UNIT-5 (PROTOZOA OF VETERINARY IMPORTANCE)

Topic

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

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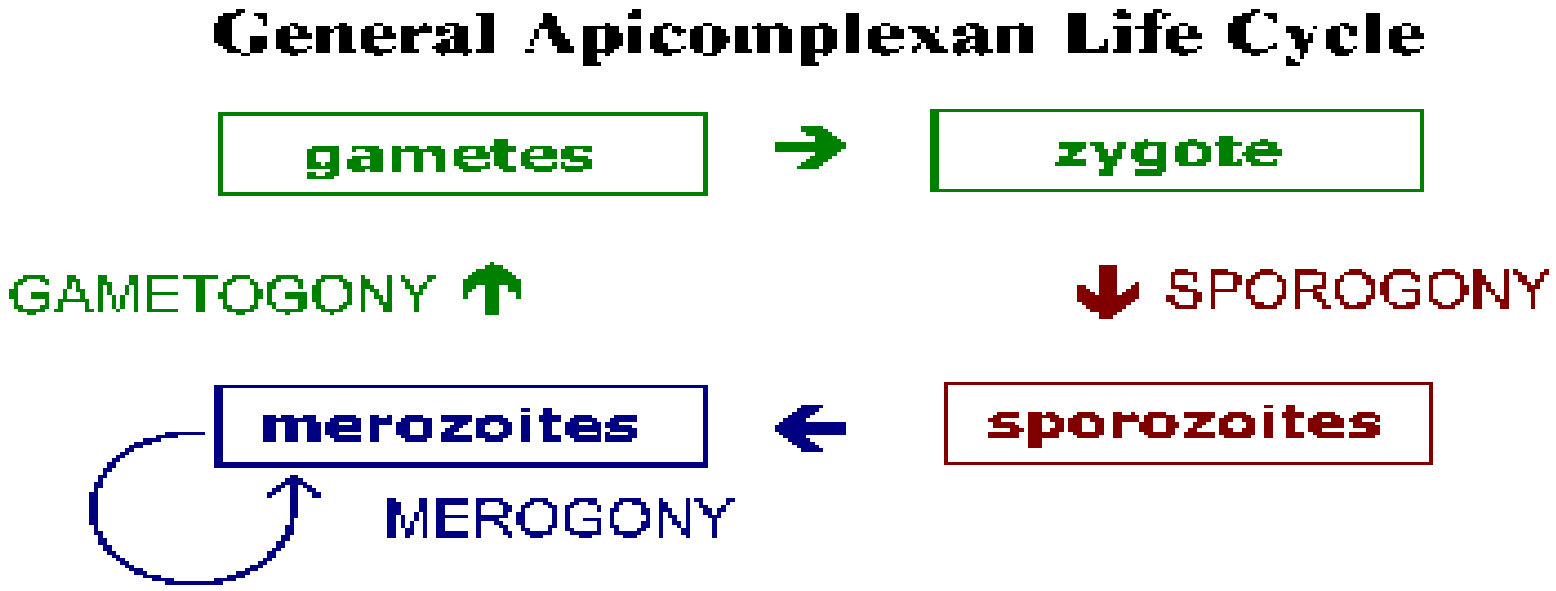
Phylum Apicomplexa
Class Sporozoa
Order Piroplasmida
Family Babesidae
Genus Babesia



- The genus *Babesia* was named after the Romanian bacteriologist **Victor Babes**, who in 1888 attributed "hemoglobinuric fever" of cattle to inclusions he detected within erythrocytes. Believed to be a bacterium, the name was later changed to ***Babesia bovis***
- **Theobald Smith** later identified the causative agent of bovine red water fever (Texas fever) in 1893 as *Babesia bigemina*, accurately described the parasite's life cycle, and demonstrated for the **first** time the arthropod-borne transmission of an infectious disease to a mammal.

Inside the vertebrate host cells

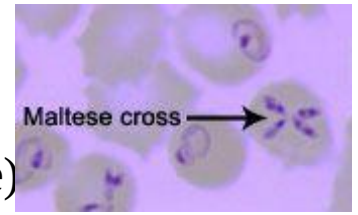
- Pear shaped appearance is ideal morphological feature of the organisms
- When stained with Romanowsky stain, cytoplasm takes **blue** colour and nucleus takes **red** colour.

