UNIT-5 (PROTOZOA OF VETERINARY IMPORTANCE)

Topic

Morphology, epidemiology, pathogenesis, clinical signs, diagnosis and control measures of protozoan parasites belonging to the families: Theileriidae

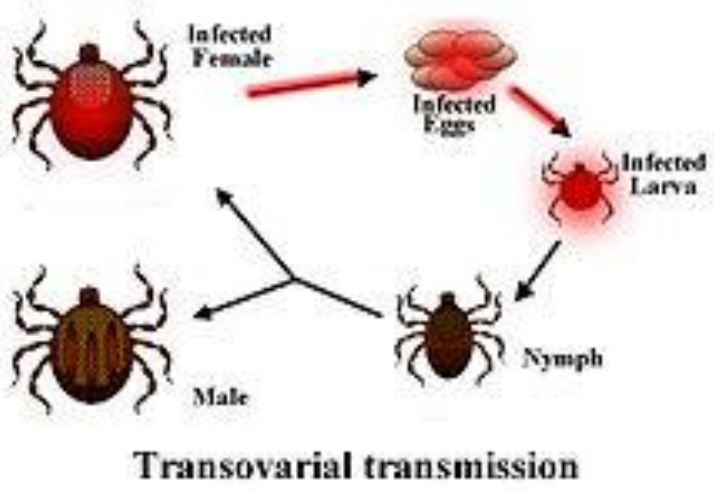
Dr. Rupesh Verma
Assistant Professor, Deptt. of Veterinary Parasitology
College of Veterinary Science & Animal Husbandry (NDVSU), Jabalpur MP

Phylum Apicomplexa
Class Sporozoa
Order Piroplasmida
Family Babesidae
Genus Theileria

Species	Vector	Disease	Host	Distributions
Theileria annulata	*Hyalomma anatolicum anatolicum*	Bovine Tropical Theileriosis or Mediterranean Coast Fever (MCF)	Buffaloes & Cattle	Mediterranean basin and pars of Asia
Theileria parva	***R. appendiculatus***	**East Coast Fever (ECF)**, Classic disease, Bovine theileriosis (**January disease**) (Turning sickness)	Buffaloes & Cattle	Central, East and Southern Africa
Theileria mutants	*R. evertsi*	Benign bovine theileriosis, (Turning sickness)	Buffaloes & Cattle	Africa, Asia, Australia Russia & USA
Theileria lawrenci	*R. appendiculatus*	Corridor disease	Buffaloes & Cattle	East & central Africa
Theileria lestoquardi (formerly Theileria hirci)	*Hyalomma anatolicum anatolicum*	**Malignant ovine / caprine theileriosis (Small ruminants theileriosis)**	Sheep & Goat	Northern and East Africa, and middle Asia
Theileria ovis	*R. bursa & Haemophysalis spp.*	Ovine theileriosis	Sheep & Goat	USSR & India
Theileria equi	*Hyalomma spp., Rhipicephalus spp*	Equine biliary fever	Horses, donkeys, giraffes	
T. bicornis	*R.evertsi evertsi*		Black, white and Indian rhinoceros	
T. buffelis	*Haemophysalis spp.*	Benign theileriosis	Buffaloes	
Theileria camelensis	*Hyalomma dromederii*		Camel	Somalia Egypt
T. orientalis	*Haemophysalis spp.*	Being theileriosis	cattle	cosmopolitan

1. Transovarial or **transovarian transmission**

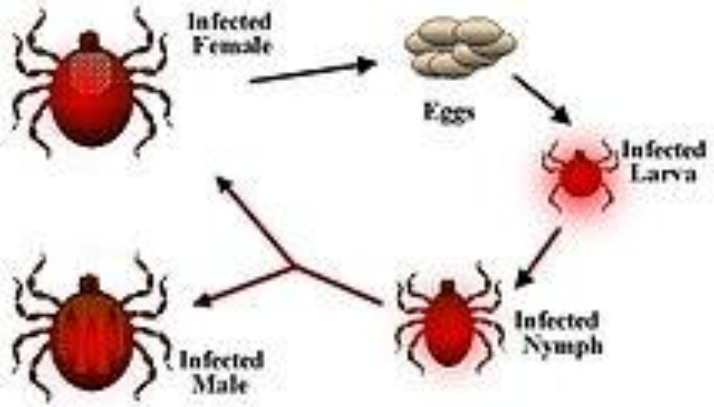
- Transmission of parasites from parent to offspring via the ovaries.
- E.g. one host ticks (*Babesia* infection only)



Transovarial transmission

2. Transstadial transmission

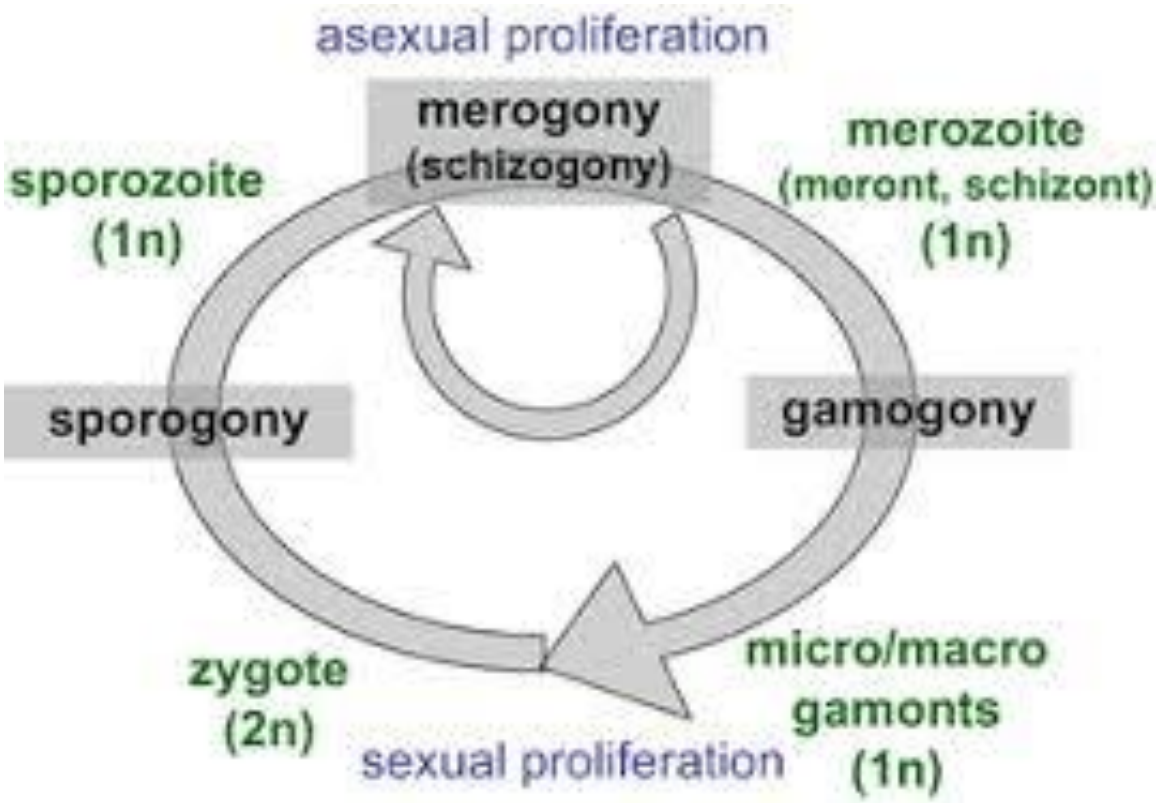
- Transmission of the parasites from one stage to next stage (through the molt to the next stage(s) or stadium)
- E.g. three host ticks (*Babesia* & *Theileria* infection)



