

- Trypanosomosis is a disease caused by *Trypanosoma* which affects humans as well as domestic and wild animals

- Trypanosomes are unicellular blood parasites belonging to:
  - Phylum: Sarcomastigophora
  - Order: *Kinetoplastida*
  - Family: *Trypanosomatidae*
  - Genus: *Trypanosoma*

Cont…

- Species of trypanosomes infecting mammals fall into two distinct groups:

i. Stercoraria - contaminative (posterior) transmission

- **Trypanosoma cruzi – bugs (triatoma) vector**
  - **South American human trypanosomosis (Chagas disease)**
  - Extremely serious characterized by myocarditis, Megaoesophagus and Mega colon
  - Vectors: Bugs of Hemiptera, often called **"kissing bugs"**
- T. theleri - cattle – Tabanid, T. melophagium- sheep - sheep ked
  - Both are non pathogenic

ii. Salivaria - inoculative (anterior) transmission: tsetse and other biting flies

Salivarian group of Trypanosomes

- The disease caused by salivarian trypanosome is considered to be the single most health constraint to livestock productivity in sub-Saharan Africa:
  - Direct annual production losses in cattle - US$ 1.2 billion/year
  - About three million cattle die each year
  - Farmers spend 35 million US $ per year - trypanocidal drugs
  - Annual loss in livestock and crop production - US$ **4.75** billion/year

❖ **Cyclical and Non-cyclical transmitted trypanosomosis**

History

- African animal trypanosomosis or Nagana is a disease caused by *T. vivax, T. congolense and T. brucei* transmitted by tsetse flies.
- In domestic animals, these parasites cause a severe, often fatal disease while in wild animals the parasites cause relatively mild infections.

- As the illness progresses the animals weaken more and more and eventually become unfit for work, hence the name of the disease "Nagana" which is a Zulu word that means "powerless/useless“.

Cont…

- The species of trypanosomes causing Nagana were discovered by different scientists in the beginning of the 20th century.
- **Bruce (1895) discovered T. brucei as the cause of cattle trypanosomosis**
- **T. congolense were discovered in 1904 by Broden**
- **T. vivax were discovered in 1905 by Ziemann**

- T. vivax and T. evansi are also transmitted mechanically by biting insects, such as tabanids and stomoxys, in areas outside the tsetse belt as well as in South America and Asia
- T. equiperdum is transmitted sexually and has a wider geographic distribution

Cont…

- Infections by these trypanosome species are not confined to cattle since they infect a wide range of domestic animals such as horses, camels, donkeys, mules, water buffalo, pigs, goats and dogs

- African trypanosomes also affect humans, causing **Sleeping sickness** or human African trypanosomosis
- These parasites are:
  - *T. b. gambiense* found mainly in West Africa
  - *T. b. rhodesiense* located mainly in East Africa

- The South American trypanosome known as *T. cruzi* is transmitted by triatomas, and causes **Chagas disease** in humans

Cont…

Tsetse transmitted trypanosomes (Cyclical)

- *T. congolense*
- *T. brucei*
- *T. b. brucei*
- *T. vivax*
- *T. simae*

Nagana in animals

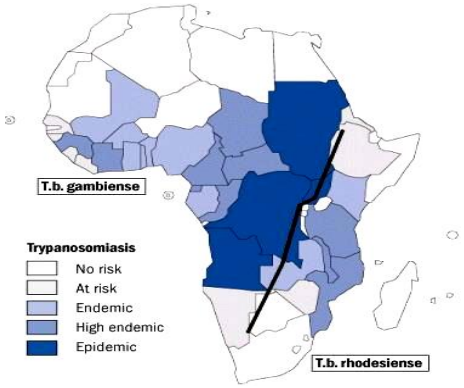
- ***T. b. rhodesinse*: acute and severe disease in Southern Africa; reservoir = wildlife**
- ***T. b. gambinse*: less severe and more chronic in West Africa; reservoir = man**
  - **Sleeping sickness in man**

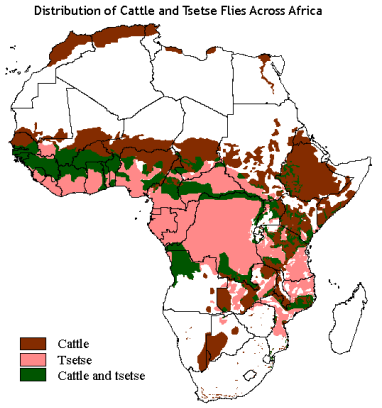
Cont…

Non-tsetse transmitted trypanosomes (Non-cyclical)

- World wide distribution
- T. evansi - camel
- T. vivax - most domestic animals
- T. equiperdum – horse- veneral transmission

African trypanosomiasis





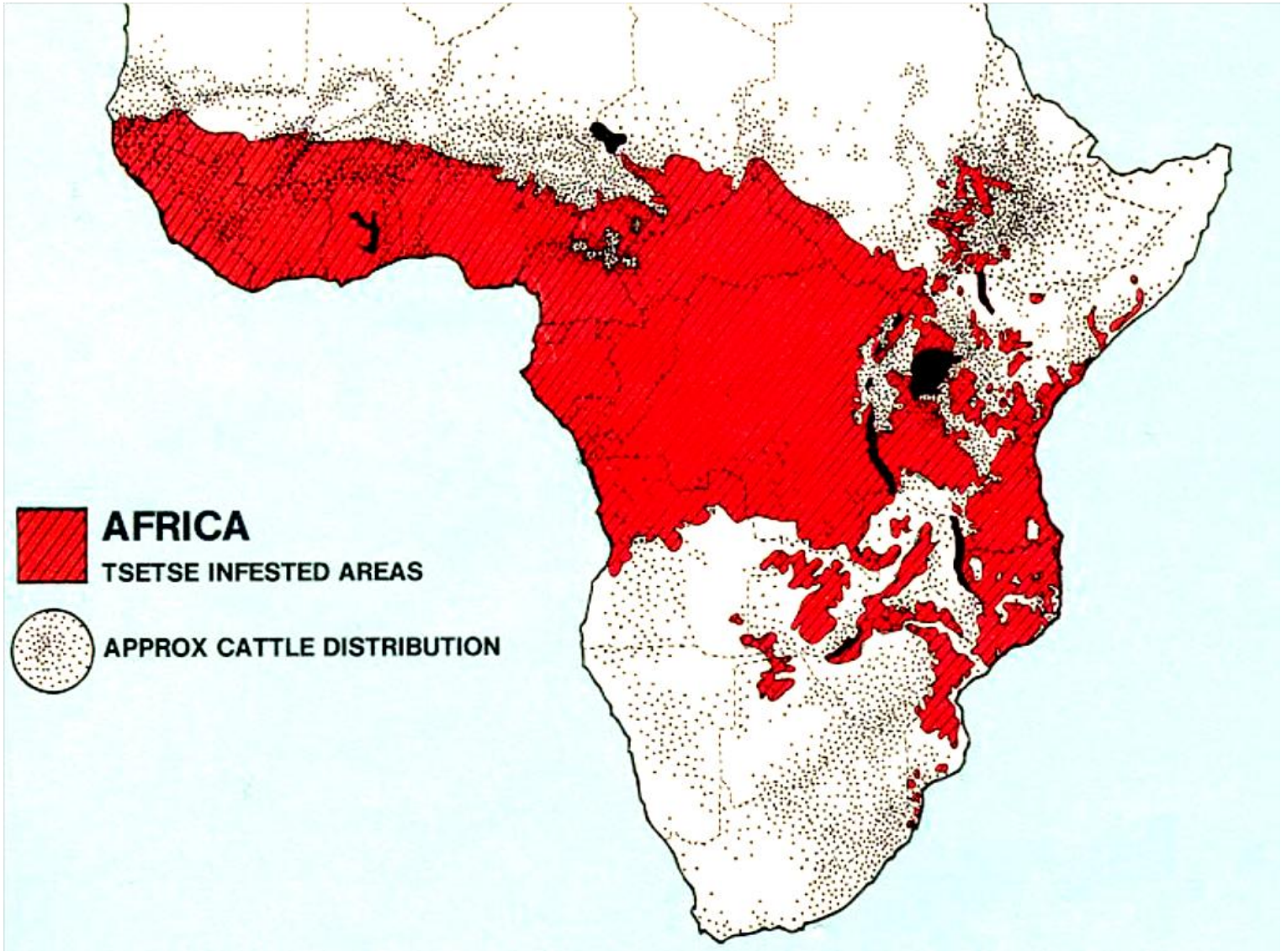
Human African trypanosomiasis

- 300,000 cases/year
- approx. 50,000 deaths/annum
- 100% mortality, if untreated

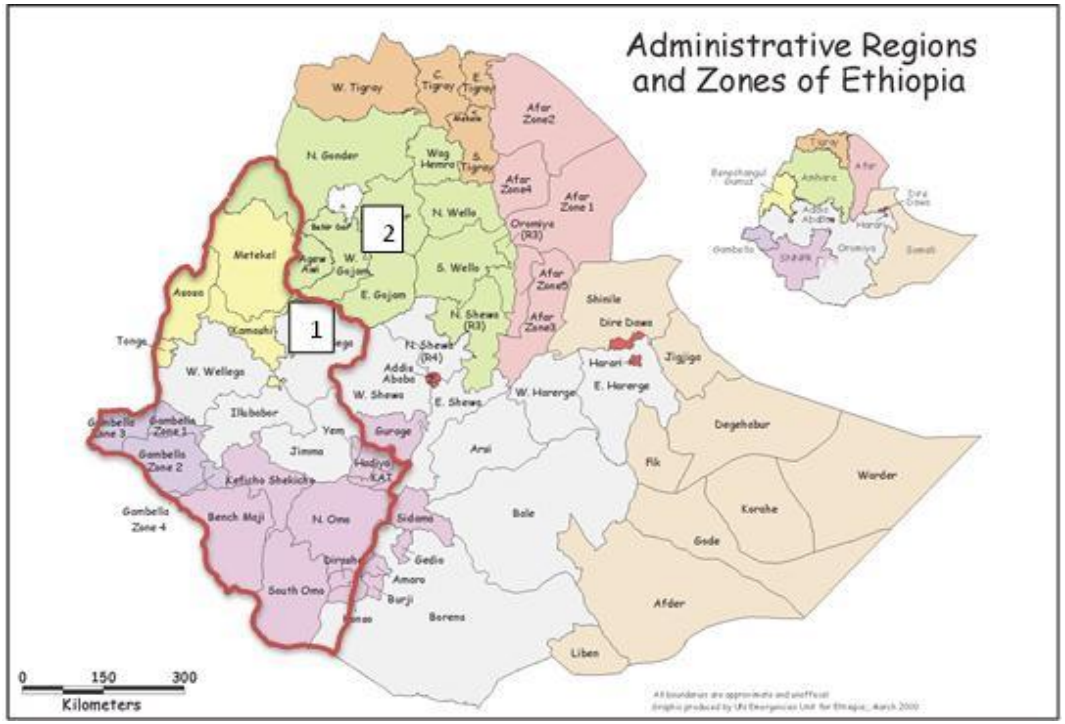
Livestock trypanosomosis

- 37 sub-Saharan countries
- 3 million cattle deaths annually. Also small ruminants and equidae
- Annual loss $ 1.0 - 1.2 bn cattle production, $ 4.75 billion agricultural Gross Domestic Product
- 166 million poor livestock keepers (30% of world total) in sub-Saharan Africa

Distribution of tsetse transmitted trypanosomosis



Distribution of trypanosomosis in Ethiopia

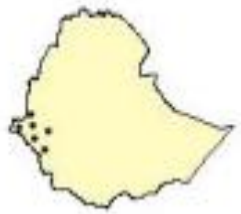


T. congolense

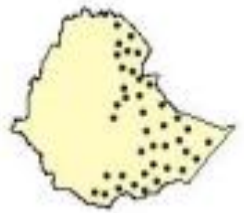
T. brucei

T. vivax

T. equiperdum



T. rhodesiense



T. evansi

The red bounded area in the map of Ethiopia is the tsetse infested and the remaining area is non-tsetse infested region