In the vector

- Round shape, ring like, spindle shape or cigar shaped organisms are found in vector

Two forms of *Babesia*

- **large form** with an average length of more than 3µm and **small forms** less than 2.5µm. The paired large forms generally lie with their narrow ends at an **acute angle** while small forms lie at obtuse angle. Generally the infection with large forms can be successful treated with **trypan blue.**e.g. - *B. bigemina, B.motasi, B. canis, B. Caballi, B. trautmanni* **(BMC2T)**
- In general small *babesia* species are **highly pathogenic** eg. *B. bovis, B. ovis and B. gibsoni.* (Moderately high pathogenic *B. bigemina* & *B. canis*)
- **Maltese cross-** *B.equi, B. microti, B.felis, B. canis* (16 organism some time)



- **Signet Ring stage of RBC-** *B. gibsoni, B. bovis*
- *Babesia* spp. which are closely **related to *Theileria* -** *B. (Theileria) equi, B. (Theileria) microti*
- **CNS symptom** – *B. bovis, B. canis* (clumping of RBC) *& B. caballi*
- **Chronic form** - *B.gibsoni and B. caballi.*

Immunology

- **An Inverse age resistance -** young animals being naturally resistant while older animals are fully susceptible. The passive transfer of maternal antibodies *via* the colostrum is probably responsible in part for this resistance.
- The natural resistance of the young calf to infection usually disappears at 9-12 month of age.
- *Babesia* and *Anoplasma* persist infection. (Exception *B. canis* & *B. divergen*)
- **Pre- immunity**
- When infected animal develop long time immunity against Re-infection with same species of infection.

Spleen

- The spleen play important role in maintaining the immune state of *babesia* infection, since immunity may be broken down by removal of spleen.

Breed of Animal

- ***Bos indicus*** has been suggested to be more resistant than ***Bos Taurus*** **(exotic and cross breed)**

Bovine babesiosis (BB)

Disease: Cattle tick fever, Red water fever, Texas fever (North America), Splenic fever Piroplasmosis,
Host: Cattle and Buffalo
Distribution: Tropics and Sub tropics.

The principal species of *Babesia* are: *Babesia bovis*, *Babesia bigemina* and *Babesia divergens*. Other Babesia that can infect cattle include *B. major*, *B. ovata*, B. *occultans* and *B. jakimovi*.

Tick vectors of *Babesia bigemina* (India)
B. bigemina transmitted by feeding of adult and nymphal stages of one-host *Rhipicephalus* spp. ticks. *Rhipicephalus microplus* (formerly *Boophilus microplus*) and *Rhipicephalus annulatus* (formerly *Boophilus annulatus*); *Rhipicephalus decoloratus*, and *Rhipicephalus evertsi* are also competent vectors

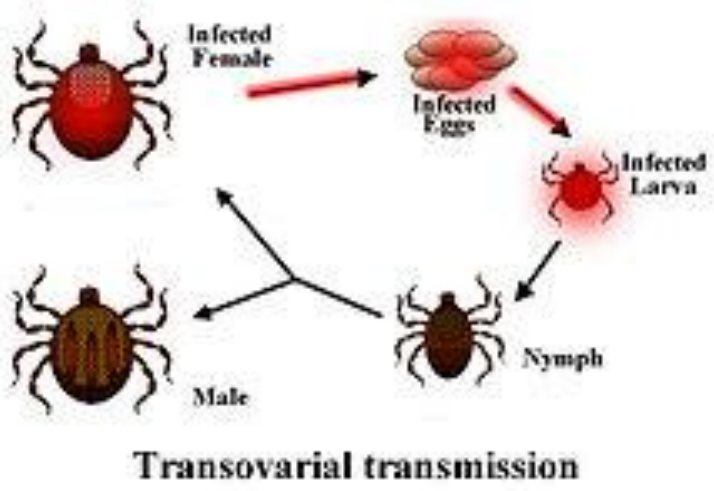
Tick vectors of *Babesia bovis*:
B. bovis transmitted by feeding of larval stages of one-host *Rhipicephalus* spp. ticks
Rhipicephalus microplus and *Rhipicephalus annulatus*; *Rhipicephalus geigyi* is also a competent vector.