Transstadial transmission

1. Sexual multiplication (Definitive Host)- invertebrate animals (Ticks)

2. Asexual multiplication (Intermediated Host) Vertebrates animals



Life cycle of *Theileria*

1. Schizogony

Ticks having sporozoites feed on vertebrate host for 2- 4 days

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Sporozoites in salivary glands of ticks will mature and become infective

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Sporozoites ingested into vertebrate host

↓

Sporozoites enter into lymphocytes and develops into schizonts in the lymph node (In case of *T. parva*)

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In *T. annulata* infection, they invade **macrophages or monocytes** to form schizont rather than lymphocytes

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Causing lymphocytolysis which leads to immunosuppression, pulmonary edema and may be anemia

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Infected lymphocytes transformed into lymphoblasts which continue to divide having schizonts

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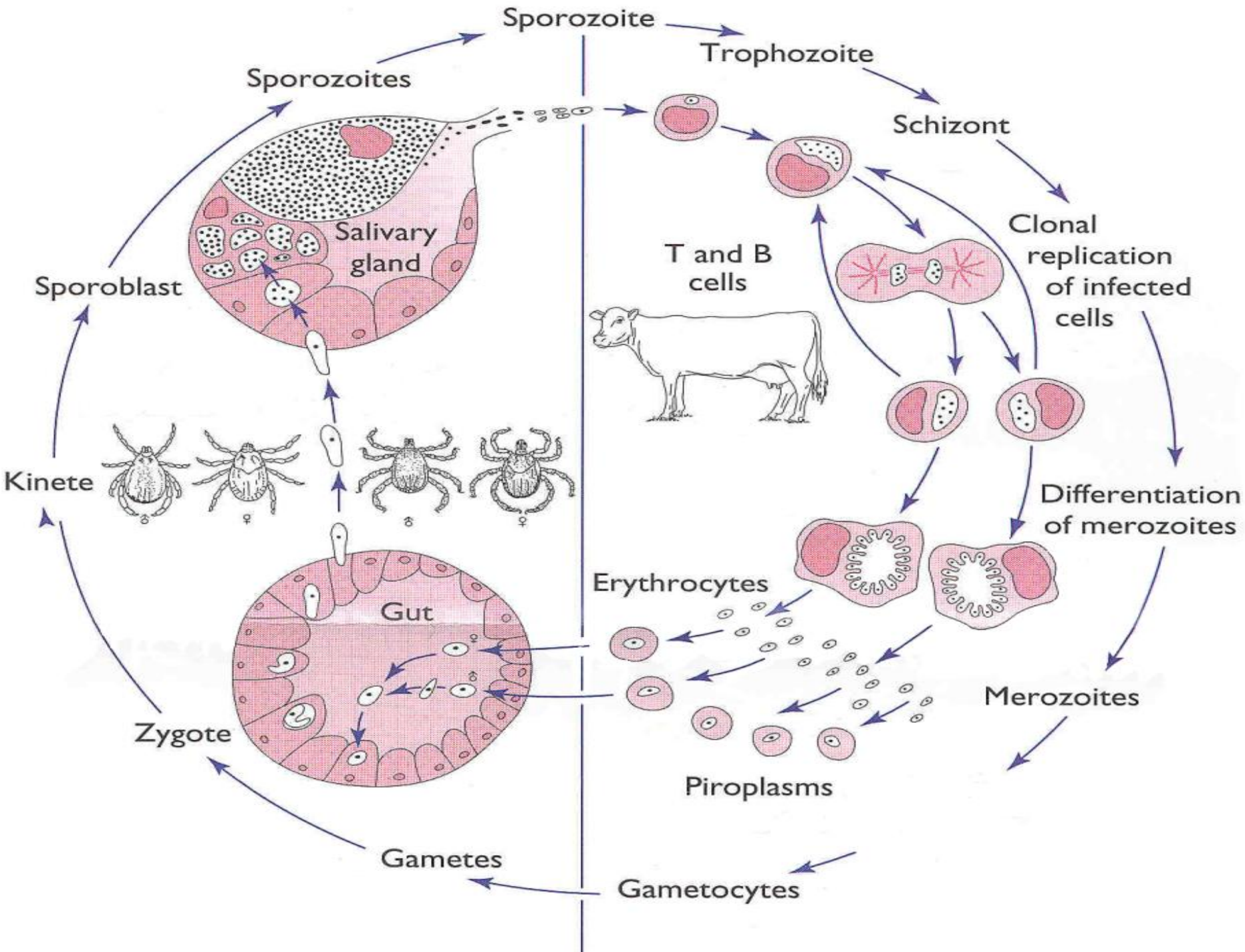
Eventually infected lymphoblasts are disseminated throughout the lymphoid system and non lymphoid organs


Sporozoite
Trophozoite
Sporozoites
Schizont
Salivary gland
Clonal replication of infected cells
T and B cells
Sporoblast
Kinete
Differentiation of merozoites
Erythrocytes
Gut
Merozoites
Zygote
Piroplasms
Gametes
Gametocytes

There are two types of schizonts

- **Macroschizont**: Lymphocytes with large schizonts, commonly known as ***Koch's blue bodies***, appear a few days after onset of symptoms. One with large chromatin granules gives (8-16 macromerozoites).
- **Microschizon**t: Later, lymphocytes infected with microschizonts appear. One with small chromatin granules gives (50-120 Micromerozoites) and they infect to RBCs (Sexually differentiated)
- Infection of RBCs is important for transmission and infection of lymphocytes is important for pathology. Damage mainly by **schizonts**

2. Merogony

Later some schizonts differentiate into merozoites

↓

They are released from lymphoblasts and invade erythrocytes

↓

In erythrocytes they are referred as **Piroplasms**

↓

Merozoites develop into trophozoites which further asexually divide into merozoites

↓

Merozoites are then released by rupture of the host red blood cells and invade healthy erythrocytes

↓

Sexual multiplication of the parasite starts by gametocytes appearing in the host red blood cells

3. Gamogony

↓

During blood uptake by ticks, gametocytes develop into gametes that mature in the tick midgut lumen

↓

Inside these, the zygote undergoes a meiotic division and results in the formation of kinetes (Vermicules/ ookinetes), which are released to the haemolymph.