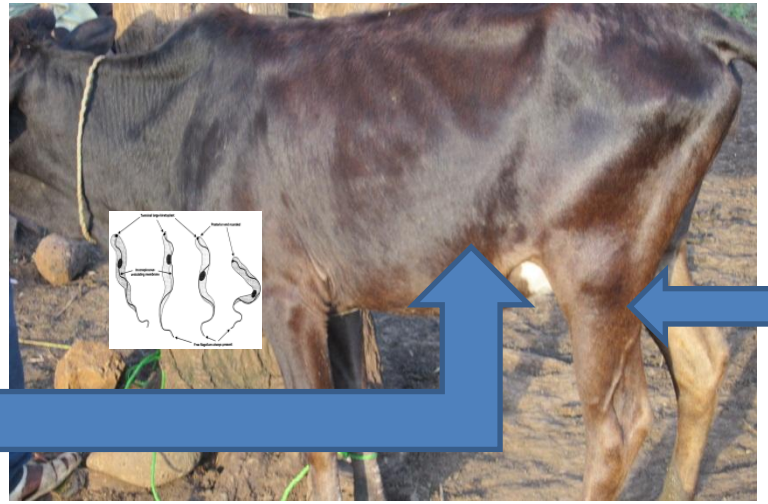
Transmission types of Trypanosomosis

Cyclical transmission
T. congolense
T. brucei
T. vivax

Transmission

Mechanical transmission
T. evansi
T. vivax

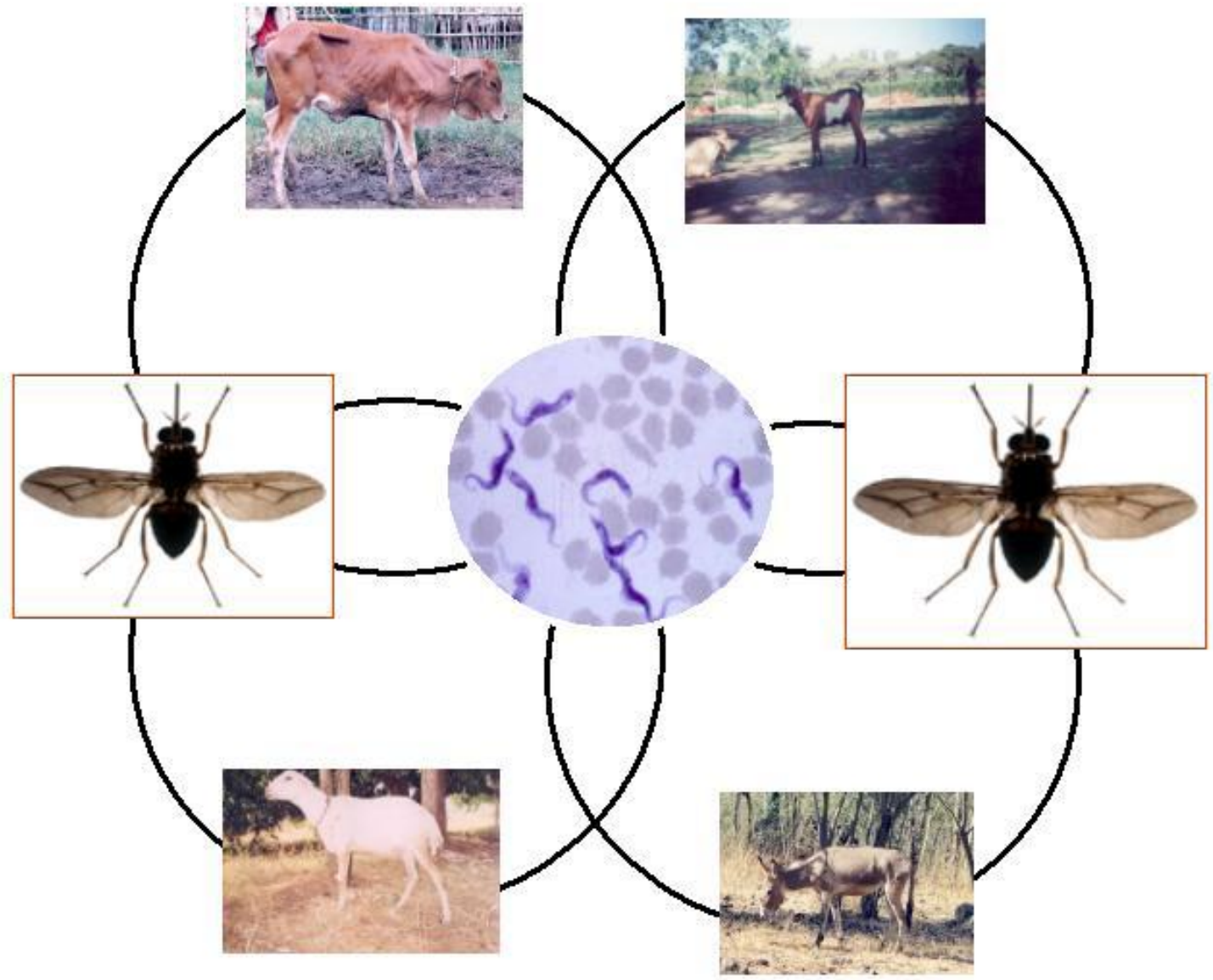
Tsetse



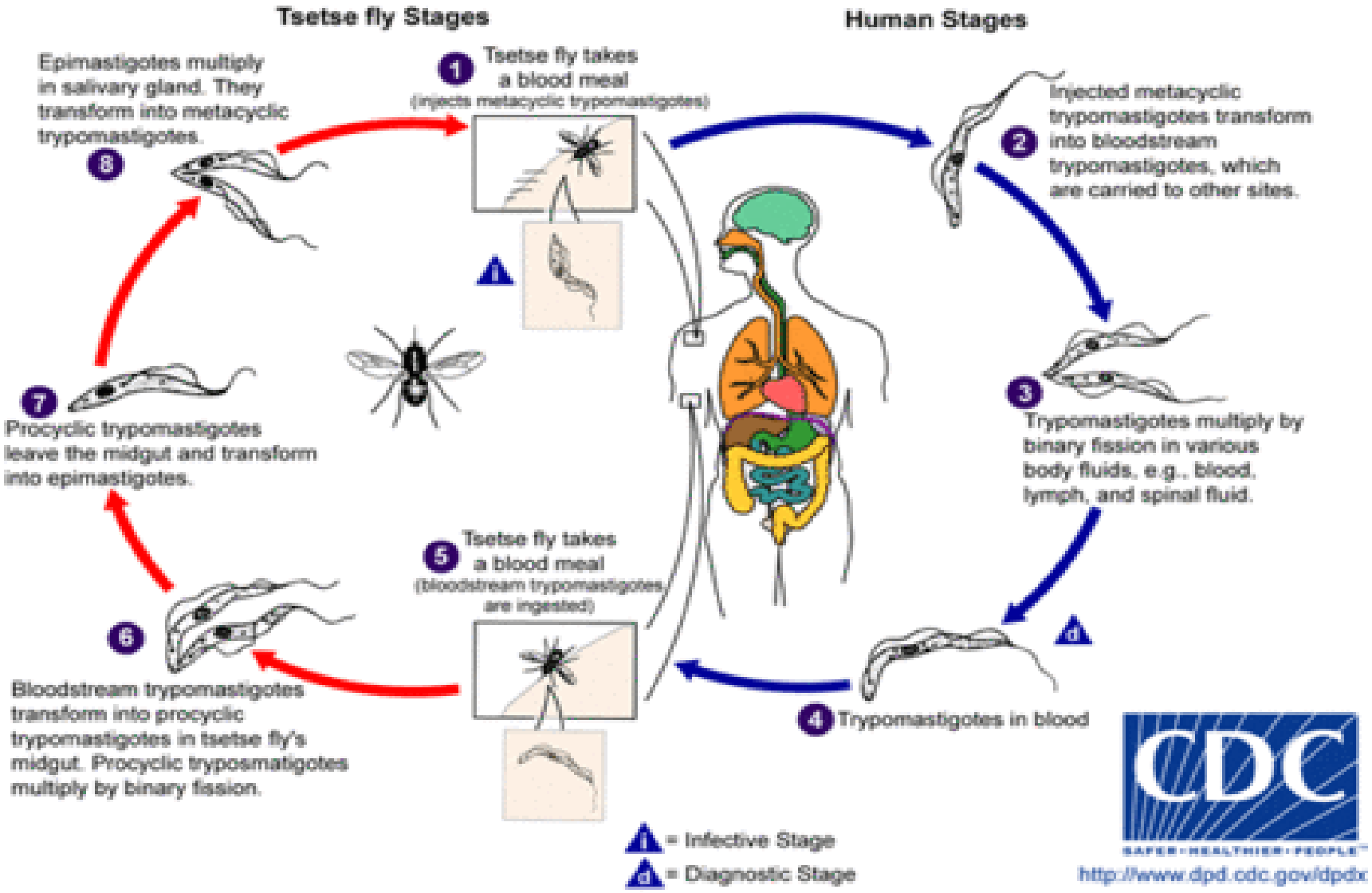
Tabanus

Venereal transmission
T. equiperdum

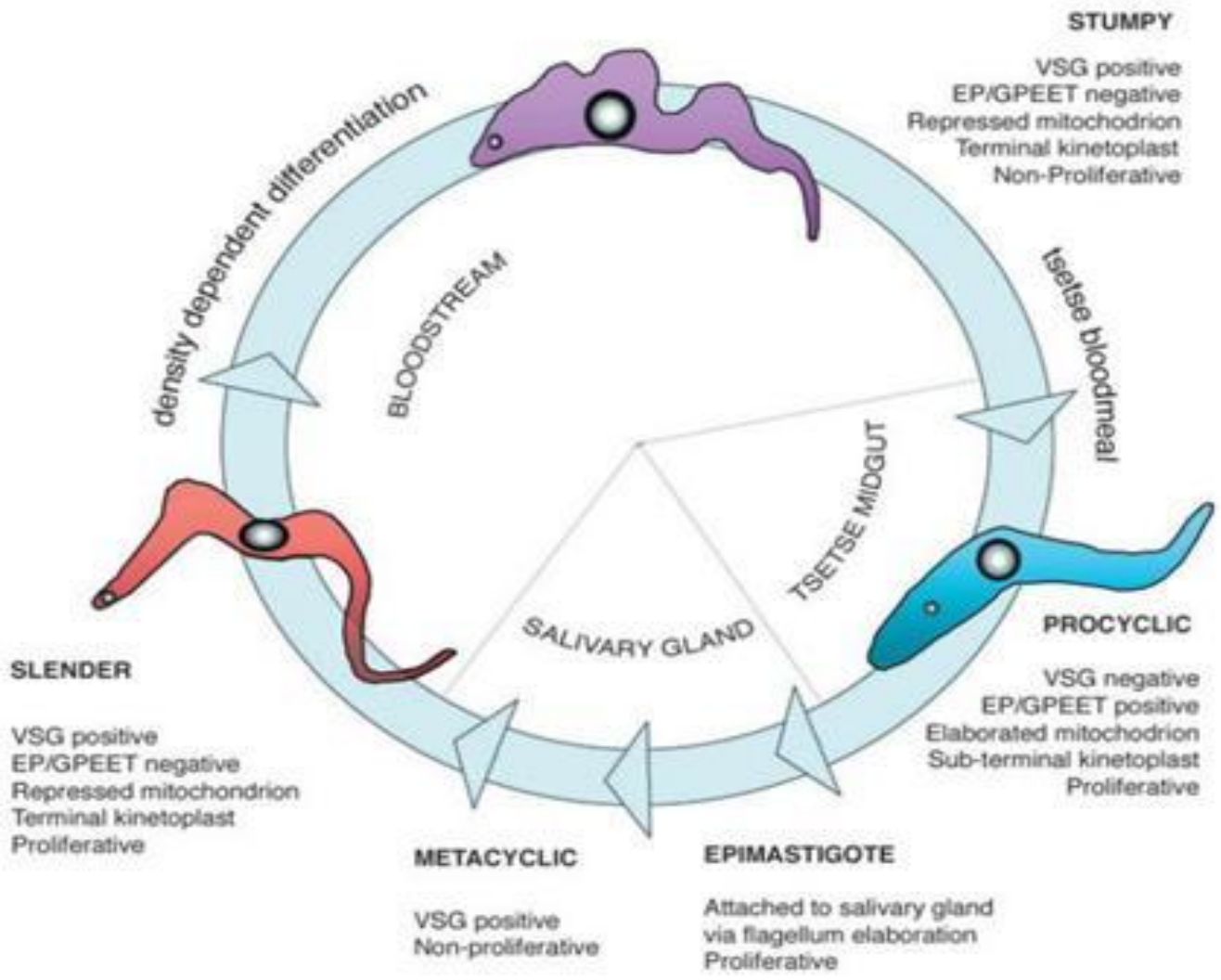
The life cycle of tsetse transmitted trypanosomes in different species of livestock



Life Cycle


Tsetse fly Stages
Human Stages
Epimastigotes multiply in salivary gland. They transform into metacyclic trypomastigotes.
8
1 Tsetse fly takes a blood meal
(injects metacyclic trypomastigotes)
i
2 Injected metacyclic trypomastigotes transform into bloodstream trypomastigotes, which are carried to other sites.
3 Trypomastigotes multiply by binary fission in various body fluids, e.g., blood, lymph, and spinal fluid.
d
4 Trypomastigotes in blood
5 Tsetse fly takes a blood meal
(bloodstream trypomastigotes are ingested)
6 Bloodstream trypomastigotes transform into procyclic trypomastigotes in tsetse fly's midgut. Procyclic tryposmatigotes multiply by binary fission.
7 Procyclic trypomastigotes leave the midgut and transform into epimastigotes.
i = Infective Stage
d = Diagnostic Stage
CDC
SAFER·HEALTHIER·PEOPLE™
http://www.dpd.cdc.gov/dpdx

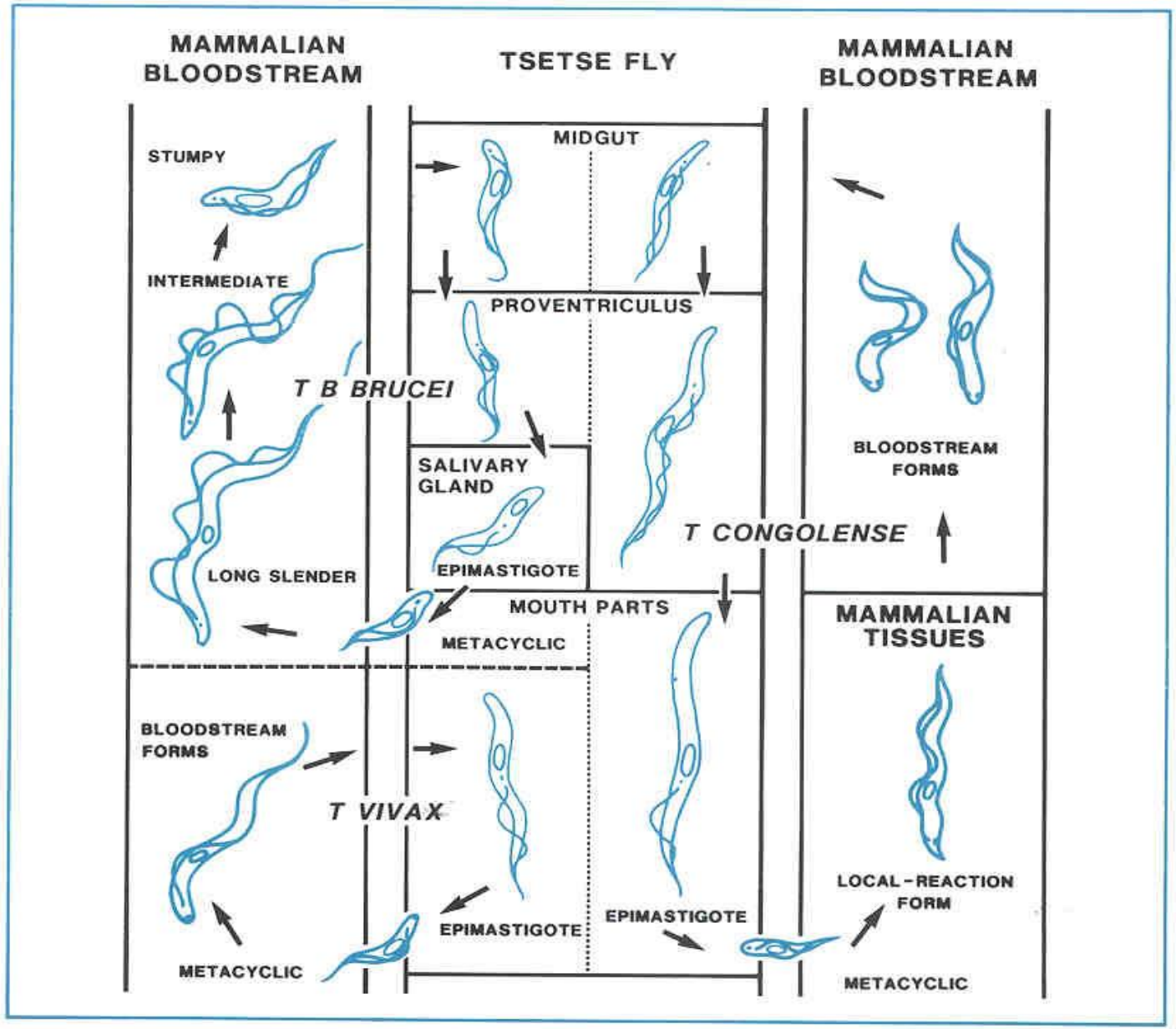
Life cycle


STUMPY
VSG positive
EP/GPEET negative
Repressed mitochodrion
Terminal kinetoplast
Non-Proliferative
density dependent differentiation
BLOODSTREAM
tsetse bloodmeal
TSETSE MIDGUT
SALIVARY GLAND
PROCYCLIC
VSG negative
EP/GPEET positive
Elaborated mitochodrion
Sub-terminal kinetoplast
Proliferative
SLENDER
VSG positive
EP/GPEET negative
Repressed mitochondrion
Terminal kinetoplast
Proliferative
METACYCLIC
VSG positive
Non-proliferative
EPIMASTIGOTE
Attached to salivary gland
via flagellum elaboration
Proliferative

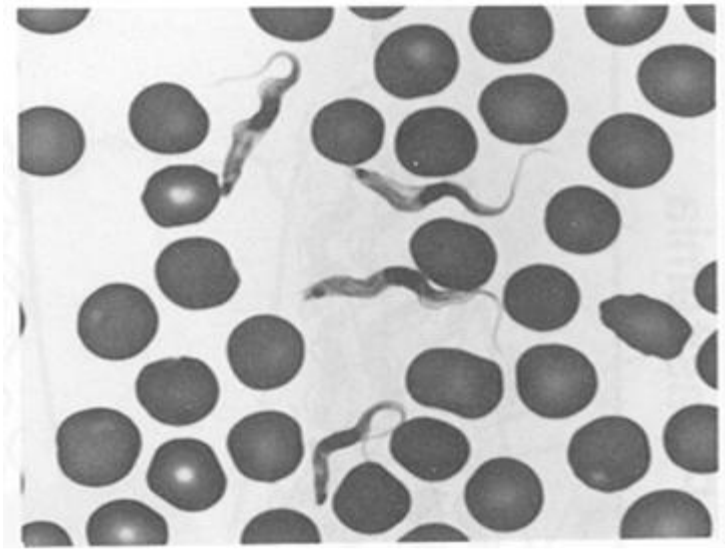
- Life cycle of *T. b. brucei*, *T. congolense* and *T. vivax*

- Heavy outlines indicate parasite forms with surface coats consisting of variable glycoprotein antigens

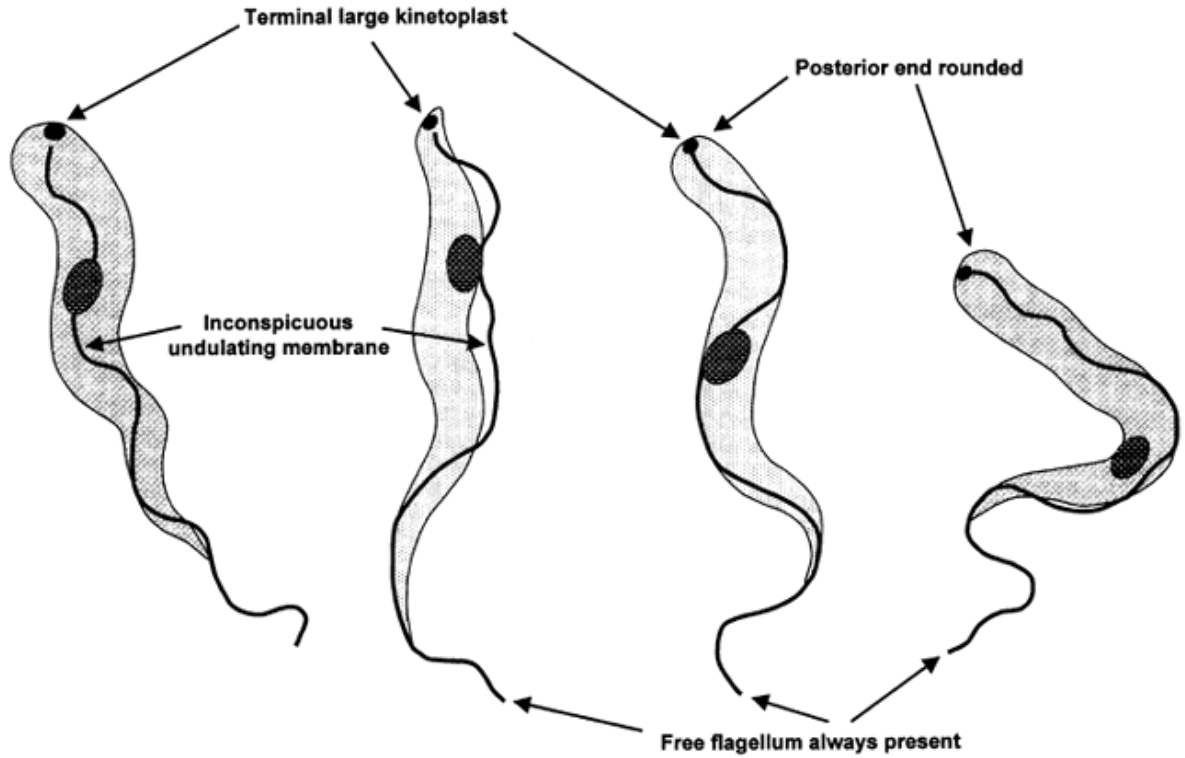
- Light outlines indicate uncoated forms which are not infective to mammals