Tick vectors of *Babesia divergens*:
principal vector is ***Ixodes ricinus*** is a three-host tick with only adult stages feeding on vertebrates (eg. Cattle)

Virulent	
B. bovis	**Bovine**
B. gibsoni	**Canine**
B. ovis	**Ovine**
Moderately high virulence	
B. bigemina	**Bovine**
B. canis	**Canine**
Moderate virulence	
B. caballi	**Equine**
B. divergens	**Bovine**
B. equi	**Equine**
B. felis	**Feline**
B. motasi	**Ovine/caprine**
B. traumanni	**Porcine**
Low virulence	
B. crassa	**Ovine/caprine**
B. foliata	**Ovine**
B. major	**Bovine**
B. microti	**Rodent/human**
B. occultans	**Bovine**
B. ovata	**Bovine**
B. perroncitoi	**Porcine**
B. taylori	**Caprine**

1. Transovarial or **transovarian transmission**

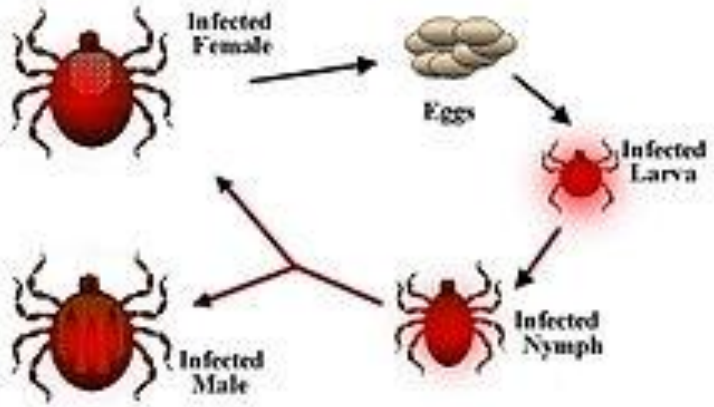
- Transmission of parasites from parent to offspring via the ovaries.
- E.g. one host ticks (*Babesia* infection only)



Transovarial transmission

2. Transstadial transmission

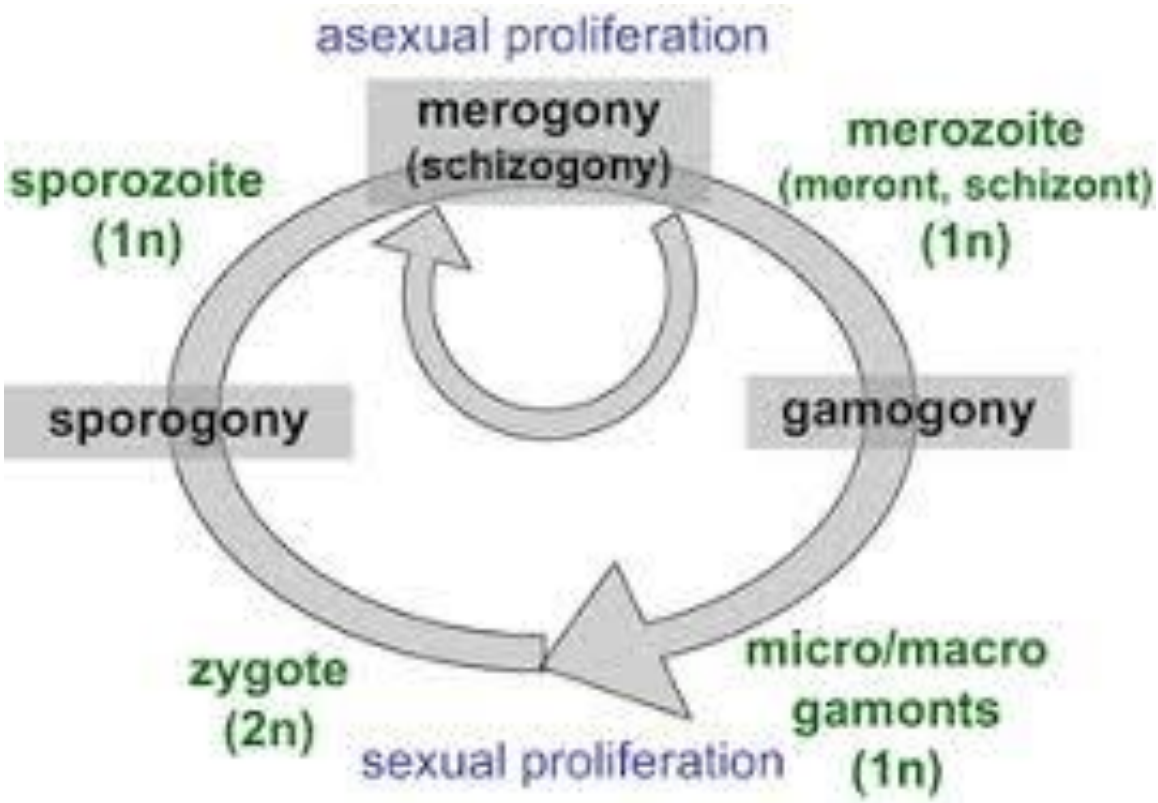
- Transmission of the parasites from one stage to next stage (through the molt to the next stage(s) or stadium)
- E.g. three host ticks (*Babesia* & *Theileria* infection)