The kinetes of *Theileria* species directly invade salivary glands (primary kinetes) but kinetes of *Babesia* parasites are subjected to two series of asexual multiplication in various tick tissues and subsequent secondary kinetes invade the tick salivary glands

4. Sporogony

↓

Sporogony starts after kinete invasion of tick salivary glands (type III acinus), which form the sporont, a polymorphous syncytium

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The sporont later evolves into a multinucleated meshwork referred as a sporoblast, which is dormant during tick ecdysis

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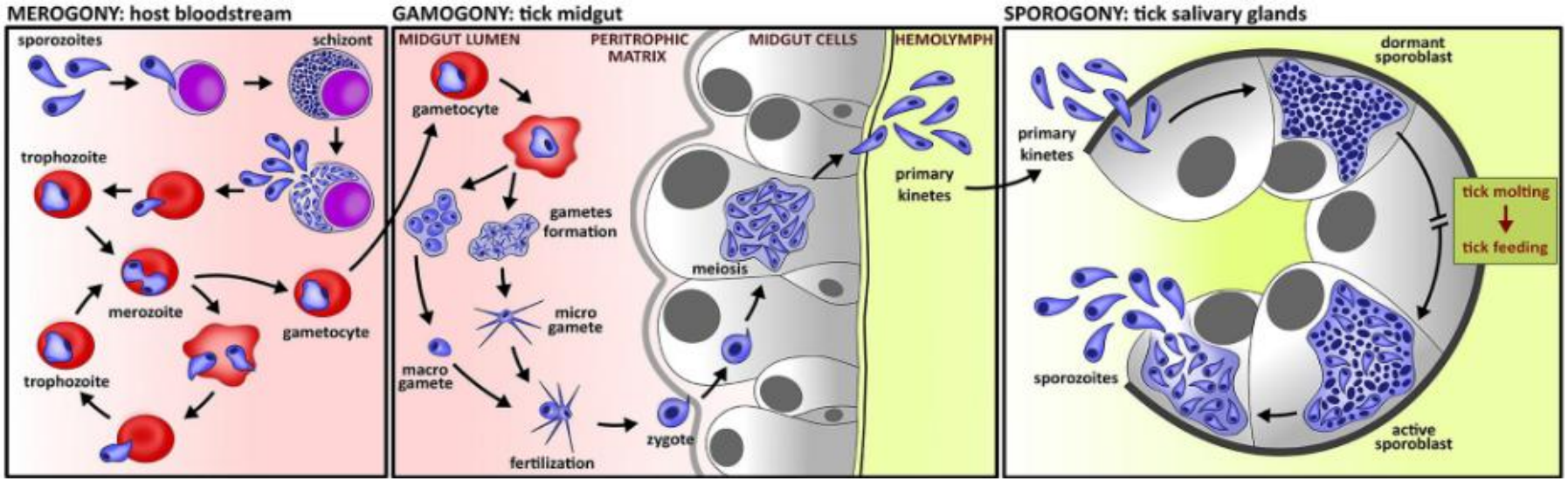
Maturation of the parasite sporoblast starts after tick attachment to the host and results in sporozoites being released into tick saliva


MEROGONY: host bloodstream
sporozoites
schizont
trophozoite
merozoite
gametocyte
trophozoite
GAMOGONY: tick midgut
MIDGUT LUMEN
PERITROPHIC MATRIX
MIDGUT CELLS
HEMOLYMPH
gametocyte
gametes formation
micro gamete
macro gamete
fertilization
zygote
meiosis
primary kinetes
SPOROGONY: tick salivary glands
dormant sporoblast
primary kinetes
tick molting
tick feeding
sporozoites
active sporoblast

Pathogenesis

- Within host leukocytes the parasite induces leukocyte cellular division, which expands the parasitized cell population.
- Infected cells disseminate throughout the lymphoid system via the lymphatic and blood vessels.
- The infected leukocyte may block capillaries, causing tissue ischemia.
- This is followed later by necrosis of infected lymphoblasts induced by cytotoxic T-lymphocytes. The severe lymphocytolysis often leads to immunosuppression.
- Later in infection some schizonts cause leukocyte lysis and release of merozoites.
- Merozoites then invade and parasitize erythrocytes, causing hemolytic anemia.

- The East Coast fever (*T. parva* infection) is characterized by a generalized lymphadenopathy due to lymphocyte infection. Hyperplastic, hemorrhagic, edematous, and necrotic lymph nodes have been observed in acute cases of the infection.
- In addition, interlobular emphysema and severe pulmonary edema have also been reported. Lymphoid cellular infiltrations appear in the liver and kidney and hemorrhages and **ulceration may be seen throughout the gastrointestinal** tract.
- 'The tropical theileriosis' (*T. annulata* infection) is characterized by macrophage infection that causes the release of cytokines (TNFα), anemia, and the presence of macroschizonts in infected macrophage-type cells.

Clinical symptoms

- Swelling of the draining lymph node, usually the parotid
- Fever 40 – 41^{o} C, maintained until death or recovery
- Nasal discharge , Lacrimation
- Swelling of the eyelids and ears
- Anemia, Jaundice, Anorexia, Heart beat rapid, dyspnea, diarrhea

❖ Poor condition and severe lymphadenopathy in heifer


Lacrimation


Rear case Corneal opacity


Nasal Discharge


Diarrhoea

➢Turning sickness: an aberrant form of theileriosis in which parasitized lymphocytes cause emboli and hemorrhagic infarcts in central nervous tissue.