Trypanosoma vivax



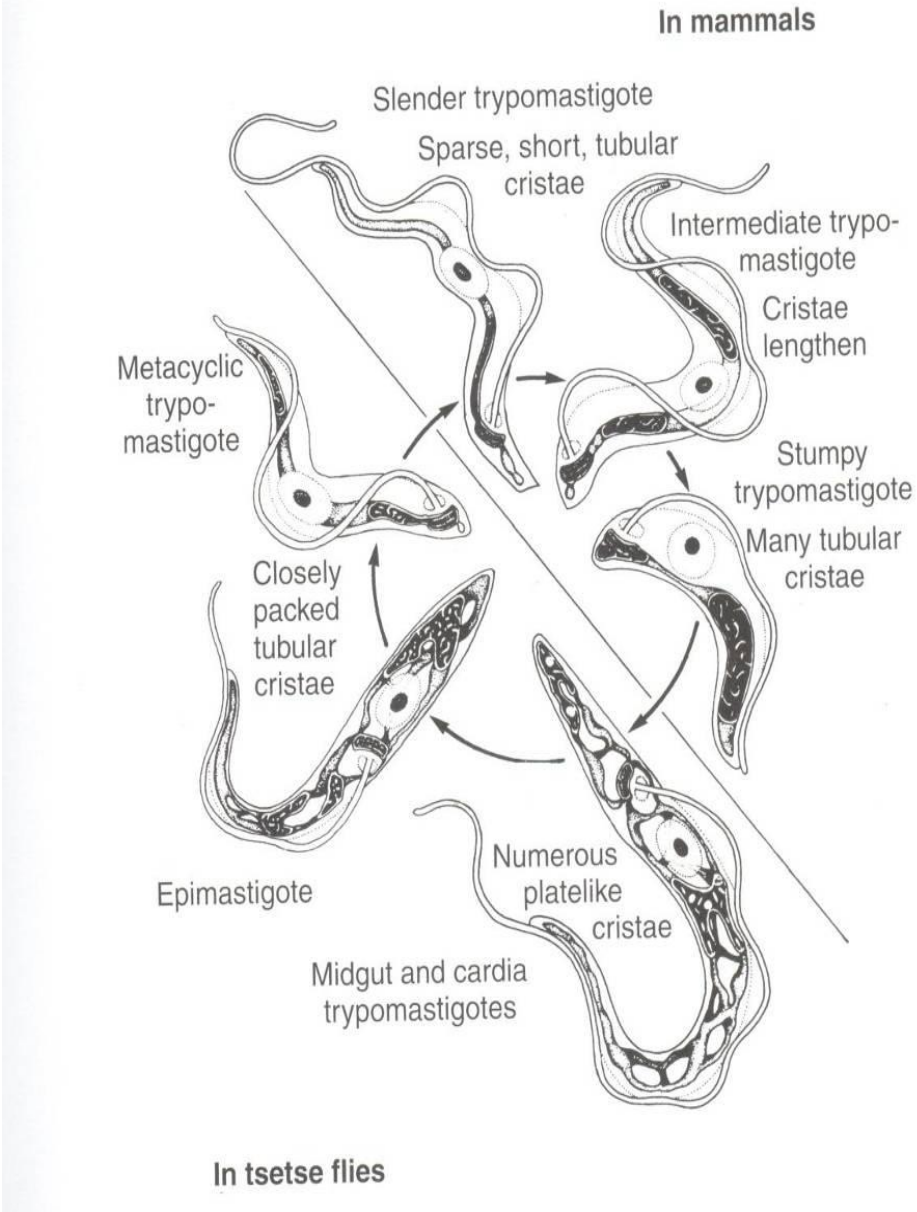
T. vivax develops in the mammalian hosts



T. vivax blood stream forms (FAO, 2006)

Trypanosoma brucei

- **In vertebrate host:**
  - Large + long flagella
  - Stumpy + short flagella
  - Lymorphic trypomastigote forms
- **In tsetse:**
  - Multiplication of trypomastigotes in midgut for 10 days
  - Between day 12 and 20: migration towards proventriculus and salivary glands and transformation in epimastigote forms; multiplication
  - Between day 15 and 35: transformation infective metacyclic trypomastigote forms (stumpy without flagella)



Trypanosoma congolense

- Only transmitted by tsetse flies
- Only found in sub-Saharan Africa
- Animal trypanosomosis only
- **Morphology in mammals:** short with a short flagella
- **In tsetse flies:**
  - Infection of midgut
    - Procyclic trypomastigote forms: multiplication starting on day 10 pi
  - Proventriculus and Proboscis infection
    - From day 20 pi
    - Transformation into epimastigote forms attached to wall
    - Release of infective metatrypanosomes (metacyclic)
- ***Trypanosoma vivax*: the whole developmental stages completed in the proboscis or mouth part of the fly may help in adaptation of mchnical transmission**

Trypanosomes: Immunology

- Antigenic variation: Striking characteristics
  - VSG: variant-specific surface glycoprotein
  - Immunity development against an immunological type (VAT) → reduced parasitaemia → generation of new VSG → increased parasitaemia → Vaccines difficult to develop

- TLF (trypanosome lytic factor): only T. b. gambiense and T. b. rhodesience can infect man (among salivarian tryps)

Cont…

- Trypanosomes among the protozoan parasites they never enter the host's cells
- Yet persist for extended periods of time in mammalian blood and tissues
- In the mammalian host, these parasites are completely surrounded by a dense immunogenic surface coat of a single polypeptide protein referred to as the **variant surface glycoprotein (VSG)** that shields invariant surface antigens from immune recognition

- **Moreover, trypanosomes constantly modify their VSG by the process of antigenic variation, resulting in the fluctuating waves of parasitaemia that characterizes African trypanosomosis**

Cont…

- Trypanosomes contain up to **1000 different genes** in their genome which afford them extensive opportunities to escape host immune responses by displaying new coat antigens

- The parasite has intrinsic mechanisms that ensure only one VSG gene is transcribed at any given time

- By switching VSG genes and expressing a new variant antigenic type, trypanosomes evade B and T-cell mediated immune responses

Cont…

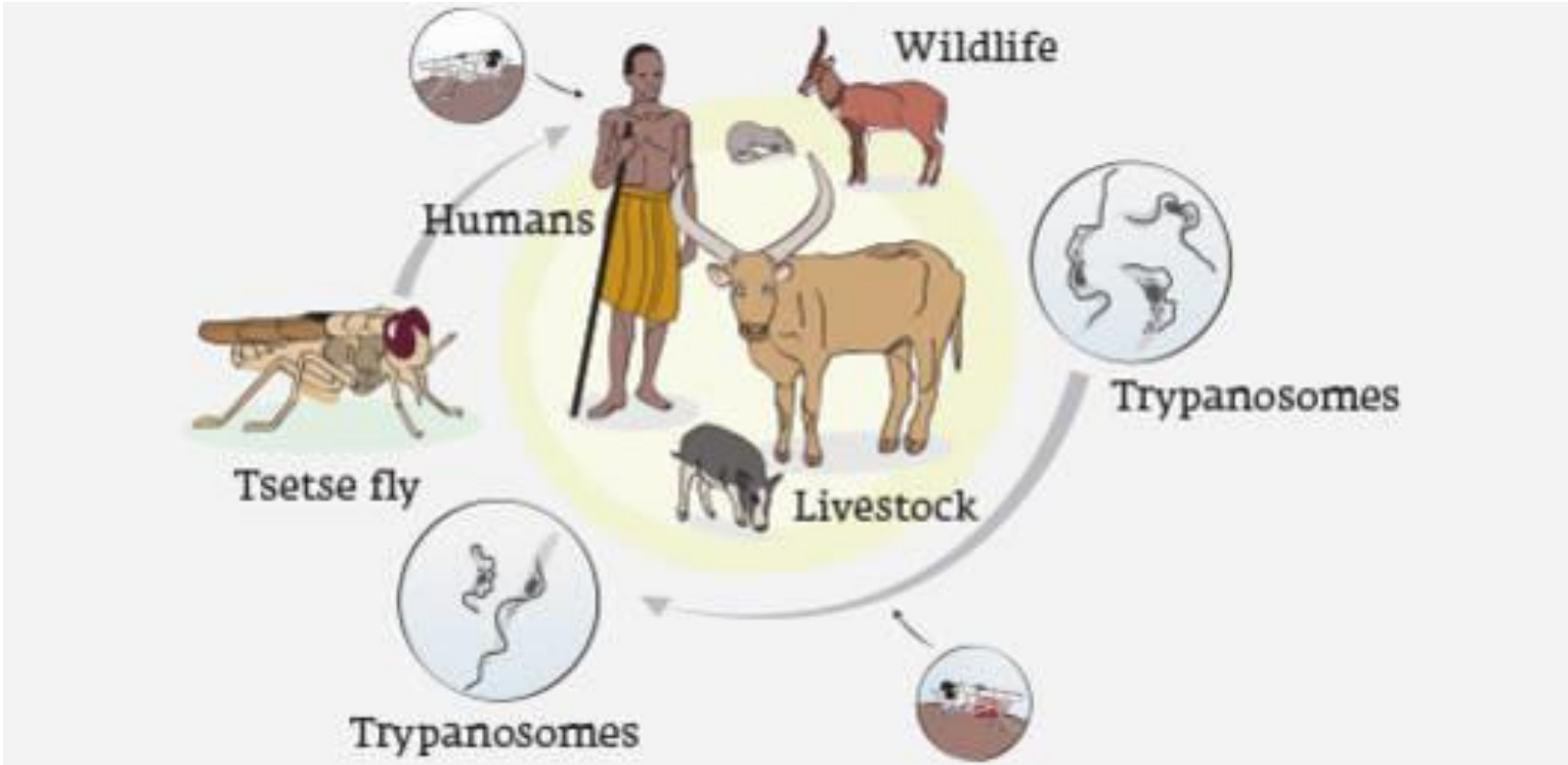
- VSG also has several effects on immune elements such as induction of autoantibodies and cytokines, in particular tumor necrosis factor (TNF)-α
- Other trypanosome components and soluble factors, such as a trypanosome-released triggering factor (TLTF) which triggers interferon (IFN)-γ production by T cells, are also involved in modulation of the immune system

- Because of the strong selection pressure from continuous contact with the host's immune system, the trypanosomes seem to have also developed several ways of evading immune killing through alteration of the host's natural and adaptive immune responses

Trypanosomes: Epidemiology

- Reservoir: wildlife, domestic or man
- Important role of tsetse flies
  - Climate and season
  - Land clearing
  - Increased susceptibility of starved or dehydrated flies

- Sensitivity of different species and breeds:
  - Wildlife
  - Trypanotolerant cattle
  - Endemic stability


Wildlife
Humans
Trypanosomes
Tsetse fly
Livestock
Trypanosomes

Trypanosomes: Pathogenicity

- Single bite: inoculation of several thousands of metacyclic trypomastigotes:

1. Chancre - skin lesion at inoculation site
2. Lymphadenopathy - host immune responses, immunosuppression
3. Anaemia - haematological changes
4. Biochemical changes - glucose, cholesterol, protein, enzymes etc.
5. Tissue damage - gross and histopathological changes in major organs: liver, heart, kidney, lung, brain

Influence of host genetics on course of disease

Genetically distinct cattle breeds suffer differing degrees of disease

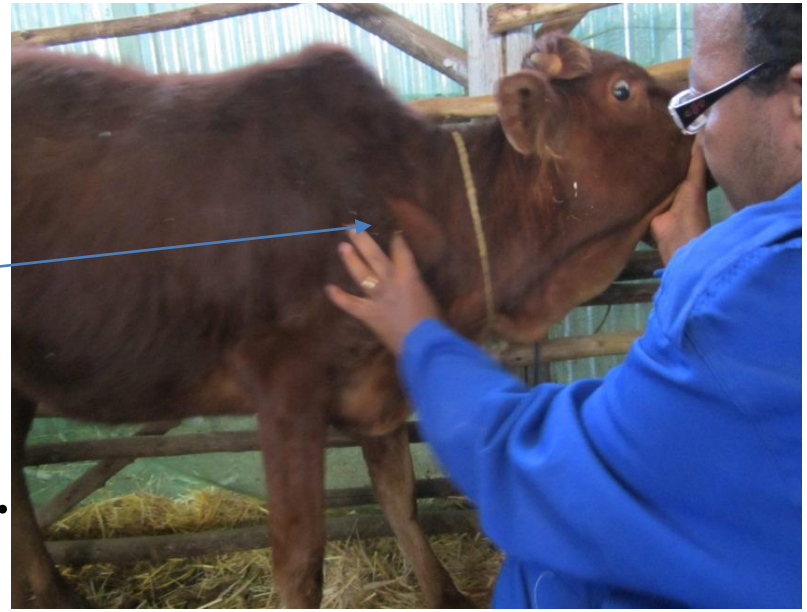
***Bos indicus* Boran**
Susceptible

***Bos taurus* N'Dama**
Tolerant

Cont…

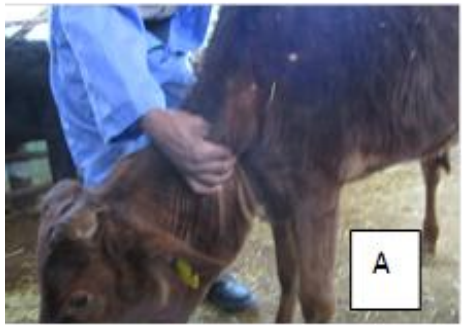
Clinical findings:

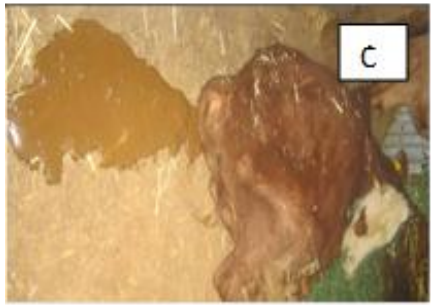
- Fever
- Parasitaemia
- Reduced feed intake
- Enlarged lymph nodes
- Rough hair coat
- Emaciation
- Diarrhea
- Oedema
- Pallor mucus membrane
- Lethargy
- Recumbence



Cont…

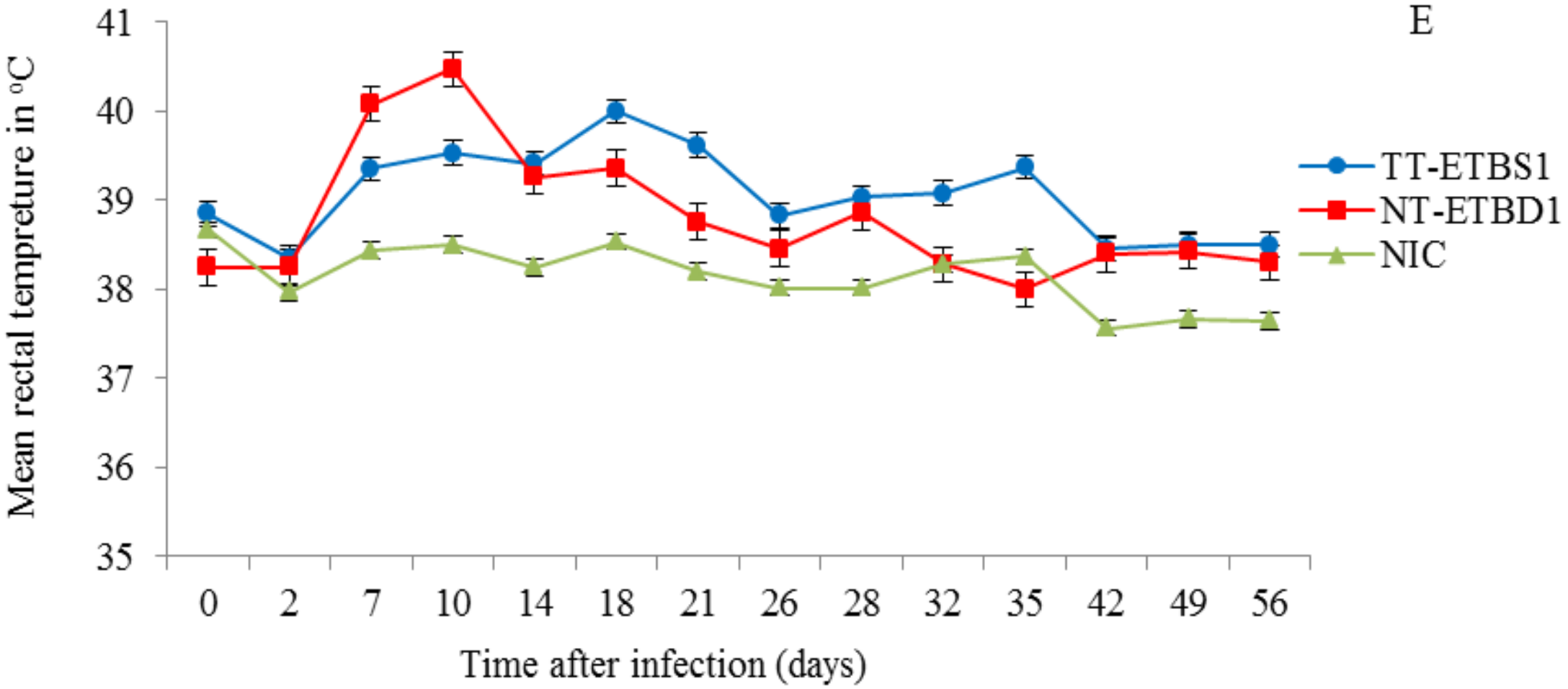
Major clinical findings in infected cattle (A-D) compared to control animals (E)