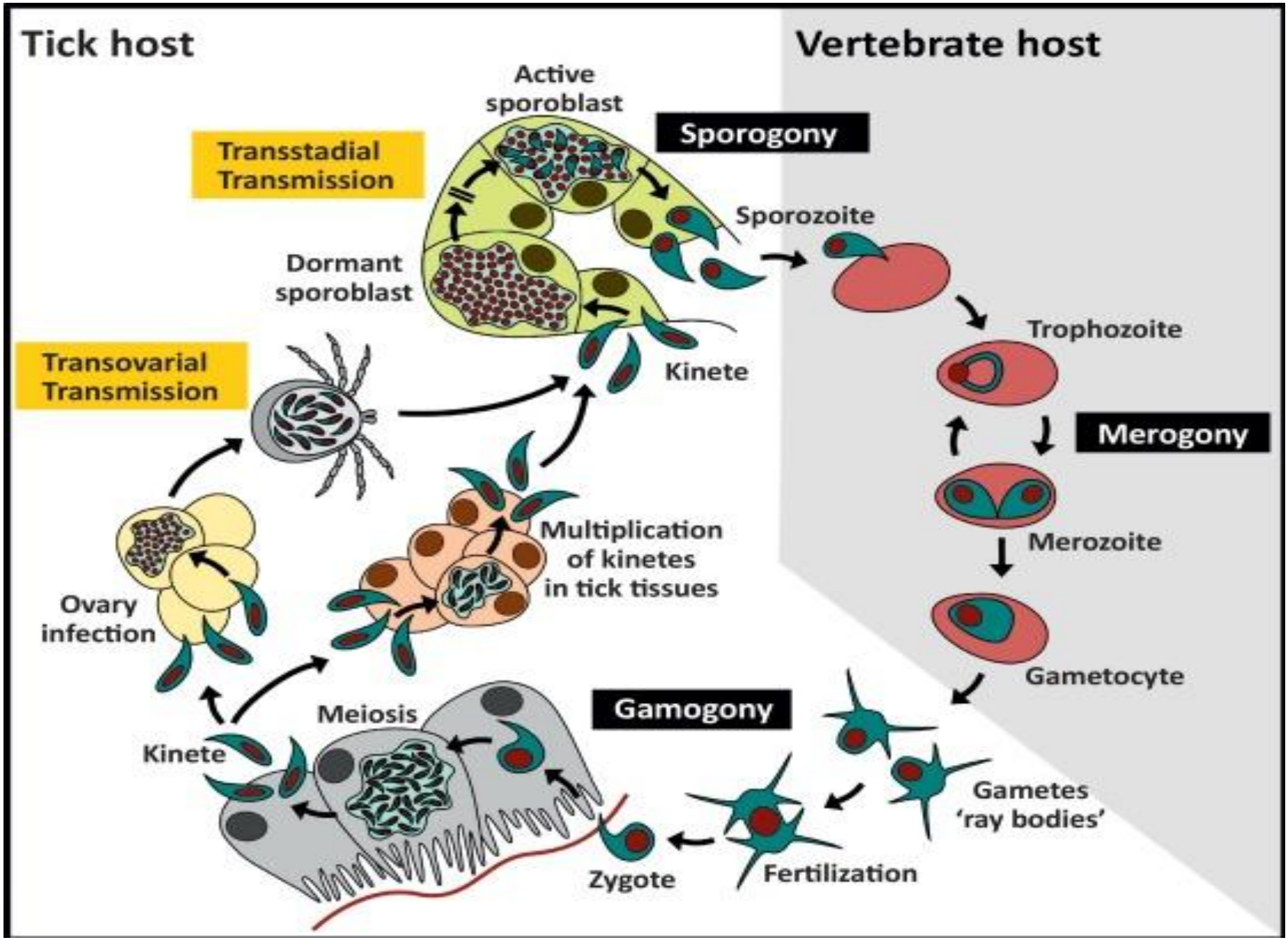


Transstadial transmission

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

2. Asexual multiplication (Intermediated Host) Vertebrates animals




Tick host
Vertebrate host
Active sporoblast
Sporogony
Transstadial Transmission
Sporozoite
Dormant sporoblast
Trophozoite
Transovarial Transmission
Kinete
Merogony
Multiplication of kinetes in tick tissues
Merozoite
Ovary infection
Gametocyte
Gamogony
Meiosis
Kinete
Gametes 'ray bodies'
Zygote
Fertilization

Life cycle

1. Merogony

Ticks during feeding inject sporozoites into blood stream of the host

↓

Sporozoites bind to RBC and enter erythrocytes and multiply by series of binary fission / budding- endodiogeny, endopolygony) / schizogony depend upon species and host involved

↓

Producing merozoites

↓

Each merozoites invade new RBC and lysis huge number of RBC

↓

Infected merozoites are lysed and only few number survive which develop into gamonts/ gametocytes

↓

2. Gamogony

Babesia infected RBC ingested by Ticks

↓

In side the ticks RBC are lysed

Gametes are set free by lysis of infected erythrocytes

↓

developed into micro and macro gametes (uninucleate **stralenkorper bodies/Ray bodies**)

↓

Ray bodies unite to form zygote

↓

The zygotes undergoes further development and multiplication and form pseudopod like structure called as Vermicules/ookinetes/ Kinetes

↓