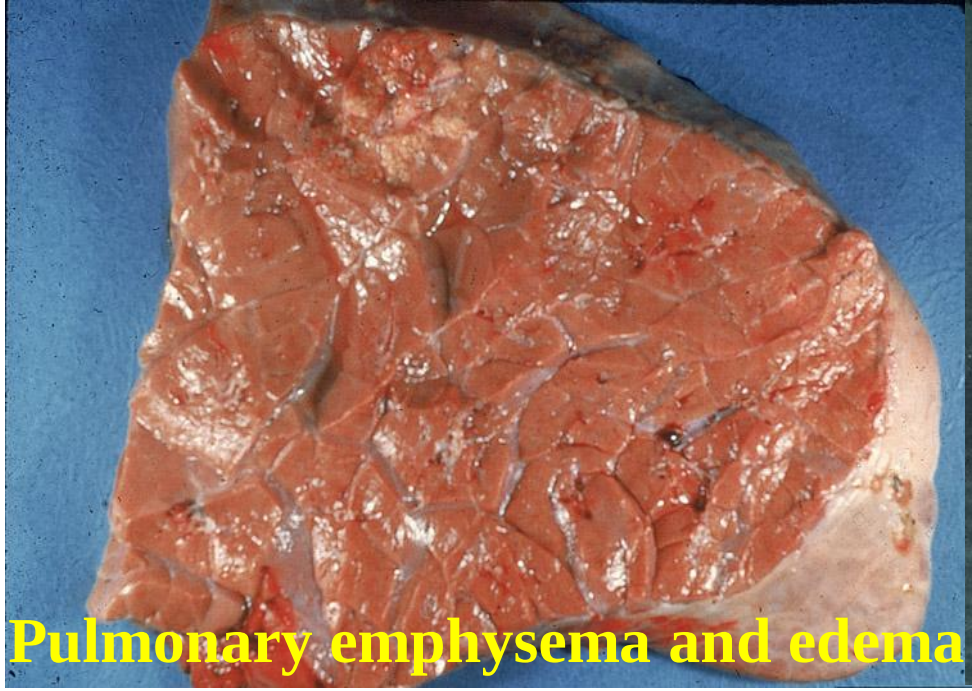
➢Occasional cases of brain involvement occur and are characterized by circling, hence 'turning sickness' or cerebral theileriosis due to the presence of schizont in the cerebral capillaries

➢*Theileria parva*
➢*Theileria mutans*

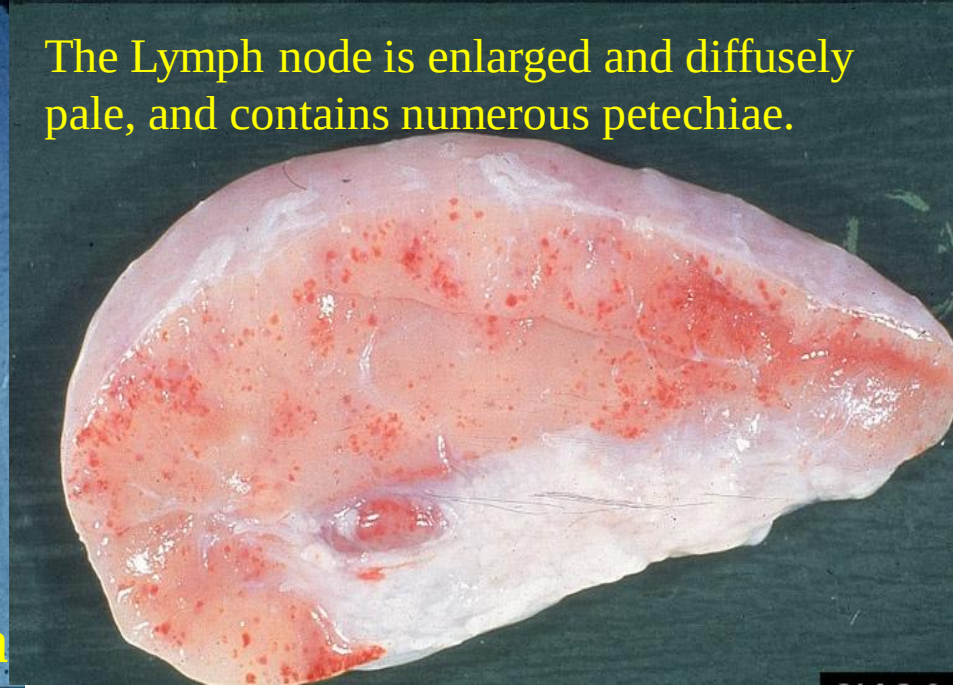
➢Animal make **CIRCLING MOVEMENT** and **ABDUCTION OF HINDLIMB**

Post mortem lesion

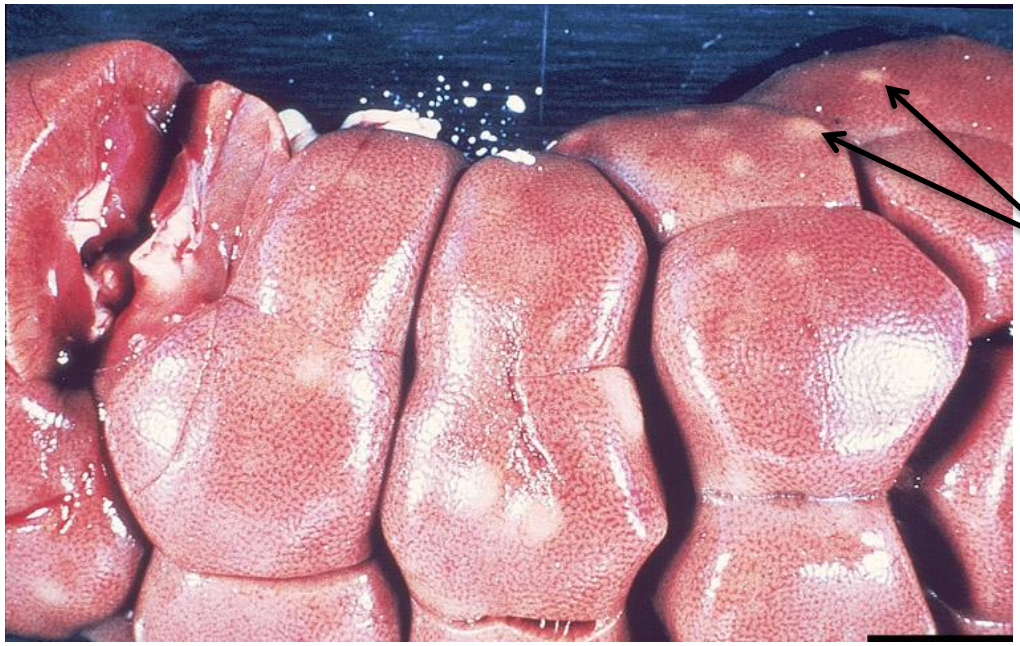
- Lymphocytes proliferate heavily invading multiple organs causing disease similar to a lymphoma (cancer of lymphocytes)
- Splenic enlargement.
- Severe pulmonary emphysema and edema along with hydrothorax and hydro pericardium.
- Generalized lymphoid hyperplasia.
- Small lymphoid nodules (the so-called pseudo-infarcts) are present in liver, kidney, and alimentary track.
- The carcass is emaciated and hemorrhages are evident in a variety of tissues and organs. Death is in most cases due to infiltration of the lung resulting in lung edema (the abnormal build up of fluid within the lung)