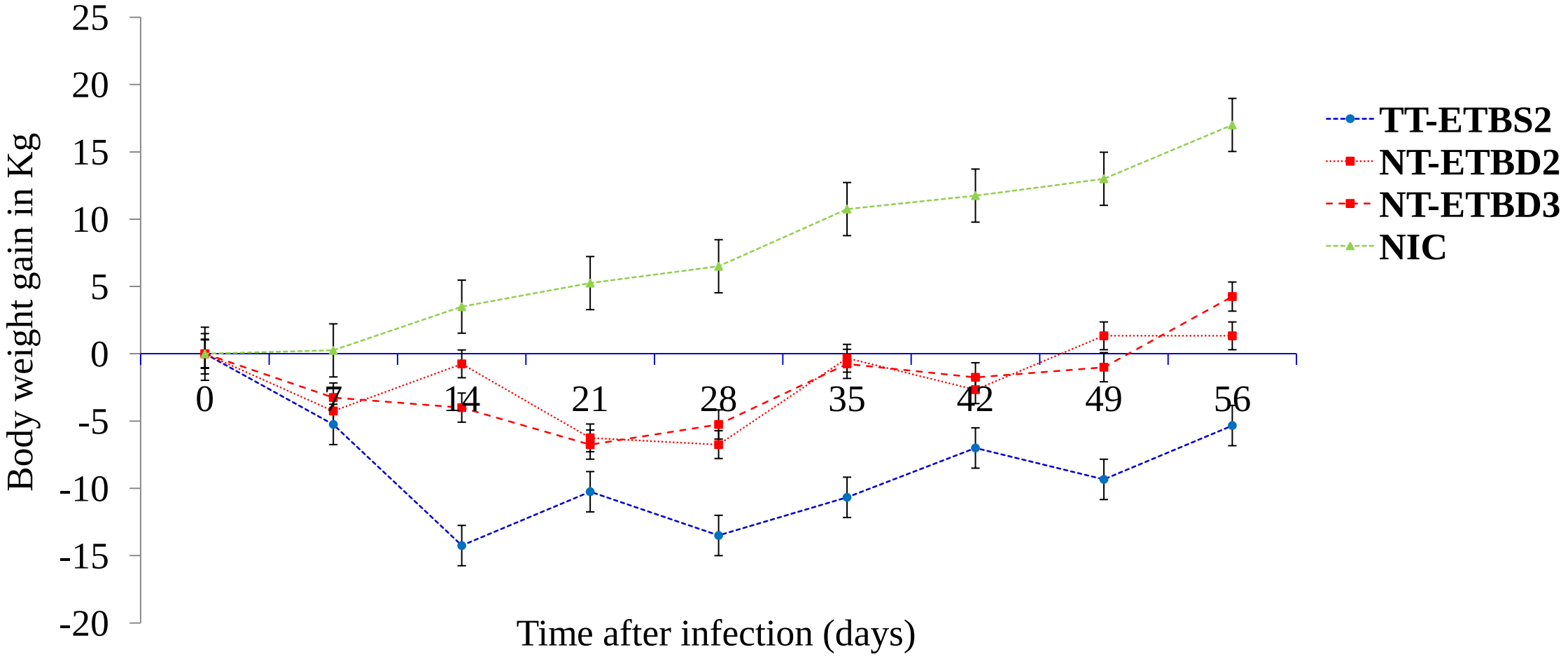


Clinical manifestations were obvious in all *T. vivax* infected animals

Rectal temprature in experimental infected cattle with T. vivax

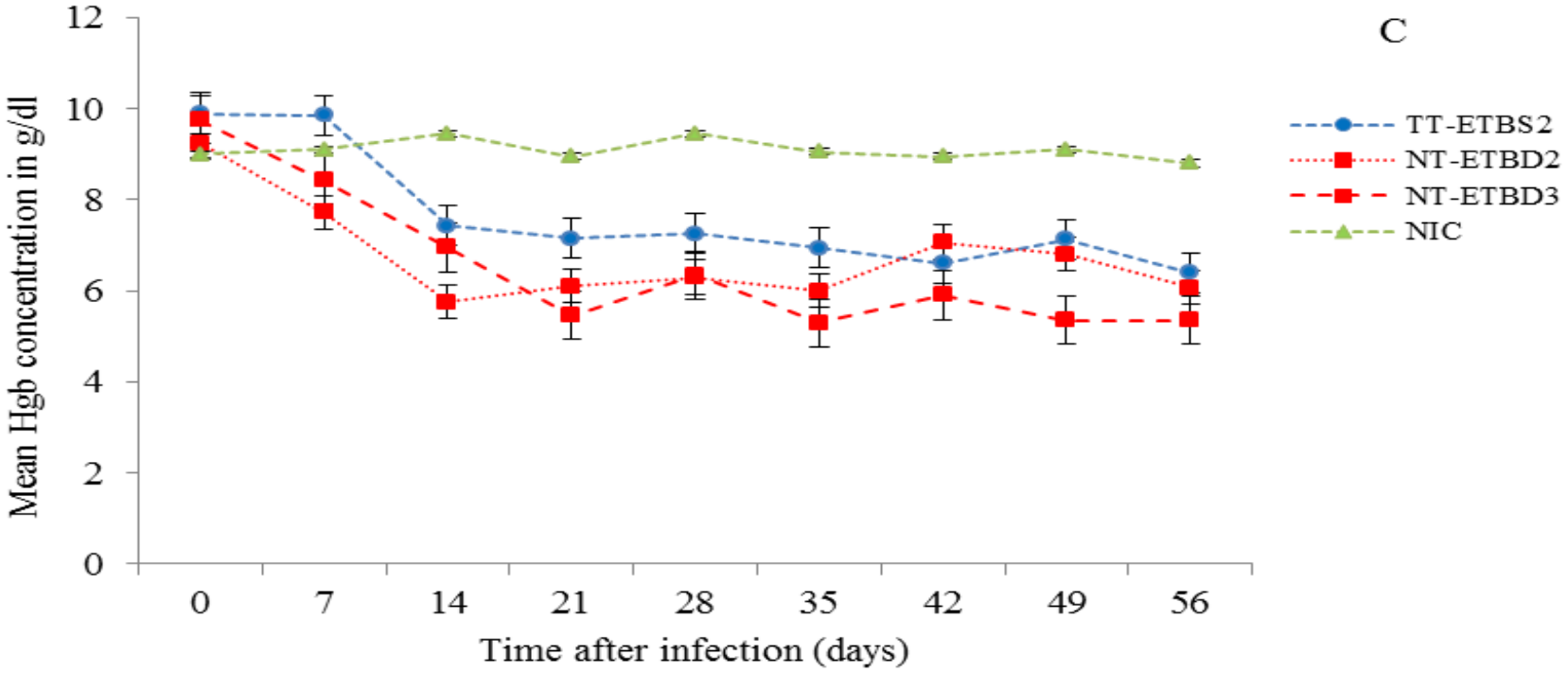


Body weight gain in experimental infected cattle with T. vivax

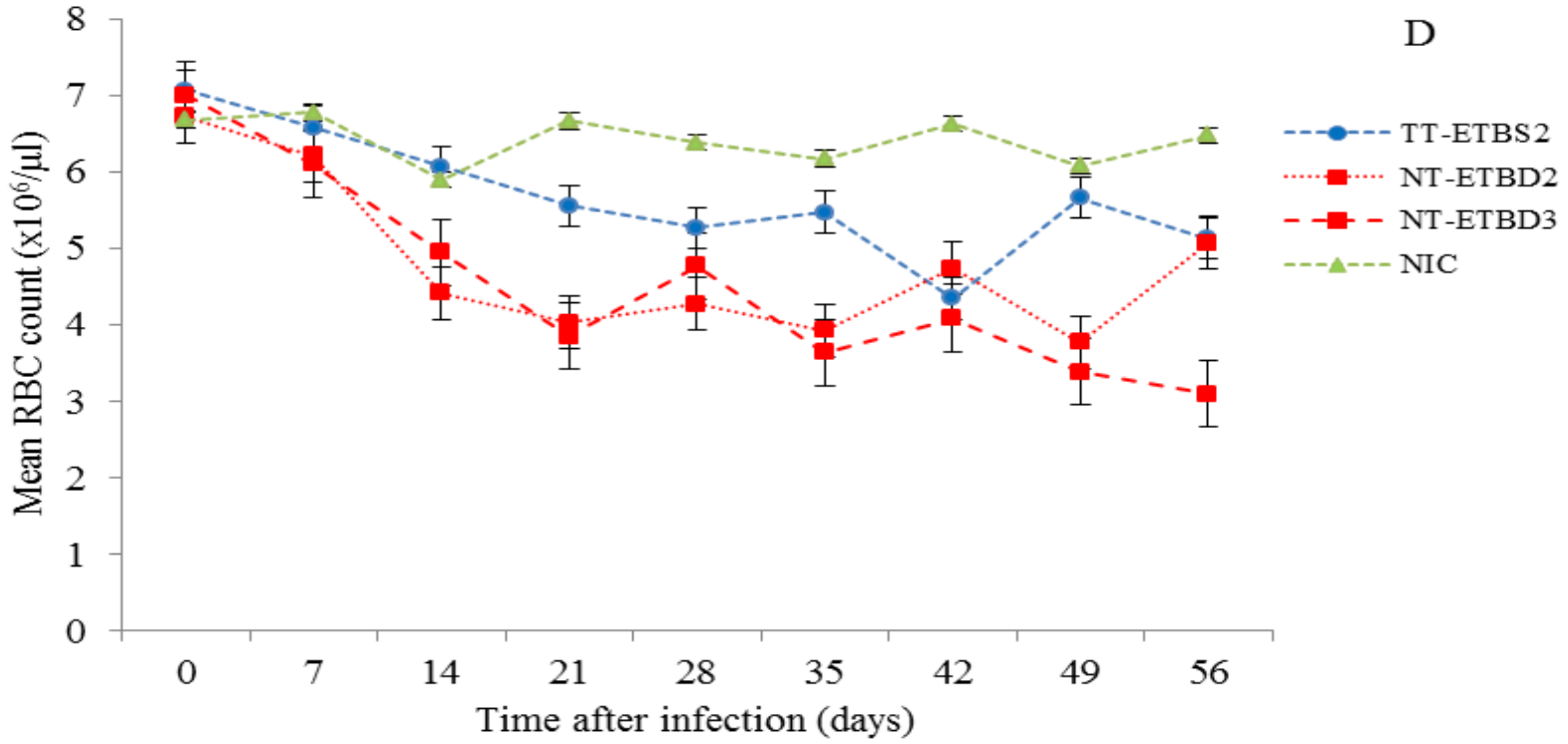


Body weight gain: significant loss of body weigh infected groups

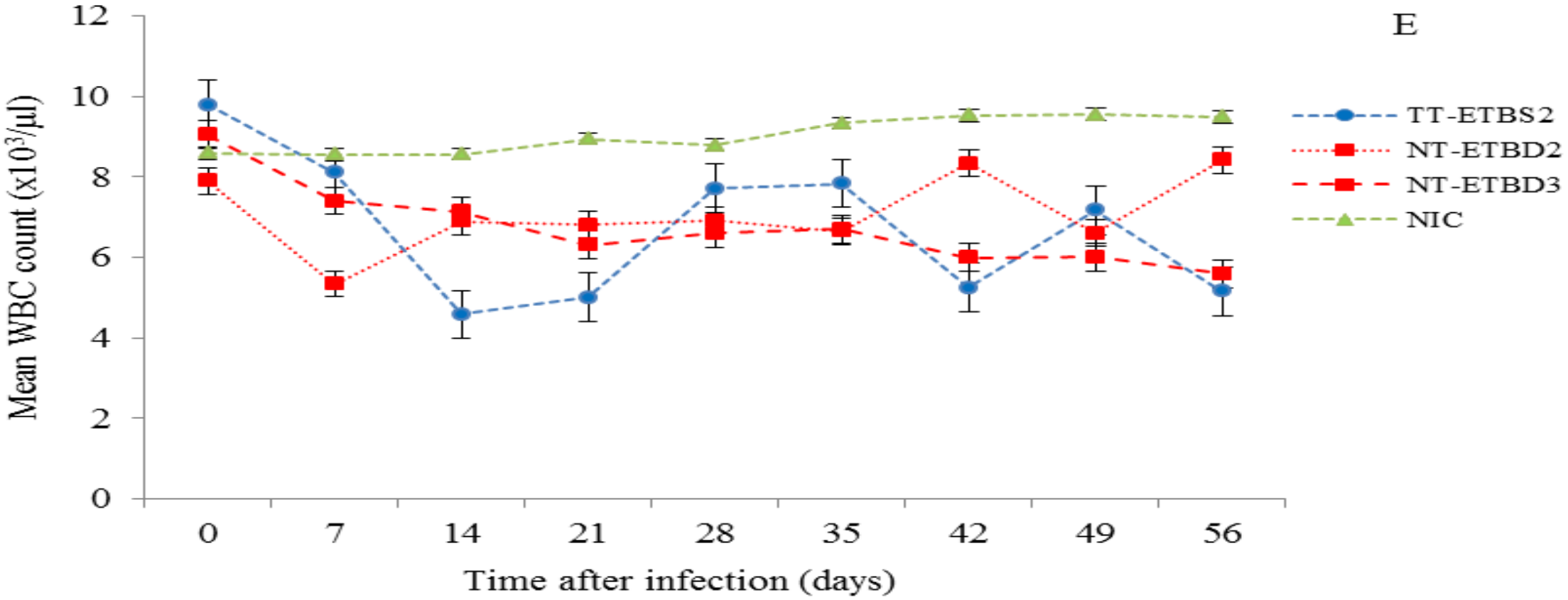
Mean Hgb conc. in experimental infected cattle with T. vivax



Mean RBC count in experimental infected cattle with T. vivax

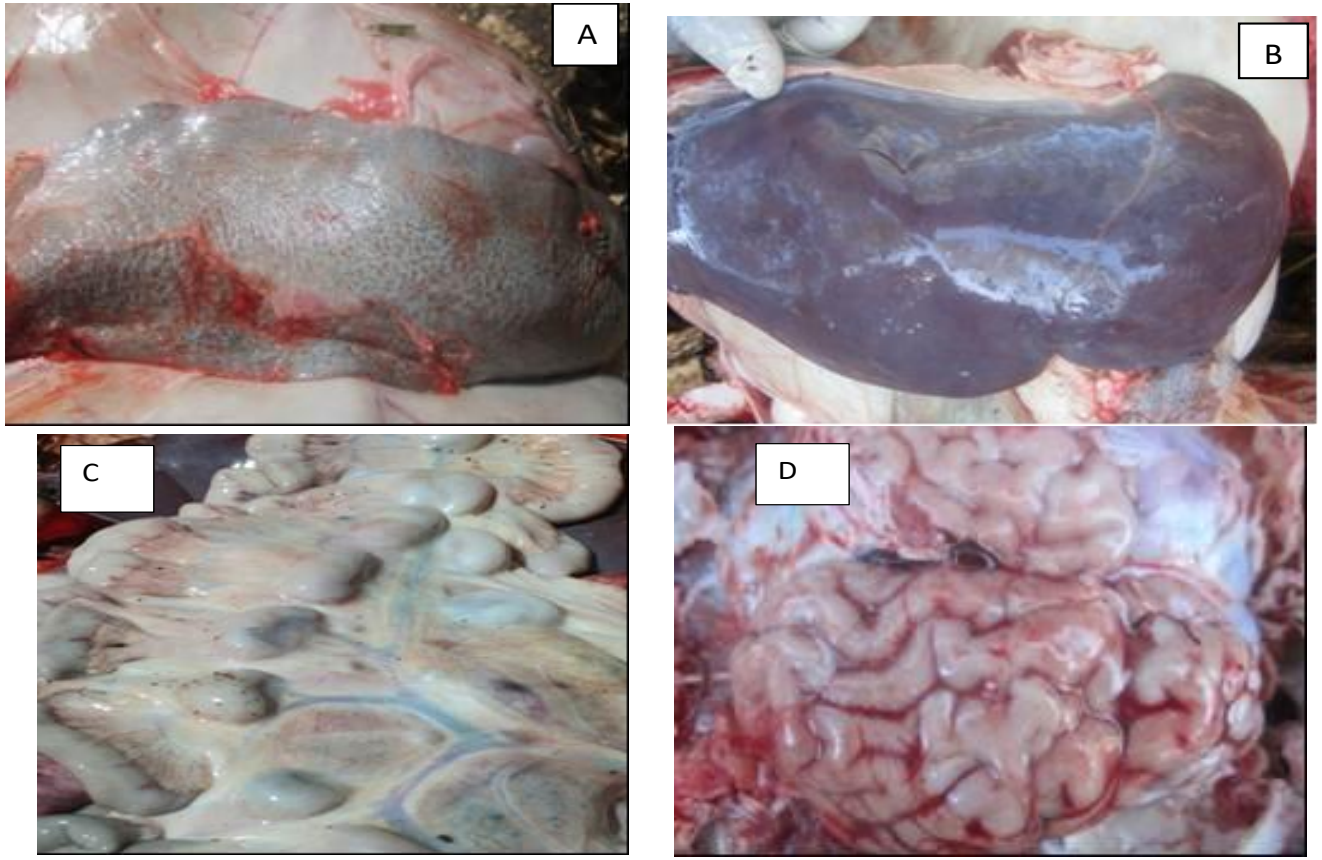


Mean WBC count in experimental infected cattle with T. vivax



Cont…

Gross pathological findings in infected cattle: pathological lesions were found in all organs examined