Kinetes entry to the epithelium cell (enteric cells) of gut tick

↓

Kinetes reinvade the enteric cells and produced new Kinetes, released by rupture of vacuoles in cells (repeating asexual multiplication)

Transovarian transmission (one host tick)

Vermicule enter into hemolymph

↓

Reach to ovary of tick

↓

Infect to ova and then larvae

↓

For further development the vermicule enter the epithelium cell of larvae

↓

Where multiply by binary fission and lysed the enteric cells

↓

Reinvade the enteric cells

↓

Enter the hemolymph when the larvae is changed to Nymphal stage

↓

Finally vermicule reach to the salivary gland and invade the acini cells and multiply

Stage to stage transmission

Vermicule enter into hemolymph and reaches muscles

↓

Tick macrophage engulf it

↓

The pseudocyst of organism occurs by 7^{th} days after Nymph drop off from infected host

↓

Upto 11 to 15 days clubbed shaped organism are present

↓

The clubbed shaped bodies are released when macrophages ruptures and reinvade muscle cells

↓

increase population and form ovoid shape

↓

Then further development occurs in recently metamorphosed adult tick when feed on host

↓

Vermicule/ parasite move to salivary gland and infect acini cells and replicate

3. Sporogony

Sporogony starts after vermicule invasion of tick salivary glands (acini cells), which form the sporont, a polymorphous syncytium