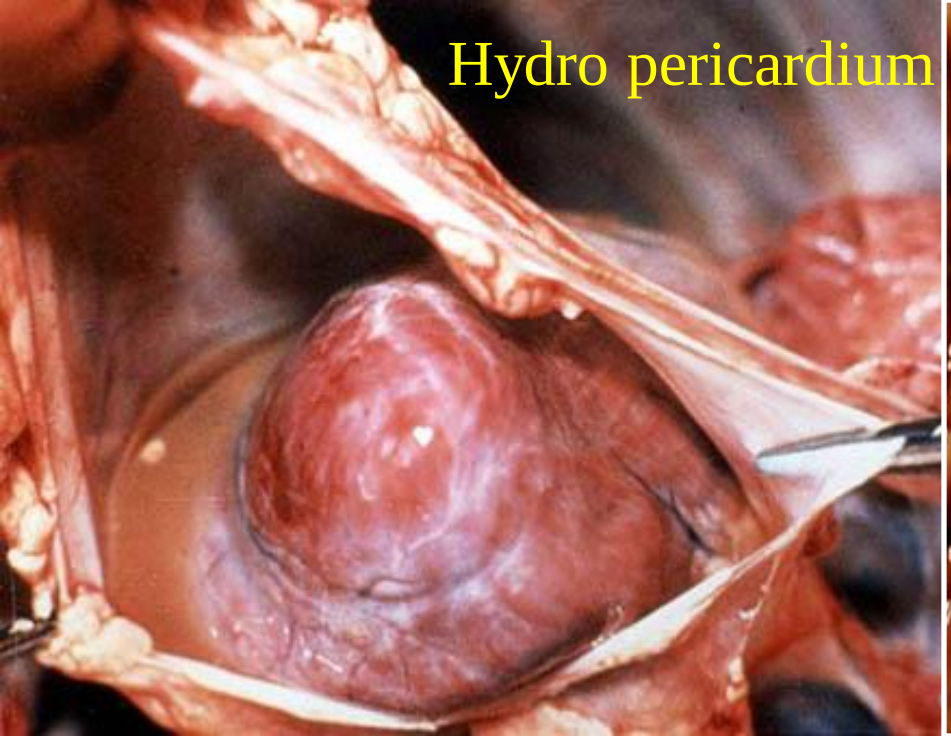
Pulmonary emphysema and edema

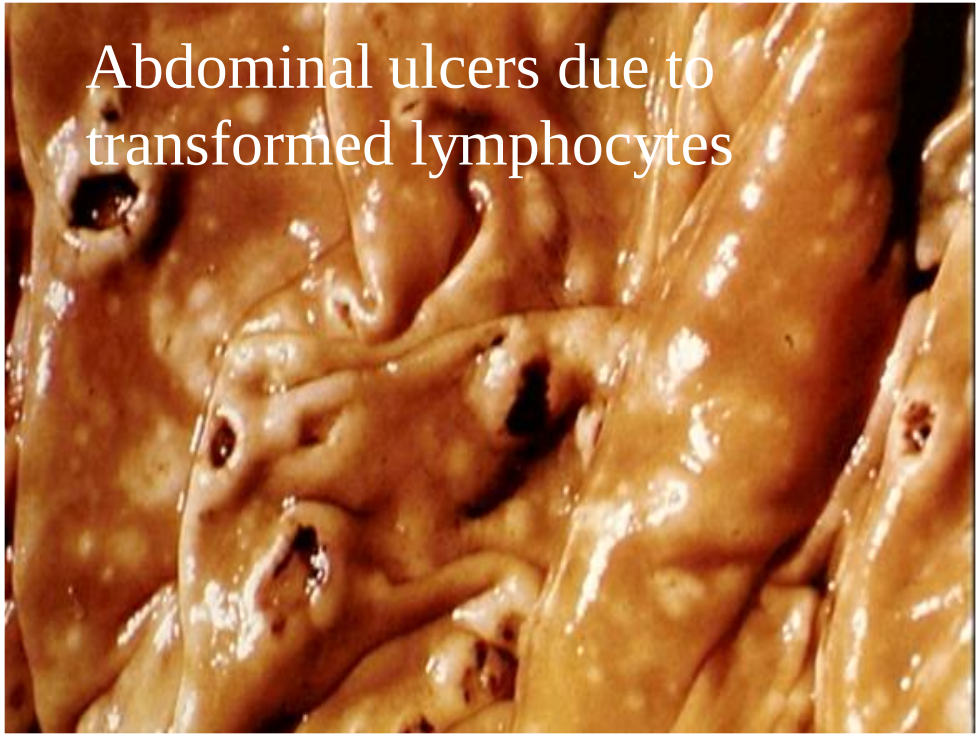


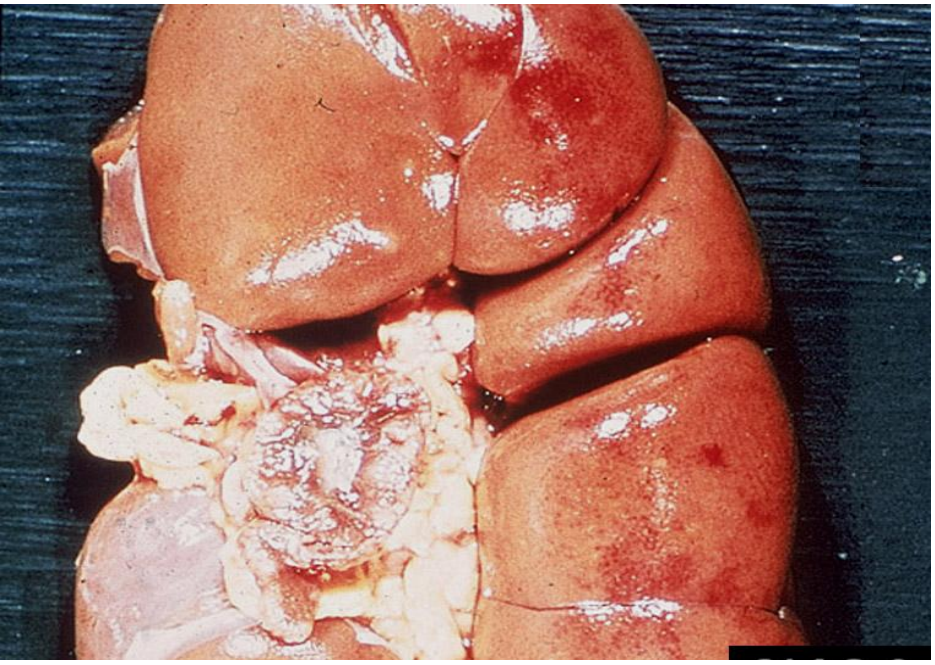
The Lymph node is enlarged and diffusely pale, and contains numerous petechiae.