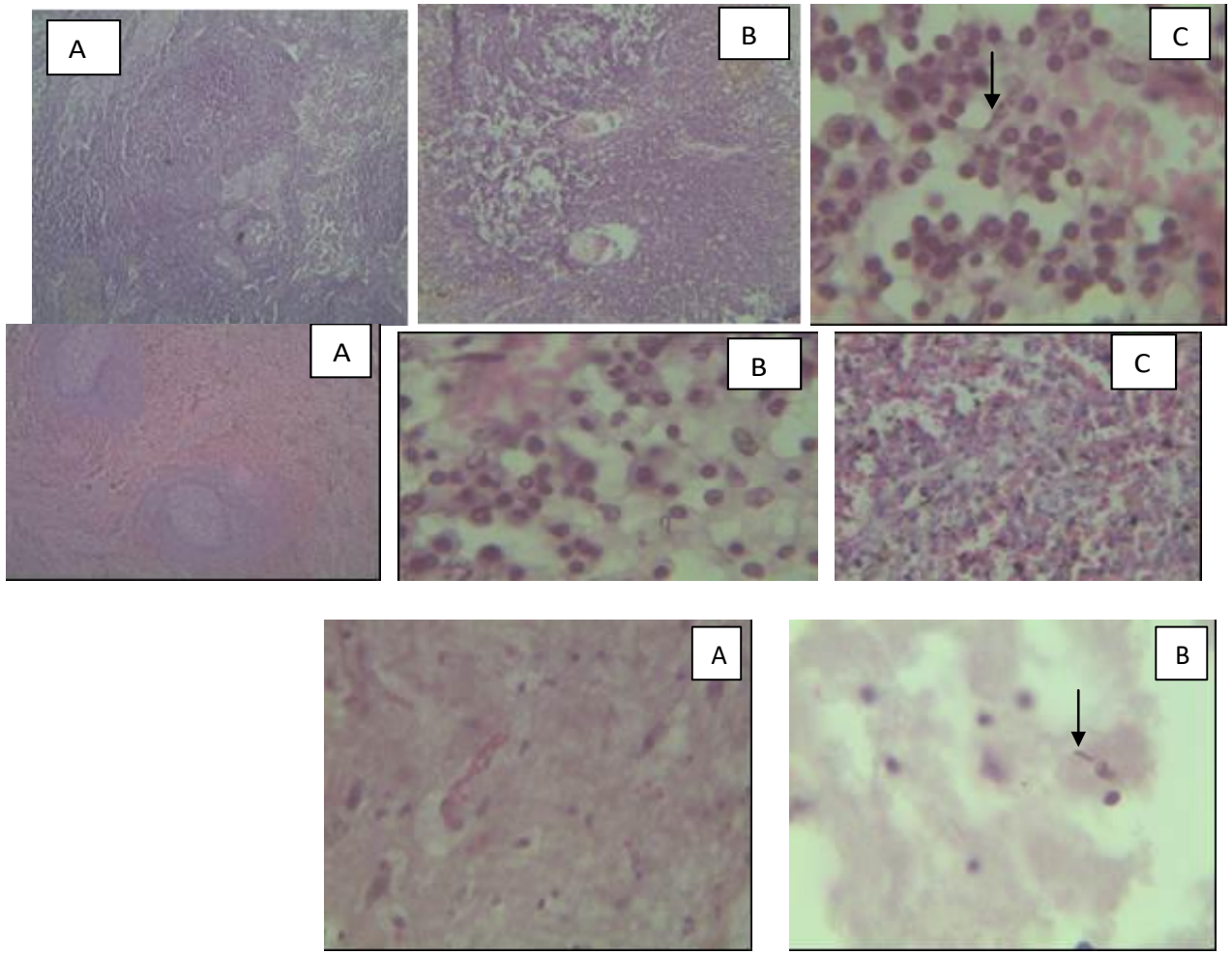


A: Spleen
B: Liver

C: Mesenteric lymph node
D: Brain

Cont…

Histopathological findings in infected cattle

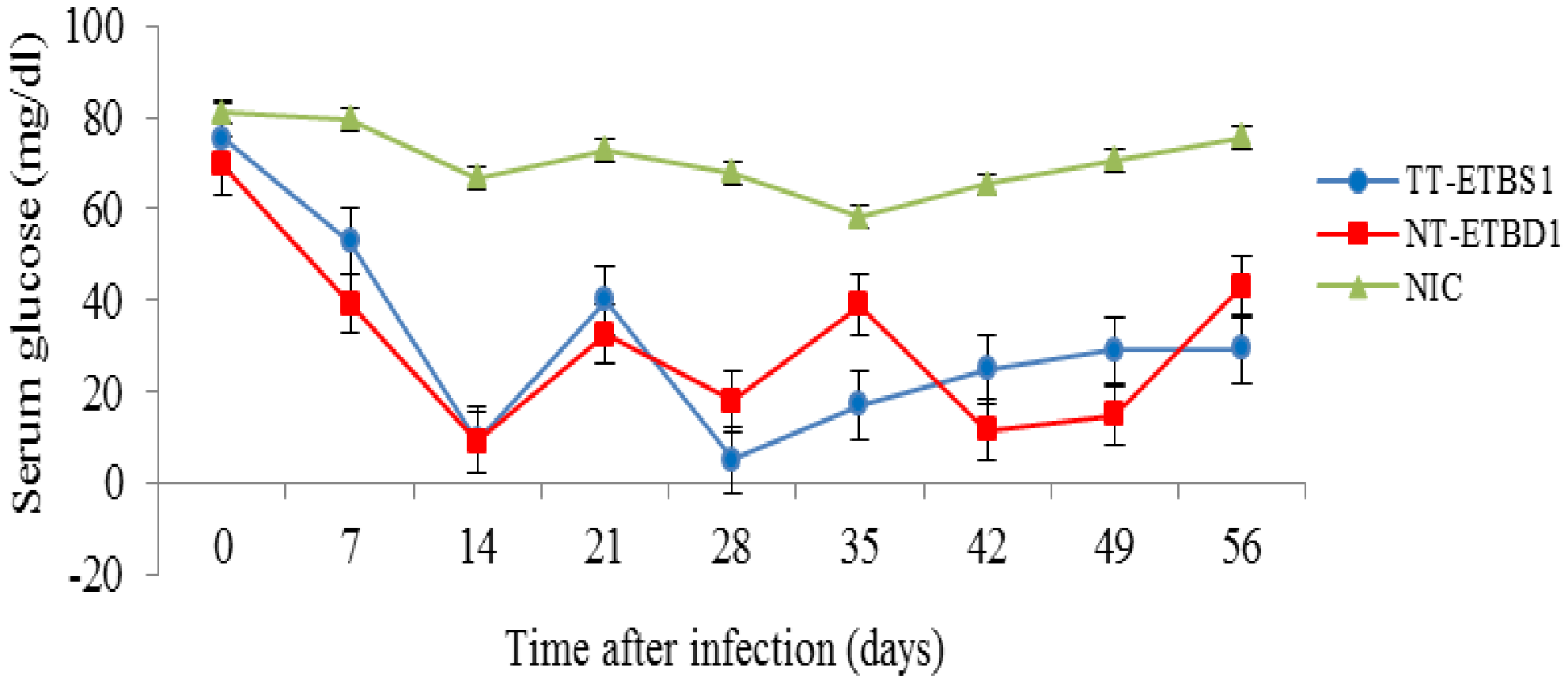


Lymph node:
Lymphatic follicle
Lymphoid cords
Infiltration
Parasite

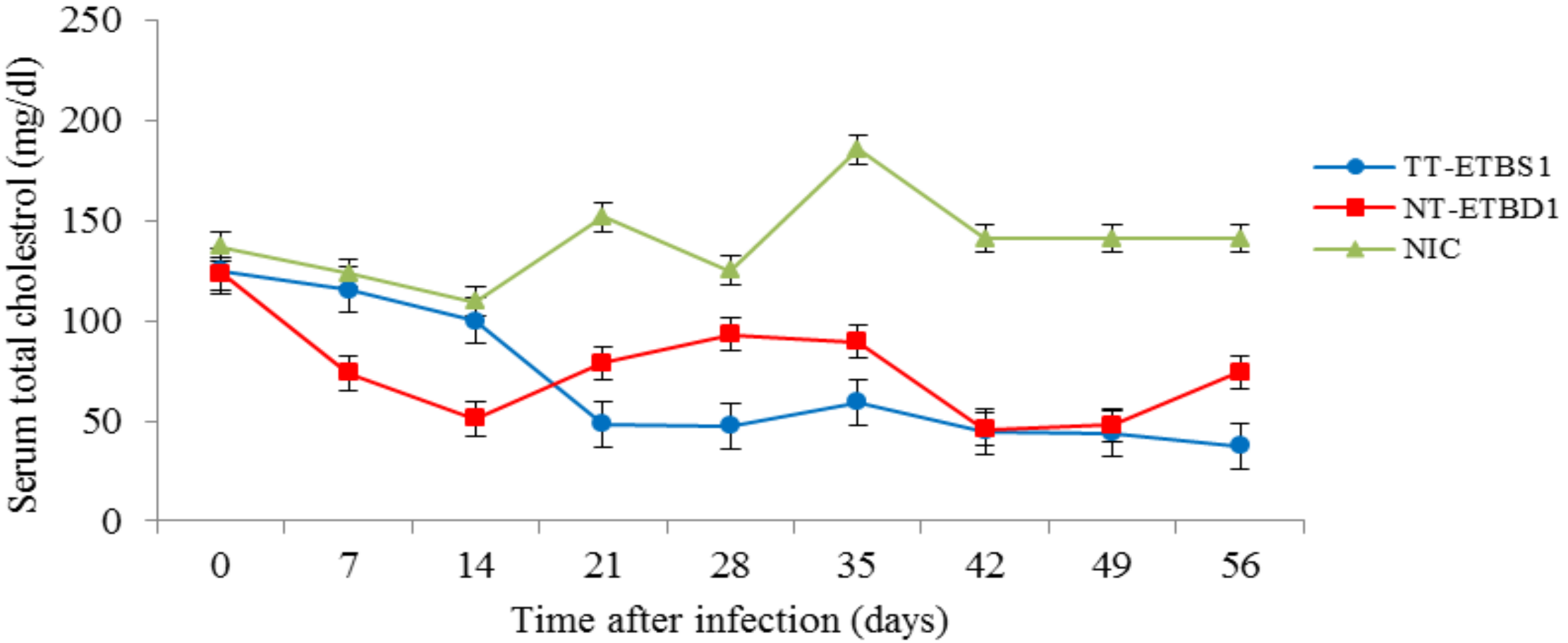
Spleen:
Haemosiderosis
Erythrophagocytosis
Infiltration

Brain:
Haemorrhagic
Oedema
Parasite

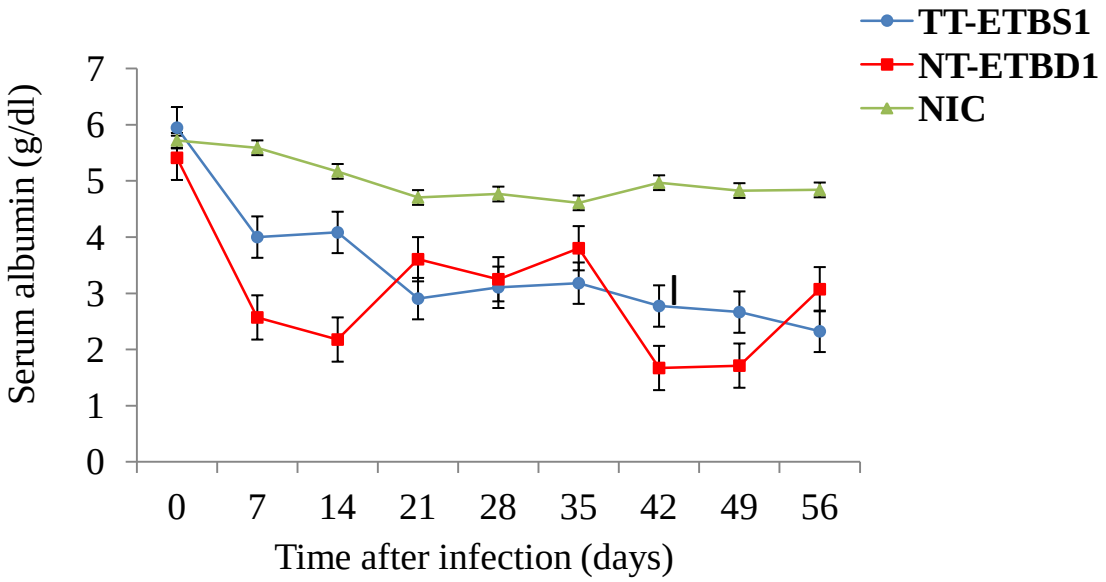
Mean glucose level in experimental infected cattle with T. vivax

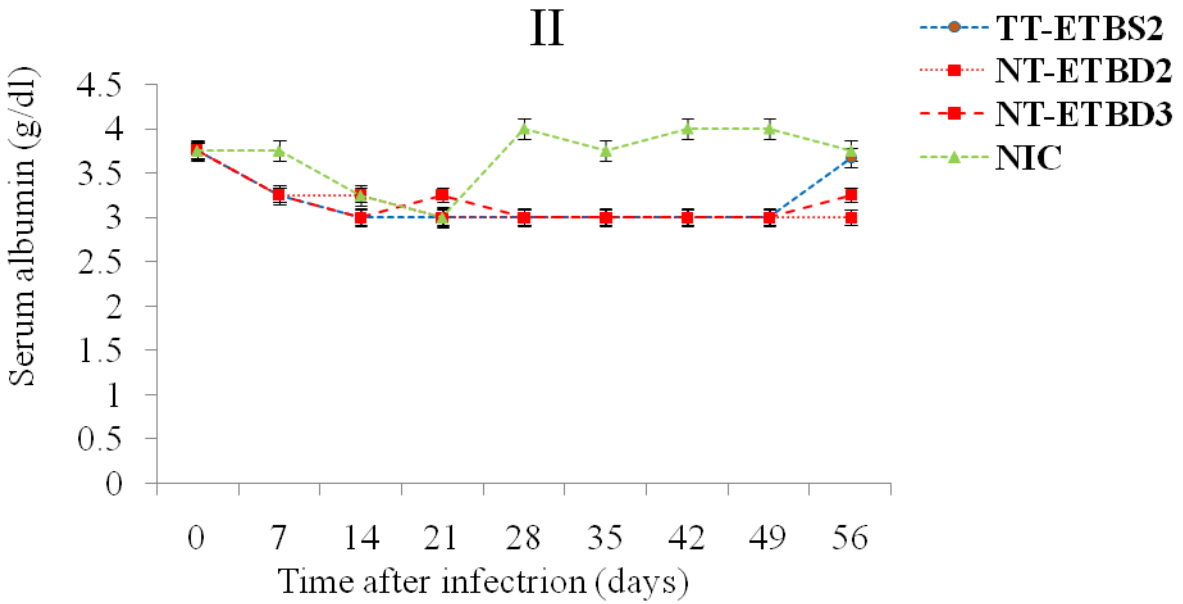


Mean serum cholestrol level in experimental infected cattle with T. vivax



Mean serum Albumin level in experimental infected cattle with T. vivax

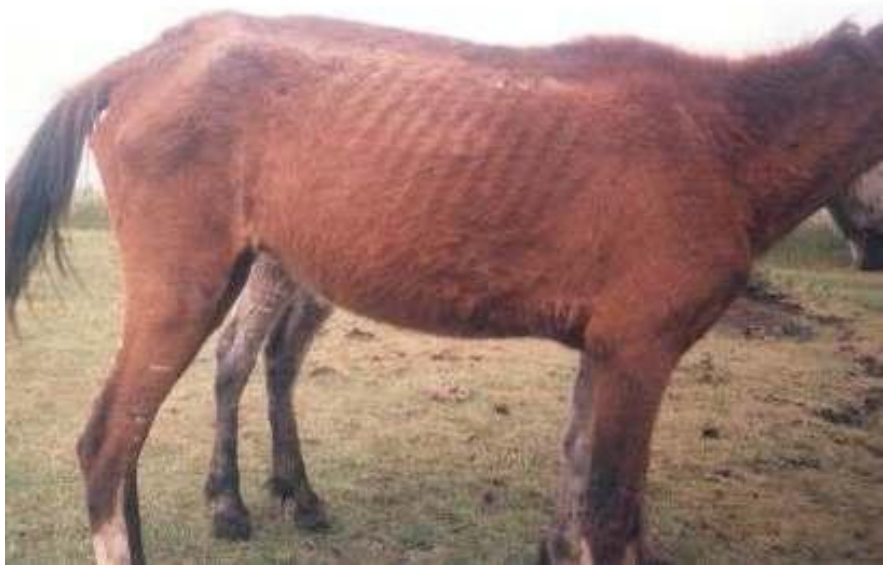




Mean serum albumin changes in cattle infected with *T. vivax:*
Significant drop in serum albumin between infected (I) as well as control groups

Figure 3.2.1. Selected serologically positive clinical cases of dourine in horses from the Arsi-Bale highlands.
A. Photo showing oedematous swelling over the ventral abdomen and penis.
B. Photo showing depigmentation over the external genitalia.
C. Photo showing depigmentation over the external genitalia and udder.
D. Photo showing poor body condition.

Trypanosoma equiperdum



Diagnosis

Vector identification

- Identification key
- Genera level
- Species level necessary for some vector-borne diseases
- Molecular tools
  - Identification
  - Genetic studies

Cont…

Microscopy

- Confirmation of clinical diagnosis
- Species identification sometimes difficult