↓

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

↓

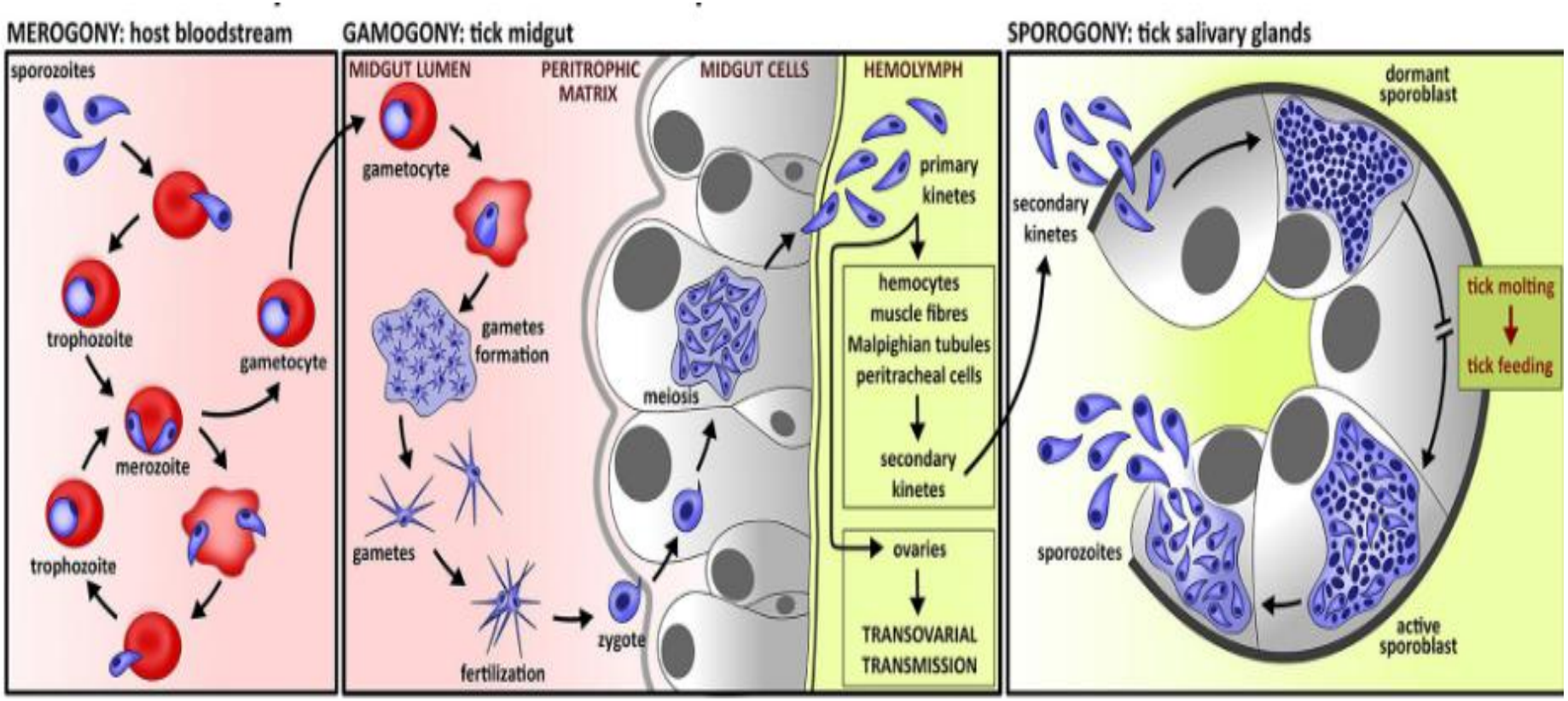
Maturation of the parasite sporoblast starts after tick attachment to the host

↓

Each cell may contain 1000s of minute parasites

↓

These form vermiform sporozoites and break up the host cell Sporzoites transfer to host during feeding of nymphal ticks ($5\text{-}10x10^3$)



Intra-erythorocytic piroplasms (replication) --> gametes --> fuse to form zygote --> migrates to hemocoel (undergo meiosis) --> ookinete --> sporokinete --> sporozoite

- The common belief that sporozoites enter erythrocytes directly (no pre-erythrocytic phase) has not been critically examined.
- The process by which extracellular merozoites invade erythrocytes (induced endocytosis) is similar to that of the plasmodia.
- In the rat *Babesia*, *B. rodhaini*, complement facilitates invasion by modification of either the erythrocyte surface or that of the merozoite; with *B. divergens*, sialic acid appears to be an important ligand for erythrocyte invasion,
- Following entry into erythrocytes, pear-shaped trophozoites (piroplasms) replicate by asynchronous budding rather than by schizogony as occurs in malarial parasites.
- During replication, double-membraned segments develop and pinch off from the parental piroplasm, resulting in both asexually reproducing merozoites and nonreplicating sexual parasites (gametocytes).

- Upon ingestion of infectious blood from a vertebrate host, babesia undergo **syngamy** and replicate in the intestinal epithelium of the tick vector and develop further in the ovaries, hemolymph, hemocytes, muscle fibers, malpighian tubules, peritracheal cells and other tissues and produce secondary kinetes and they invades to salivary glands.
- Sporozoites in salivary glands are deposited in the skin of vertebrate hosts during the tick's blood meal.