Multiple pale foci on the cortical surface of the kidney are lymphoid infiltrates.







Kidney, There are **multiple petechiae** on the surface of the cortex. The lymph node near the hilus is markedly enlarged

PUNCHED OUT NECROTIC ULCER IN ABOMASUM

Diagnosis

History
Presence of Ticks, Seasonal occurrence

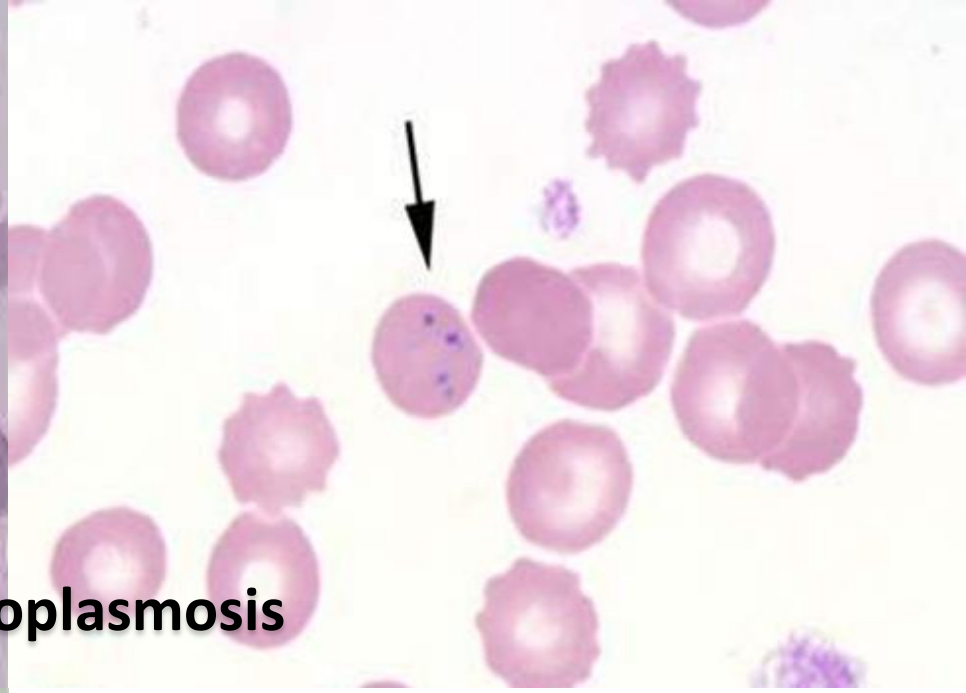
Clinical Findings
Lymph node swelling, anaemia , icterus

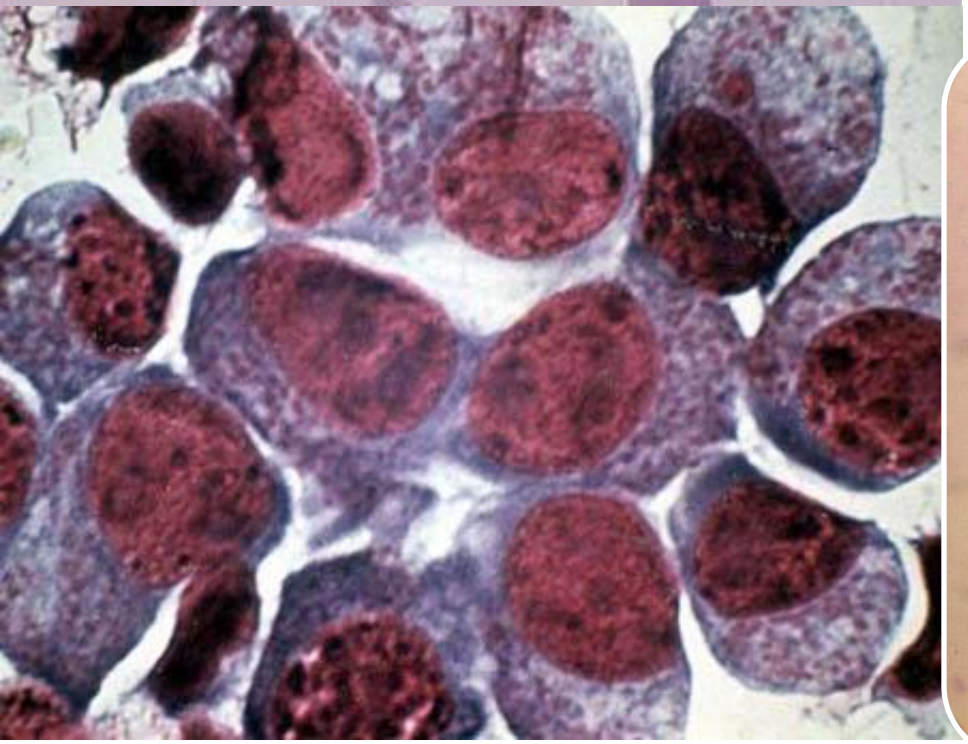
Post-mortem findings
Punched necrotic ulcer in Abomasum