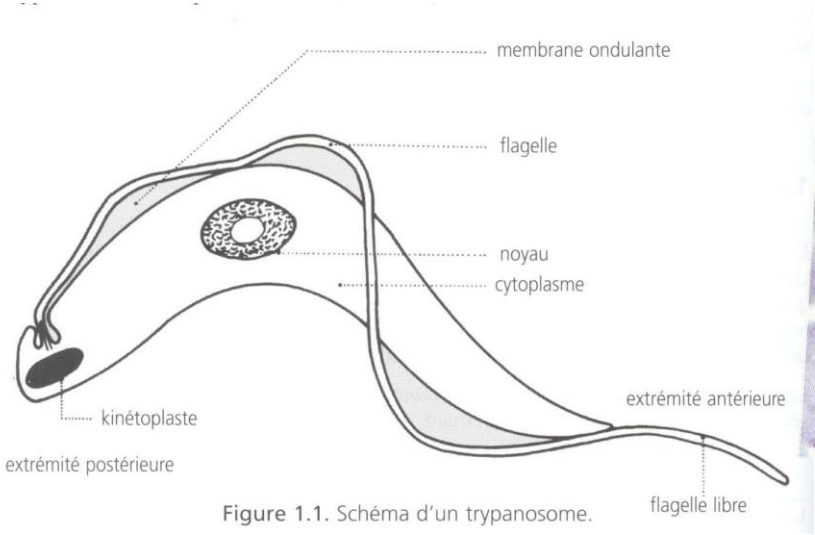
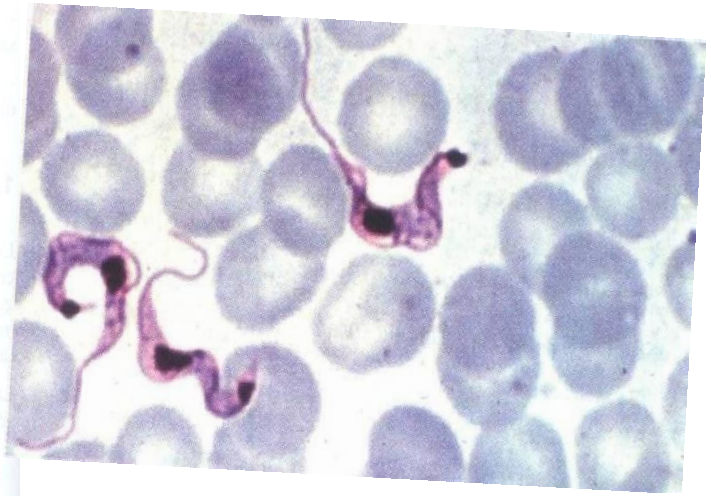
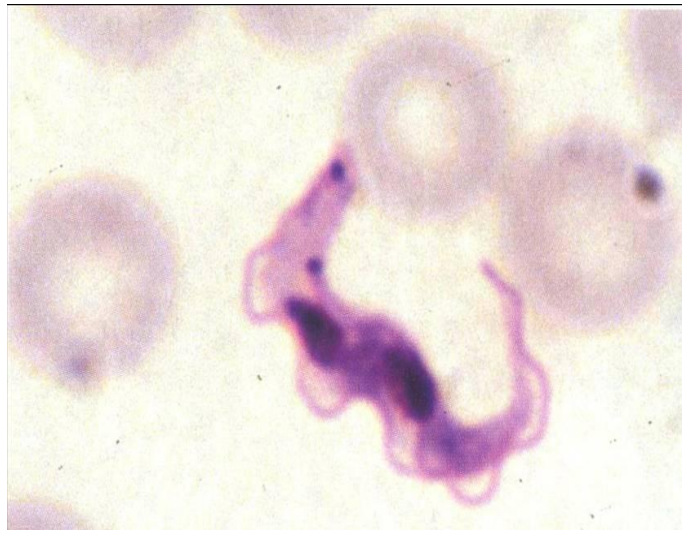
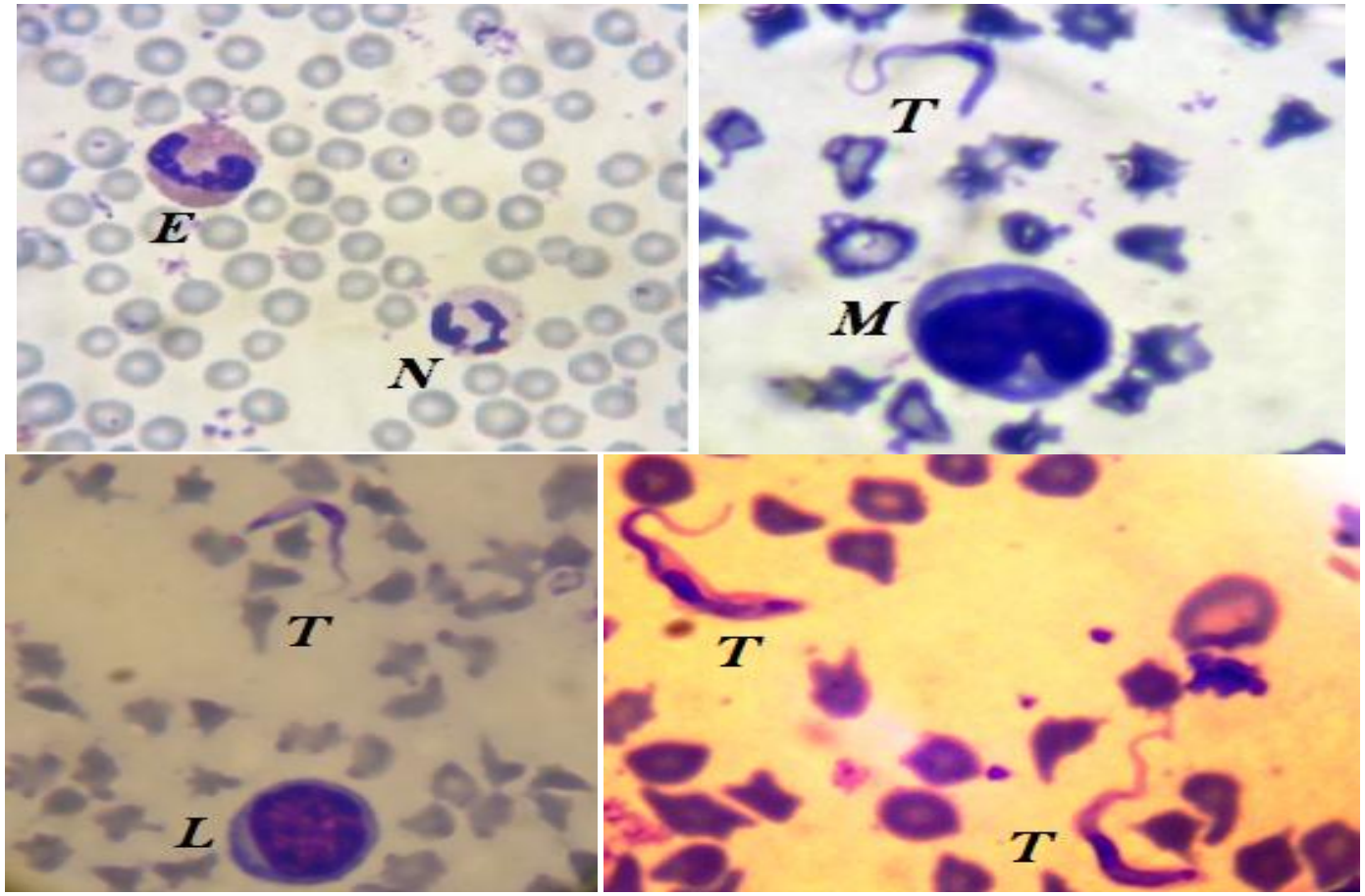


Figure 1.1. Schéma d'un trypanosome.

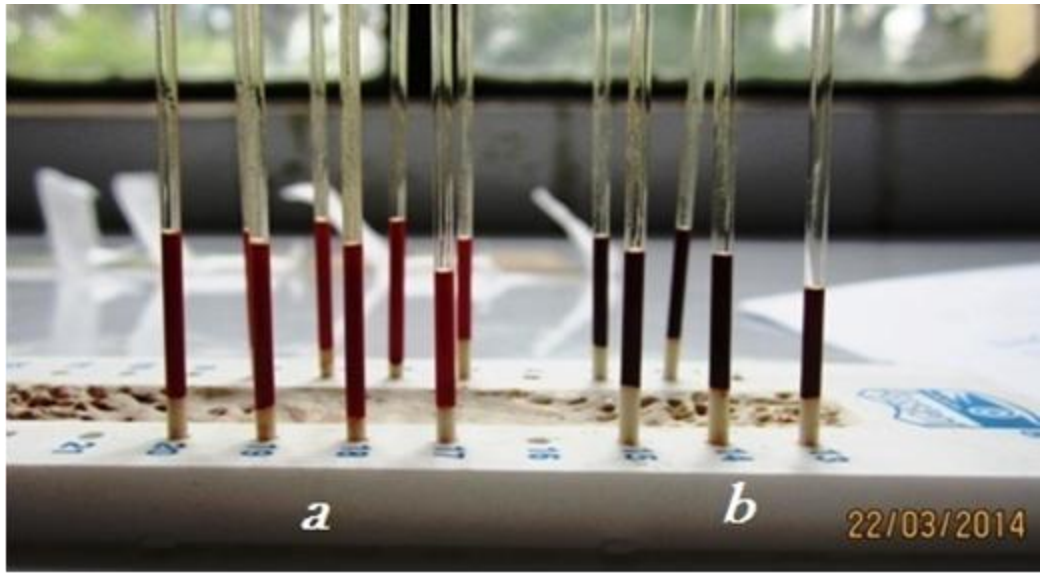




Different WBC and *T. vivax* parasite in Giemsa stained thin smear (E: eosinophil, N: neutrophil, M: monocyte, L: lymphocyte, T: *T. vivax)*



Hematocrit of non-infected control (a) and *T. vivax* infected (b) animals: Concentration technique



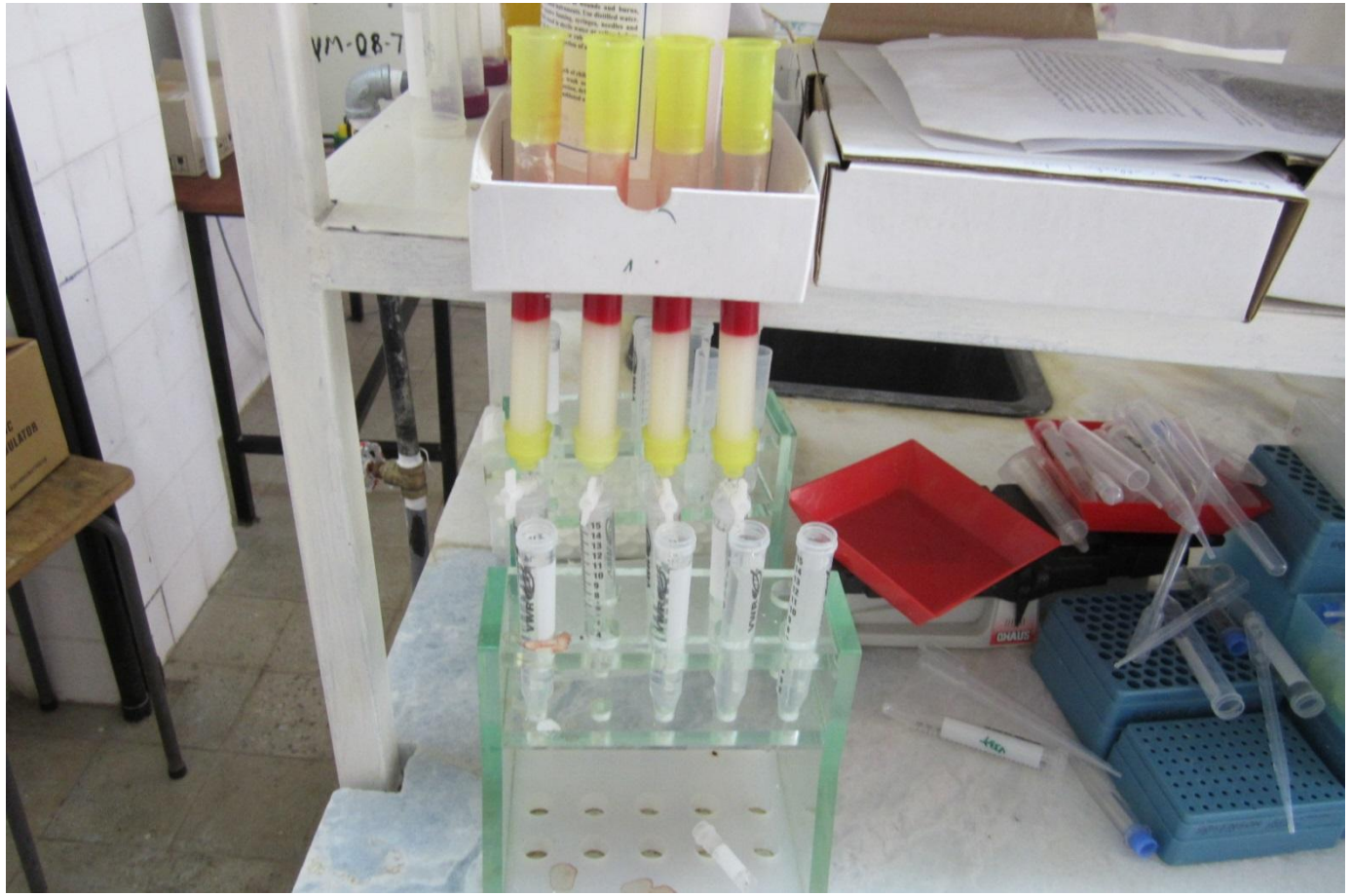
mini Anion Exchange Centrifugation Technique (mAECT): Concentration technique

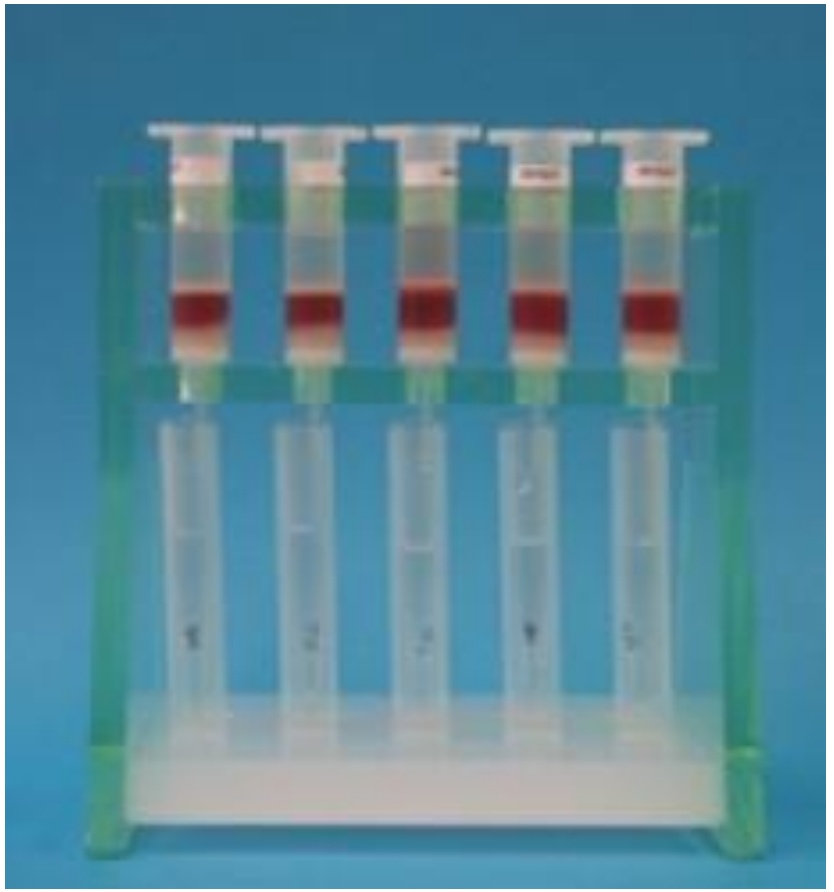
The mAECT method is carried out in two stages: chromatography then concentration and viewing

1. The parasites are separated from venous blood in a gel column by anion exchange chromatography and collected in a sealed glass tube

2. After parasites are collected in the glass tube, it is centrifuged and the tip examined by microscopy

Cont…





Cont…

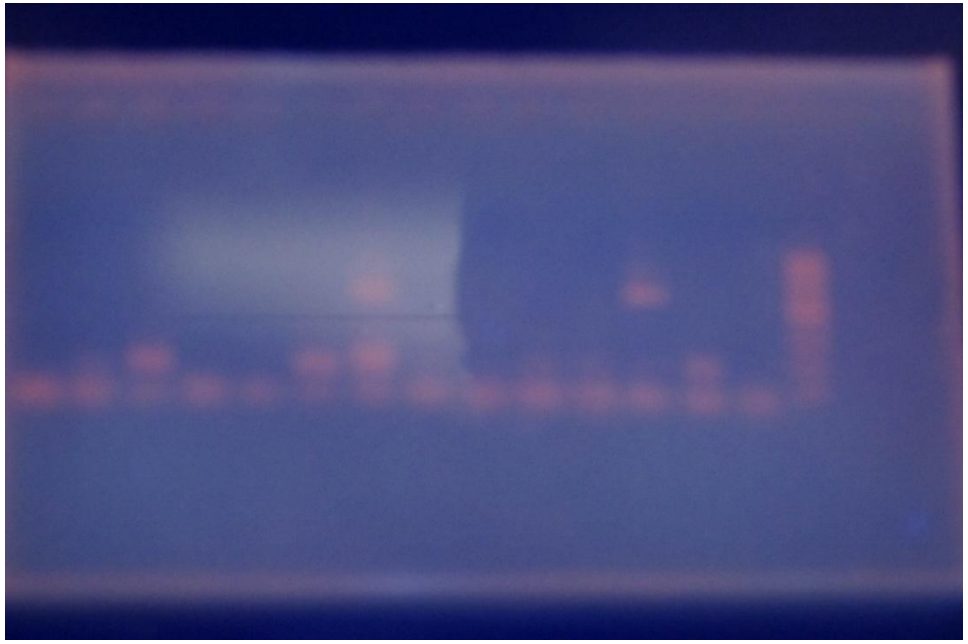
Serological tests

- IFAT
- ELISA
- No species identification
- Sensitivity and specificity (test characteristics)
- Epidemiological studies
- **CAT**

Cont…

Molecular tools

- High specificity
- High sensitivity for clinical cases
- Detection of carriers?



Cont…

Infection detection in vectors

- Dissection
  - Tsetse: midgut, salivary glands or mouth parts
  - Other biting flies: mouth parts

- Molecular tools (pooling)
- Xenodiagnosis

Control of Trypanosomosis

- **Vector control:**
  - Insecticide
  - Sterile insect technique
  - Traps etc.

- **Parasite control using trypanocidal drugs:**
  - Chemotherapy
  - Chemoprophylaxis

Challenge: Trypanocidal drug resistance

Cont…

Control of tsetse fly

- Traps
- Odor-baited and insecticide impregnated screens
- Pour-on
- Aerial and manual spraying
- Land clearing

PATTEC

SIT: Sterile Insect Technique

Zanzibar

STEP in Ethiopia

Cont…

Parasite control

- **Chemotherapy in animals**
  - Diminazene and isometamidium
  - Severe side effects in man
  - Curative or prophylactic treatment
  - **Development of resistance**

- **In man**
  - Arsenical derivatives

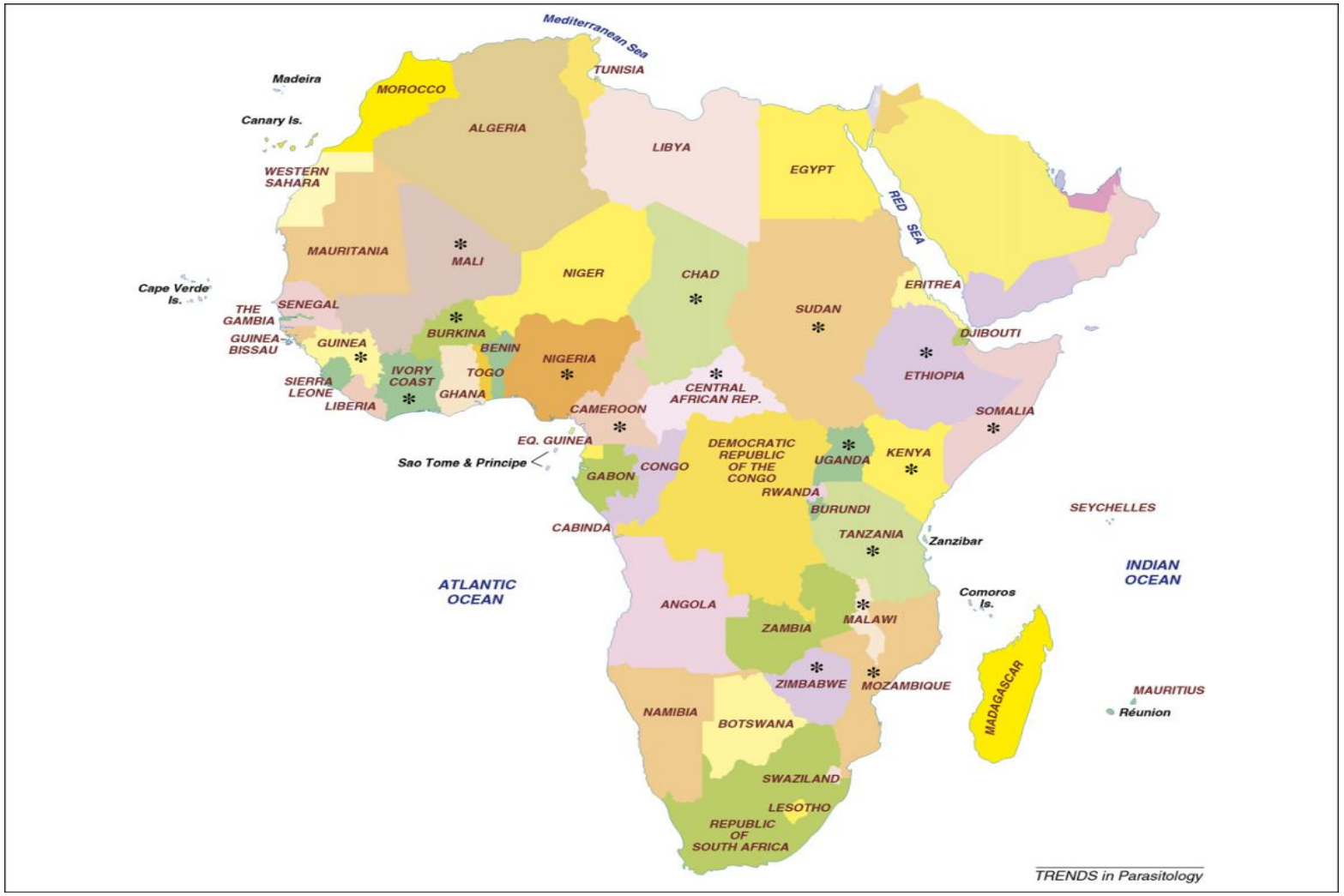
Status of trypanocidal drug resistance

- Drug resistance is defined as a loss of sensitivity by a strain of an organism to a compound to which it had been previously susceptible

- The problems of drug resistance have been reported from 17 countries in sub-Saharan Africa including Ethiopia as shown in the following figure

- The control of New World *T. vivax* relies heavily on drug therapy and resistance to diminazene aceturate has been reported

African countries with reported resistance to trypanocidal drugs



A star indicates that resistance to trypanocidal drugs has been reported in animal trypanosomes in that country

Questions

- Antigenic variation in trypanosmes?
- Trypanotolerance vs trypanoresistance?
- The role of vectors in the epidemiolgy of tryps?
- The futrue of trypanosmosis (cyclical vs mechanical): perspectives?
- Is tryps neglected or non-neglected disease?

Future Research Areas

1. Vaccines against trypanosomes
2. Vaccines against fly infestation
3. Make use of trypanotolerant breeds and to fly infestations resistant animals

Leishmaniasis

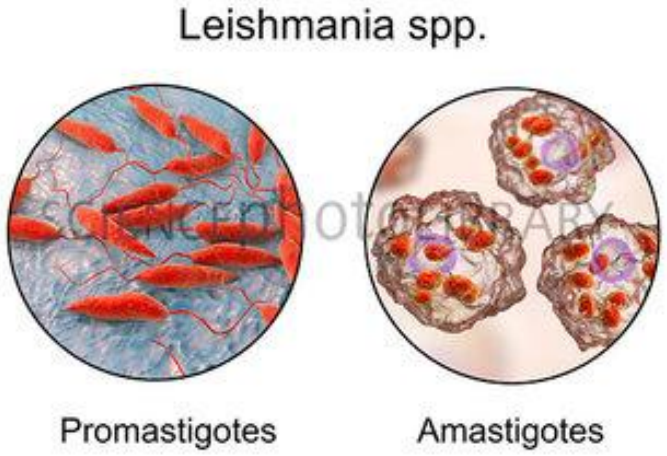
- Leishmaniasis is a severe, widespread zoonotic disease that occurs worldwide
- Leishmania is primarily a parasite of rodents, carnivores, marsupials, edentates, insectivores, and secondarily of dogs and humans
- Severe disease develops in the man and in the dog, which is characterized either by skin lesions or a general visceral involvement
- The dog is the reservoir for visceral leishmaniasis
- The disease is transmitted by the blood sucking sand fly (genus *Phlebotomus* in the Old World and genus *Lutzomya* in the New World)
- The disease is caused by several species of protozoa in the genus *Leishmania*.

Cont…

Etiology

- Visceral forms of Leishmania are caused by:
  - *L. donovani*
  - *L. infantum* in the Old World
  - *L. chagasi* in the New World
- Leishmaniasis is a group of infections caused by protozoan of the genus *Leishmania*
- In nature all existing species of *Leishmania* are transmitted to humans and other mammals by the bite of infected female sandflies
- In the Americas, human leishmaniasis can be divided in two broad categories:
- Cutaneous
- Visceral leishmaniasis

Cont… Morphology of Leishmania



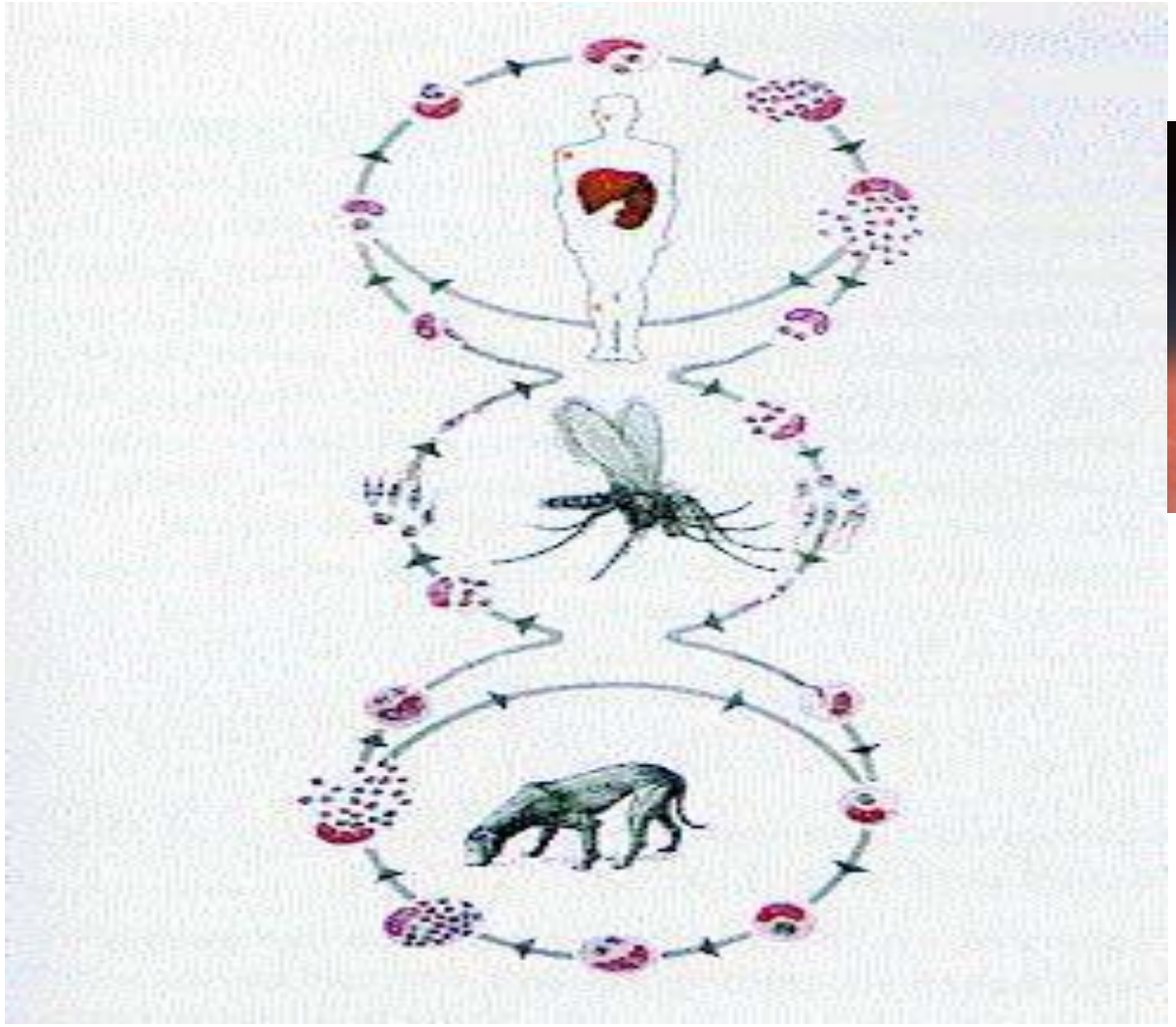
Cont…

- In the mammalian host, *Leishmania* is an obligate intracellular parasite and exists in the amastigote form inside cells of the mononuclear phagocytic system
- The amastigote forms are characterized as circular, about 5 microns in diameter, having a nucleus, kinetoplast and rudimentary flagellum
- It multiplies by binary fission, repeatedly until the host cell is destroyed
- Inside the gastrointestinal tract of the sand flies, the amastigote transforms into promastigotes and promastigotes-elongated, flagellated, motile forms, which have a central nucleus and terminal kinetoplast
- The transmission of the disease to vertebrate hosts occurs predominantly by inoculation of the infective promastigotes form during a bite of the sand fly.

Cont…

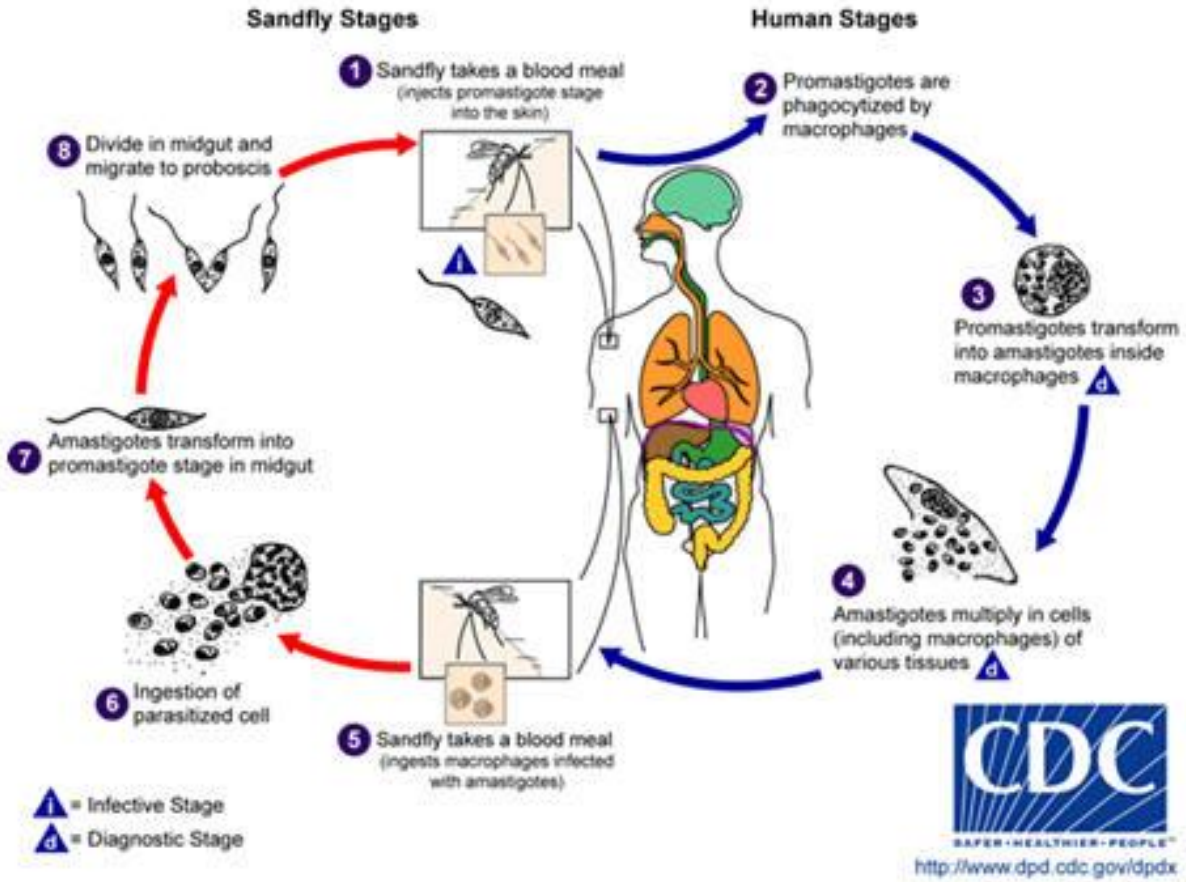
- However, other possible routes included direct contact, transplacental, venereal and blood transfusions
- After inoculation in mammalian hosts, the infective promastigote attaches to macrophages through different cellular receptors, and it is then localized into a vacuole which fuses with lysosomes
- The parasite survives phagocytosis and undergoes different metabolic transformations, becoming amastigote forms, which lyse the host cells and then infect other phagocytic mononuclear cells
- Visceral leishmaniasis in humans is known as **Kala-azar**, which means lethal disease in Hindu

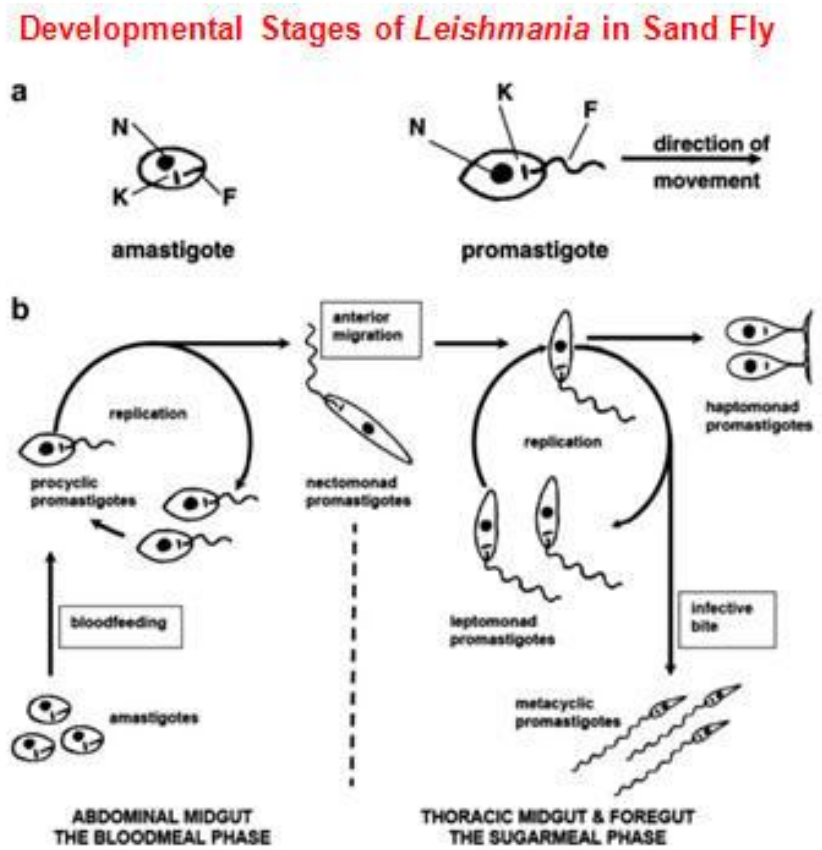
Life cycle of Leishmania indicating zoonotic importance



A sandfly vector of Leishmania parasites taking a blood meal through human skin.

Cont…


Sandfly Stages
Human Stages
1 Sandfly takes a blood meal (injects promastigote stage into the skin)
2 Promastigotes are phagocytized by macrophages
3 Promastigotes transform into amastigotes inside macrophages
4 Amastigotes multiply in cells (including macrophages) of various tissues
5 Sandfly takes a blood meal (ingests macrophages infected with amastigotes)
6 Ingestion of parasitized cell
7 Amastigotes transform into promastigote stage in midgut
8 Divide in midgut and migrate to proboscis
= Infective Stage
= Diagnostic Stage
CDC
SAFER·HEALTHIER·PEOPLE
http://www.dpd.cdc.gov/dpdx


Developmental Stages of Leishmania in Sand Fly
a
N
K
F
amastigote
promastigote
direction of movement
b
anterior migration
replication
procyclic promastigotes
nectomonad promastigotes
haptomonad promastigotes
bloodfeeding
leptomonad promastigotes
infective bite
amastigotes
metacyclic promastigotes
ABDOMINAL MIDGUT
THE BLOODMEAL PHASE
THORACIC MIDGUT & FOREGUT
THE SUGARMEAL PHASE

Cont…

Epidemiology

- Visceral leishmaniasis is currently considered an emerging and reemerging disease in both rural and urban areas.
- According to the World Health Organization (WHO), in 1990 there were 12 million cases with 400 thousand new cases every year.
- The WHO has currently pointed out the increase in the number of cases of co-infection HIV/visceral leishmaniasis, especially in southern Europe.
- This is due to primarily to sharing of needles and syringes.
- This route of infection has been extending to the Nordic countries.