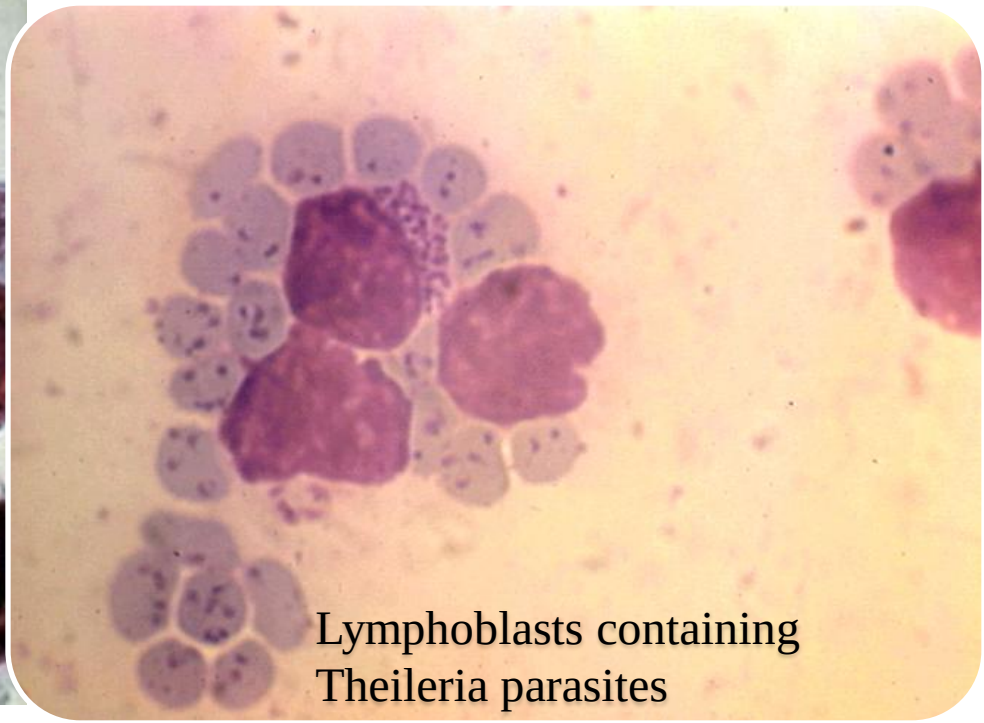
Blood smear exam
Presence of Piroplasm stage in Blood smear some time KBB(Common in *T. parva*)
T. parva schizonts in Lymphoblasts & *T. annulata* schizont in macrophages/monocytes

Lymph node biopsy
lymph nodes, spleen and liver biopsy presence of KOCH BLUE BODIES