Cont…

- Leishmania is largely distributed in tropical and subtropical areas
- Leishmaniasis is considered the second most important protozoal disease in the world, with its incidence being lower than malaria only
- In humans, visceral leishmaniasis affects mainly children and immunosuppressed individuals characterized by:
- Long periods of irregular fever
- Weakness
- Weight loss
- Pancytopenia
- Hepato and splenomegaly
- Hypergammaglobulinemia
- Hypoalbuminemia which progresses to chronicity or
- Death in the absence of adequate treatment

Cont…

Clinical Signs

- Syndromes range from mild self-limiting local skin lesions to systemic fatal diseases.
- Visceral leishmaniasis starts as a cutaneous lesion and the infection spreads systemically
- Organs particularly affected are spleen, liver and bone marrow
- The main dermatologic signs of visceral leishmaniasis consist of:
- Emaciation
- Keratitis
- Scaling of skin seborrhea
- Ulceration
- Alopecia

Cont…

Cont…

- Some heavily infected dogs don´t have any clinical signs
- With the visceral form, the disease can be severe, causing clinical signs in many organs.
- There are:
  - Extreme weakness
  - Wasting
  - Diarrhea
  - Epistaxis
  - Lameness
  - Anemia
  - Renal failure
  - Edema of the feet
  - Dermal ulceration
  - eye inflammation leading to blindness
  - Lymphadenopathy and hepatosplenomegaly
  - Body temperature may fluctuate but is usually normal or subnormal
  - Immunosuppression may promote the occurrence of concomitant infections

Cont....

Histopathologic and Necropsy Findings

- Infection with *Leishmania* is typically demonstrated by identification of the amastigote stage of the parasite within macrophages
- This can be achieved through biopsies from a wide variety of tissues or aspirates from lymph nodes or bone marrow stained by Giemsa
- The amastigotes, which are approximately 2 micrometers in diameter, is usually found within macrophages and other phagocytic cells
- Upon necropsy
- Findings may include severe emaciation
- Enlargement of the liver, spleen, and lymph nodes
- Skin lesions and overgrowth of the claws

Cont…

Diagnosis

- The diagnosis of Leishmania is difficult because the clinical signs are variable, the histopathology is non-specific, and the microscopic lesions are also observed in other immune-mediated diseases and infections

- The diagnosis is divided into three main categories:

1. Parasitologic diagnosis
2. Immunologic diagnosis
3. Molecular methods (amplification of parasite DNA)

Parasitologic Diagnosis

- This is the simplest and most commonly performed procedure
- It is based on cytologic or histopathologic observation of amastigotes in Giemsa stained smears of bone marrow or lymph node aspirates
- Immunoperoxidase staining improves the detection of amastigotes in the infected tissues

Cont…

Immunologic Diagnosis

- This is the detection of antibodies (mainly IgG and especially IgG1) against Leishmania parasites or a specific cell-mediated response
- The four main serological tests performed are IFAT, ELISA, DAT, and Western Blot
- IFAT is considered the gold standard of tests, it has a high specificity and high sensitivity
- IFAT uses the whole organism, which gives more repeatable and reliable results, rather than those using soluble Ag, such as complement fixation
- ELISA is more sensitive, but less precise than IFAT
- ELISA also cross reacts with *Trypanosoma cruzi* and *Babesia*

Cont…

Molecular Methods (detection of Leishmania DNA)

- PCR uses primers from the rRNA gene to identify parasites from a variety of samples, including canine and human bone marrow, lymph nodes, skin biopsies, and heparinized whole blood.

- PCR is useful in the diagnosis of Leishmania, for follow-up patients pre and post treatment, and for the identification of parasitic species

- PCR is a very sensitive test, but is not always available

Cont…

Differential Diagnosis:

VL:

- Brucellosis, leprosy, schistosomosis, trypanosomiasis (African), leukemia, lymphoma, malaria, typhoid, liver diseases, other entities

CL:

- Numerous primary and secondary skin diseases/conditions such as:
  - Tropical ulcers
  - Impetigo
  - Infected insect bites
  - Leprosy
  - Lupus vulgaris
  - Tertiary syphilis
  - Yaws
  - Blastomycosis
  - Skin cancer

Cont…

Treatment

- Treatment has traditionally been unsatisfactory because of drug toxicities, poor responses, multiple disease syndromes, and other factors including the emergence of antimony-resistant strains
- Orally administered miltefosine 100 mg/day (2.5 mg/kg/day) for four weeks has recently shown great promise in the treatment of Indian visceral leishmaniasis
- Other treatments include sodium stibogluconate (a pentavalent antimony compound) 20 mg/kg/day IM or IV once daily for 40 days
- Second line treatments are amphotericin B total IV dose of 6-20 mg/kg over 20 doses or L-ampho 3.0 mg/kg IV day 1, day 5, day 10
- Second line treatments include IM pentamidine 2-4 mg/kg/day for 15 days or IM aminosidine 15 mg/kg/day for 30 days

Trichomonasis

- It is a venerally transmitted protozoa

- It is multiflagellated organism of the reproductive tract of cattle
- In bulls, infection is inapparent, but in pregnant cows it produces early fetal death
- It is usually recognized as an infertility problem
- The most important pathogen in this genus is T. foetus and T. gallinae in the esophagus and crop of pigeons

Cont…

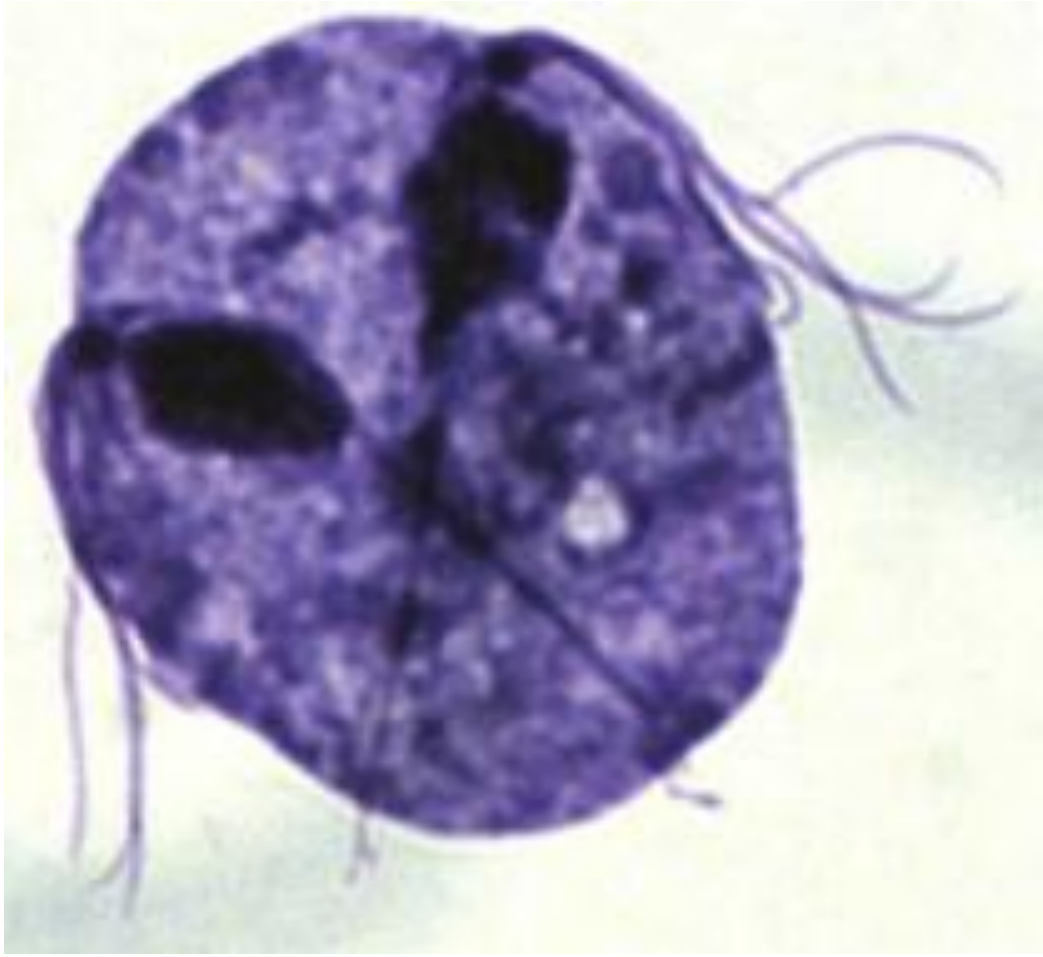
Trichomonas foetus

- **Hosts**: cattle
- **Site**: uterus and intermittently the vagina, preputial cavity
- **Distn:** WW but dramatically reduced in areas where AI utilized

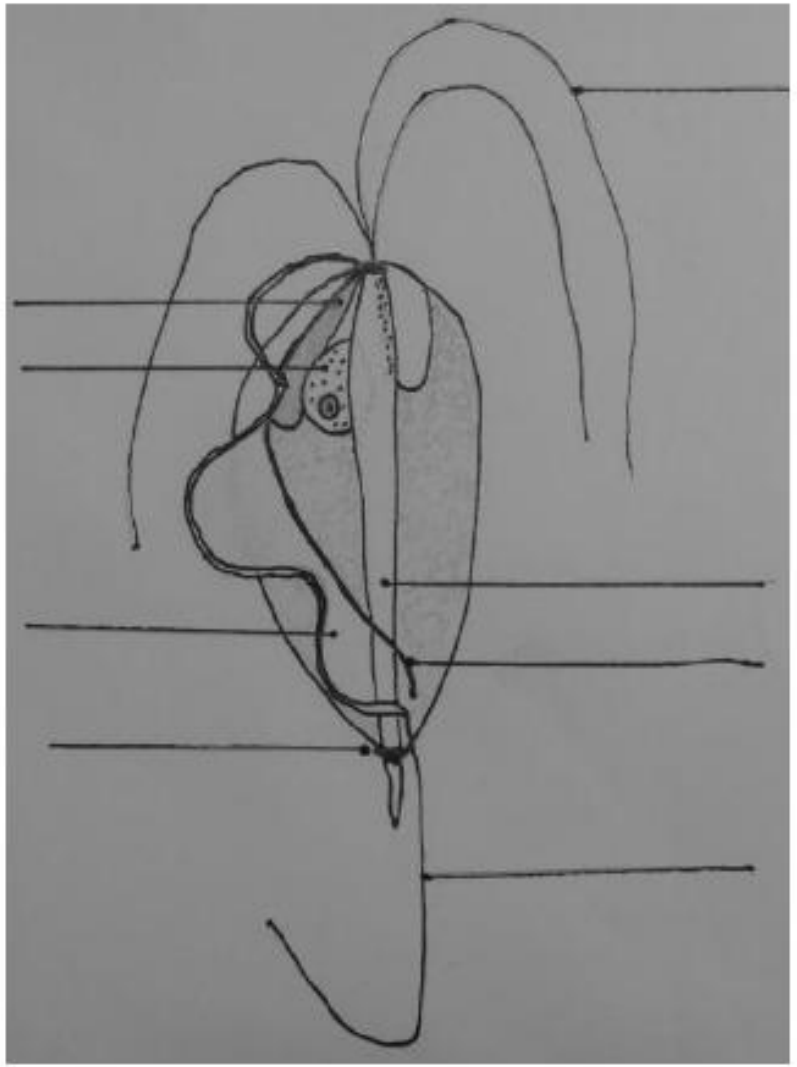
- **Identification**:
- Pear shaped
- 20x10 Nm length
- Single nucleus and four flagella
- Three of the flagella are free anteriorly
- The fourth extends backwards to form undulating membrane along the length of the organism posteriorly
- In fresh preparations, the organism is motile and progresses by rolling jerky movements

Morphology of trichomonas organism

- Trichomonas is a pear-shaped protozoan with 5 hair-like ‘tails’ called flagella (four anterior and one posterior)
- Flagellate protozoa use their flagellae to move the cell around
- The presence of the five flagellae and the jerky movements of the cells, when seen through a microscope, are distinctive and assist in its identification



Typical morphological structure of *T. foetus*



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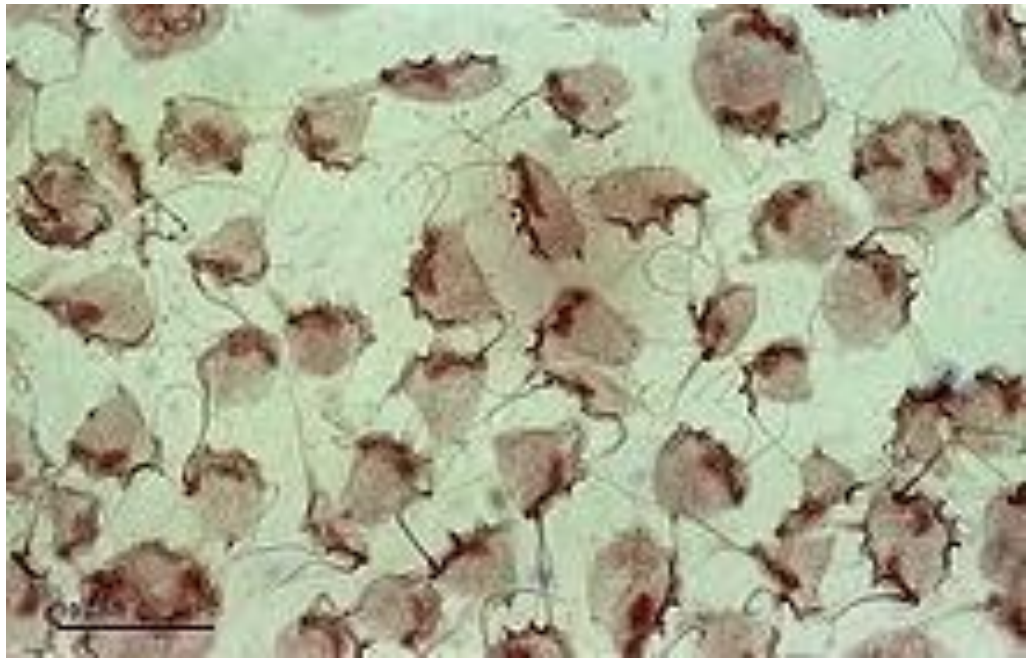

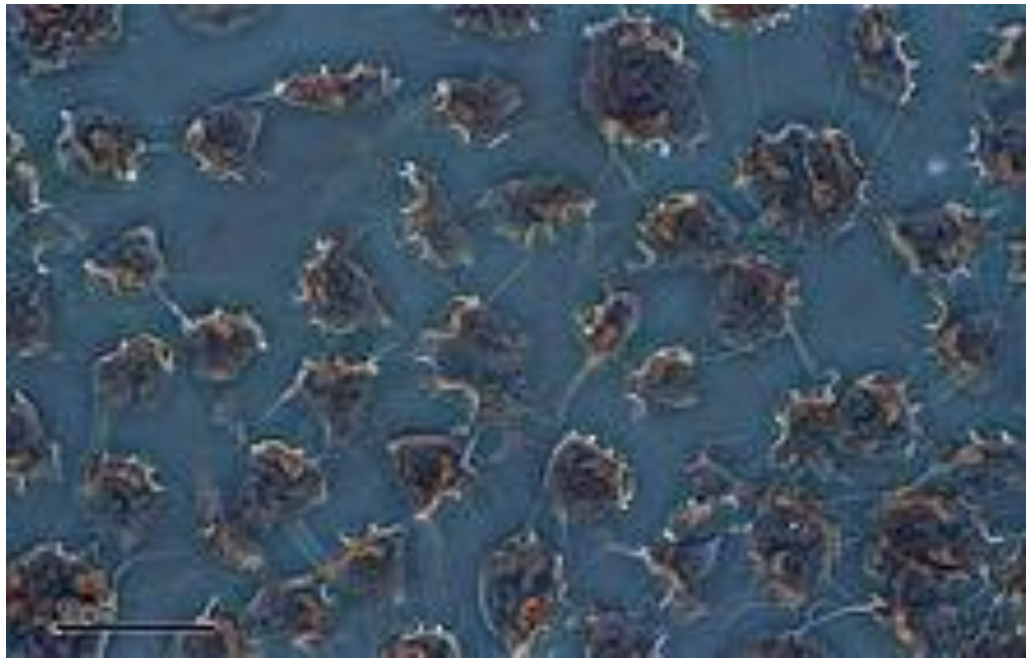

Cont…

Life cycle:

- Bulls once infected remain permanently infective
- The organism inhabits the Preputial cavity and transmission to the cow during coitus
- From vagina to uterus via cervix to produce a low-grade endometritis
- Organisms are flushed into the vagina two or three days before oestrus
- Infection is usually followed by early abortion
- Subsequently cows appear to ‘self cure’ and in most cases appear to develop a sterile immunity

Cont…

Pathogenesis:

- In the bull, a preputial discharge associated with small nodules on the preputial and penile membranes
  - There after there are no clinical signs or lesions

- In the cow, abortion before the 4^{th} month of pregnancy is commonest sequel followed by recovery
  - Persistent uterine discharge and anoestrus
  - Infrequently the CL is retained and the cervical seal remains closed
  - When a massive pyometra develops which, visually, simulates the appearance of pregnancy

Cont…

Clinical signs:

- Early abortion is characteristic
- Irregular oestrus cycle
- Purulent Endometritis
- Closed pyometra
- Permanent sterility

Cont…

Epidemiology

- The overall prevalence of Trichomoniasis to be high since bulls, which show no clinical signs, generally transmits it
- Today it is limited to areas where there are many small farms each with their own bulls or to countries where veterinary supervision is limited

Diagnosis

- Infertility
- Demonstration of the organism
- Vaginal mucus collected from the anterior end of the vagina by suction in to a sterile tube, or preputial washing from the bull-examined using warm stage microscope for the presence of the organisms
- On a herd basis, samples of vaginal mucus may be examined in the laboratory for the specific agglutinins against laboratory cultures of T. fetus

Cont…

Treatment

- In female symptomatic treatment and sexual rest for 3 months
- In bull slaughter is the best option.

Control

- Artificial insemination from non- infected donors
- Recovered cows should be disposed off since some may be carriers.

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Thank You