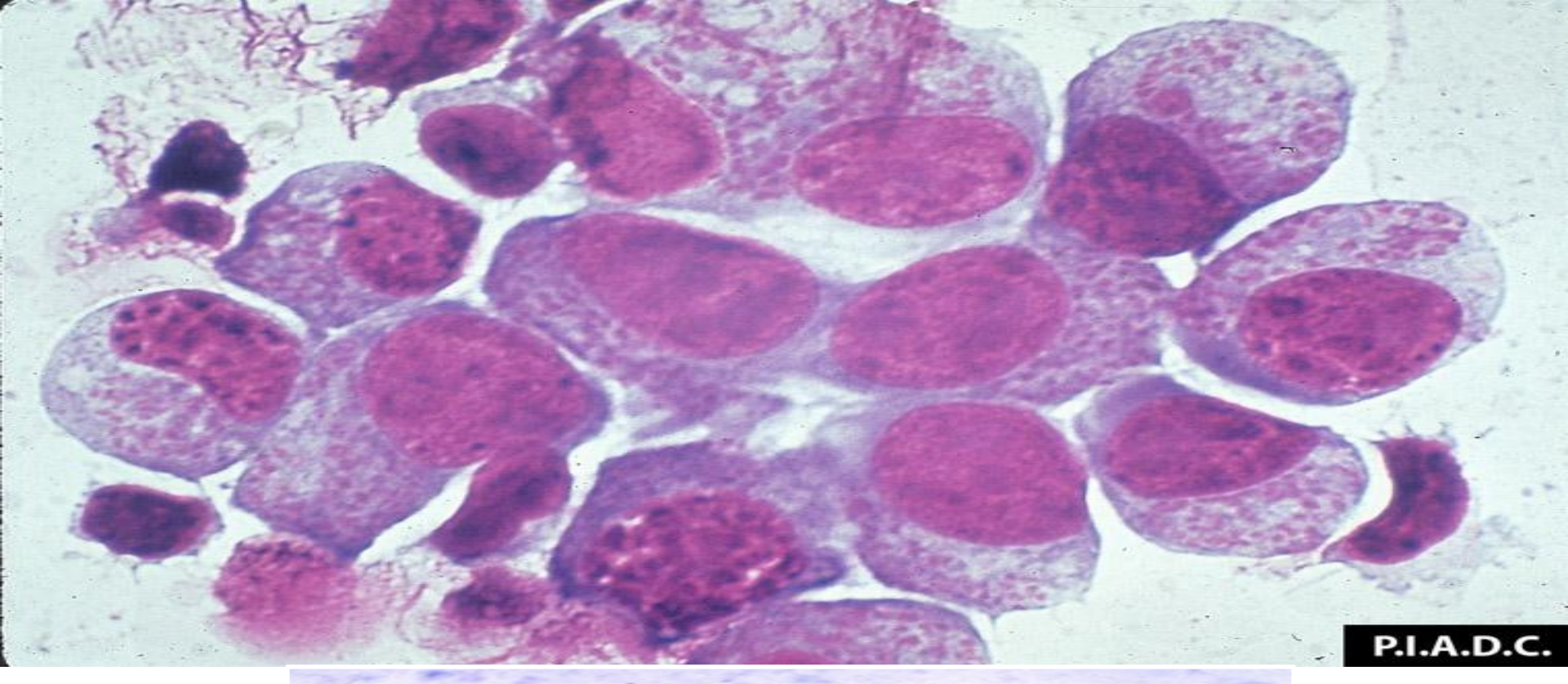
Theileria Piroplasmosis

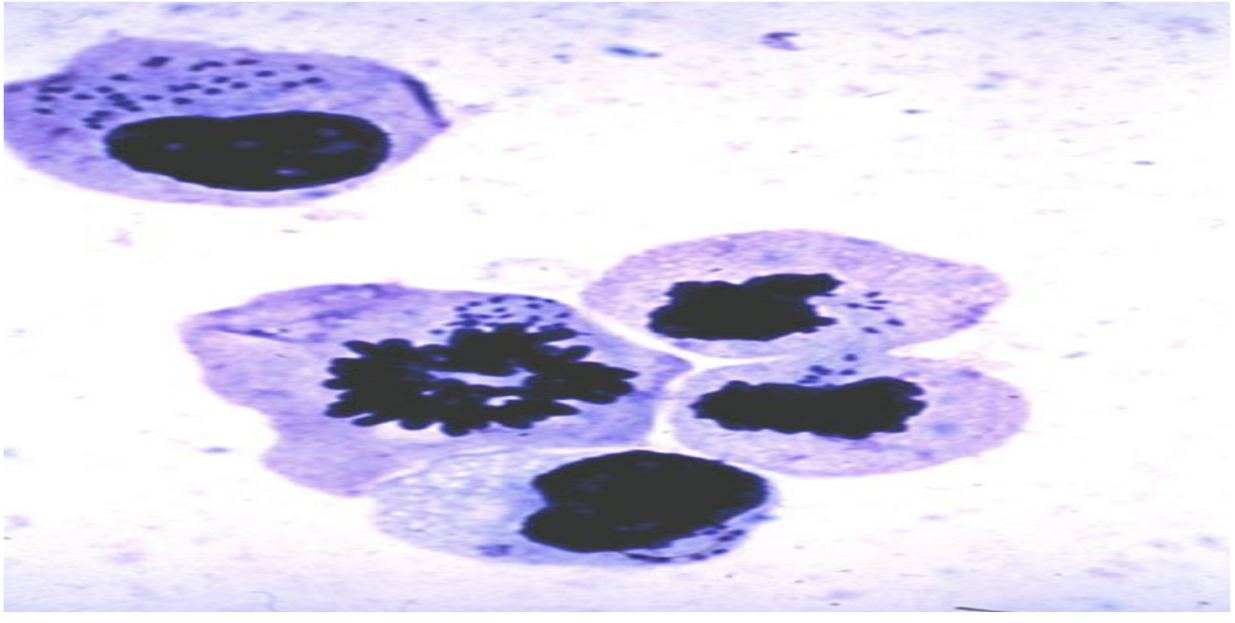






Lymphoblasts containing
Theileria parasites


P.I.A.D.C.



Xenodiagnosis

❑In *Theileria annulata* infection in salivary gland of tick use **Methyl Green Pyronin staining**

Animal Inoculation

❑In Live animals, theileriosis can be diagnosed by finding schizonts in Giemsa-stained thin smears from blood or lymph node biopsies. At necropsy, schizonts may be found in impression smears from many internal organs.

Cultivation

❑The intralymphocytic stages of *Theileria parva*, *T. lawrencei* and *T. annulata* have been cultivated for several months in tissue cultures of bovine lymphocytes associated with baby hamster kidney cells.

❑Medium RPMI-1640 supplemented with 20% foetal bovine serum (standard growth medium) resulted in optimum growth of *T. annulata* (Hisar) schizonts in vitro.

Serological test

❖Antibodies to *T. parva* and *T. annulata* can be detected with enzyme–linked immunosorbent assays (ELISAs) or an indirect fluorescent antibody test (**IFA**).

❖Tests used for *T. parva* and *T. mutans* are indirect ELISAs based on parasite-specific antigens, **PIM** and **p32**, respectively

Molecular Test

❖Fluorescence resonance energy transfer **(FRET)-**based real-time assays have also being developed for specific diagnosis of ***T. parva***

❖A reverse line blot **(RLB)** assay based on hybridisation of PCR products to specific oligonucleotide probes immobilised on a membrane for simultaneous detection of different Theileria species has been introduced.

- Conservation of the18S V4 hyper variable region and ITS-1 & ITS-2 Genes

- Recombinant Theileria annulata sporozoites surface protein **(rTaSP/TaSP**) antigen

- Sporozoites surface antigen -1 (**Spag-1**)

- Merozoites surface antigen) **(rTams**-1/**Tams-2)**

- PCR amplification of the **p33/34** genes of the T. orientalis/buffeli complex followed by restriction enzyme analysis can be used to characterize the various types

Treatment

- Tetracyclines(@5-10mg/kg B.W.)- used **earlier**

- Broad spectrum antibiotics like oxytetracycline(@10mg/kg B.W.),
- Chlortetracycline (only **against schizontal stages**)

- Parvaquone 10 mg/kg two injection at 48 hrs interval (for both **schizontal and piroplasmal** stage)

- **Buparvaquone**(**butalex**,bupaven,zubion ,50mg/ml) @**2.5**mg/kgB.W.,im, two injection at 48 hrs interval (for both **schizontal and piroplasmal** stage)**Drug of choice**

- Halofuginone lactate @1.2mg/kg b.w. ORALLY

Control

- Control of vector i.e. ticks mainly of Hyalomma spp. By application of insecticides(like deltamethrin(butox,12.5mg/ml)2-3ml/litre of water, Ivermectin(hitek,neomec,1%w/v)@0.2mg/kg, s/c) and rotational grazing(breaks the transmission cycle between cattle &tick).

- Exotic & Crossbred cows should be kept in tick free shed.

- Recovered Cattle are immune to homologous challenge

- Avoid nutritional stress

Chlortetracycline@16mg/kg B.W..orally for 8 days, or
Rolitetracycline@4mg/kg B.W., im, for 3days in calves

Immunoprophylaxis-
"Infection and treatment method"-injection of cryopreserved susp. Of sporozoites from ground up infected tick

Live vaccine efforts have been made to immune the animals by transfer of infected blood

1. Rakshavac-T vaccine

IVRI, Bareilly, have evolved tissue **culture live attenuated schizontal stage (2*106 cells)**, Indian immunologicals, @3ml, s/c, primary vaccination at 2 months age & revacc after 1 year, (1-3yrs protection). For cross bred and exotic cattle

2. Anti Vector Vaccine

- Symptomatic treatment includes **ANTIPYERETICS -** e.g.paracetamol@10mg/kgB.W. and **ANTIINFLAMMATORY DRUGS -**e.g.meloxicam@0.2-0.3mg/kgB.W, **ANTIDIARREOALS-**e.g.neblon powder@30-50g ,b.i.d.,centrogyLM@4-6boli/day

- Supportive therapy also provided **HEMATINICS -** e.g.ferritas,bolus@2boli/day &inj@1ml/50kg, imferon@0.5-1g/week

- Liver protectants and restoratives may also be given,e.g.liv-52vet@1-2bolus,bid

1. **Heartwater** because of pulmonary edema and hydrothorax. Examination of brain smears and lymph node or spleen impression smears can differentiate between the two diseases.
2. **Trypanosomiasis** because of edema, lymphadenopathy, and anemia. Blood and lymph node smear examination will normally differentiate between the two diseases.
3. **Babesiosis and anaplasmosis** because of anemia. These diseases can easily be differentiated from theileriosis on examination of blood smears.
4. **Malignant catarrhal fever** because of lymphadenopathy and corneal opacity. Examination of blood and lymph node smears will clearly differentiate between the two diseases.