Pathogenesis of Babesia infection

- The release of pharmacologically active substances and the destruction of erythrocytes play a major in pathogenesis of *Babesia* infection.
- The disease caused by *B. bigemina* resemble a **haemolytic anaemia** while with *B. bovis* infection**, kinin production** is more important.
- **Kanin- Kallikrein system** produces increased vascular permeability and vasodilatation leading to circulatory stasis and sock- (*B. bovis* and *B. caballi).*
- The initial fall in packed cell volume in *B. bovis* infection is largely attributable to this disturbances rather than erythrocyte destruction.
- Kallikrein also triggers **intravascular coagulation** and this is reflected in the changes of coagulation parameters in *B. bovis*, *B. caballi* and *B. canis* infection.

- The Anemia is associated with the emerging parasites from erythrocytes, Mechanically rupture of cell by parasites.
- Non infected erythrocytes by phagocytosis suggests that **osmotic fragility (***B. bovis* and *B. caballi)*of cells and other adsorption of circulating **antigen-antibody complexes** to surface of RBC leading to RBC removal by phagocytosis
- **Central nervous system** damage is a feature of *B. bovis* and *B. canis.*
- Selective concentration of Parasitized cells occurs in brain capillaries leading to obstruction of the blood flow.
- Infected cells stick to one another and the vessel endothelium, and the increased stickness has been ascribed to a parasite enzyme or antigen which alters surface charge.

- Babesiosis may cause intravascular and extravascular hemolytic anemia via direct red blood cell injury, and indirect through immune-mediated hemolytic anemia.
- Intravascular hemolysis due to rapid multiplication of parasites in the RBCs followed by destruction of the cells
- Extra vascular hemolysis which mostly occurs in spleen, due to phagocytosis of infected and non infected RBCs by activated macrophage system.
- This may cause hemoglobinemia, hemoglobinuria , bilirubinuria and anemia with further consequence of tissue hypoxia, metabolic acidosis hyperkalemia, hypovolemic shock and development of multiple organ dysfunction leading to death

- Destruction of RBC –Anemia-Lack of O2-Hypoxia-Cell death/ necrosis.
- Haemodilation- hypovolumic shock – death of animal.
- Osmotic pressure change - odema & Anemia –haemaglobinuria/ haemaglobinemia/ icterus.
- Hypoxia- heart rate / pulse rate/ breathing/ increase And rumination reduce – diarrhea followed by constipation.

Haematological changes

- PCV, RBC concentration, Haemoglobin- reduced by 5o%, osmolytic fragility of RBC increased by *B.bigemina* infection.
- In acute phase, anaemia is normocytic later become macrocytic and increase MCV.
- WBC decrease first, increase to 2-3 folds after recovery.
- When Haemoglobinuria is seen , the temperature becomes subnormal.
- In serum, increased SGOT,SGPT, BUN, Alkaline phosphate, later stages calcium levels decrease- Serum urea kinin for enzyme, kallikrenin activated several days before parasite reach detectable levels in peripheral vessels leads to shock, decreased PCV.
- Due to increased vascular permeability vasodilatation leading to circulatory stasis-shock

- Intravascular coagulation occurs. These effects are seen before effects of RBC destruction are seen.

- Destruction of RBC, decreased PCV, results in destruction to organ from anaemic anoxia superimposed on that cause by shock.

- Release Hb overloads kidney- red cell stroma and tissue product accelerate kinin release leading to intravascular coagulation.

- Final stage of disease, pathogenesis process that persistently originated from biological active substance of parasites are reinforced by effects of Haemolytic anaemia and produce tissue destruction.

- Apart from RBC destruction, evidence of direct removal of non-infected erythrocytes by phagocytosis, increased osmotic fraility of non- infected cells predisposes spontaneous lysis in small blood vessels.

➢Circulatory Antigens form circulatory complexes with antibody and complement which lodges in kidney and cause glomerulo nephritis.

➢This reaction depletes body which disposes anaphylotoxin- augment shock.

Symptoms

➢The symptoms are more marked in exotic breeds. The incubation period is 1-2 weeks.

➢Fever-41-45.5^0 C marked dullness, listlessness, inappetance, severe anaemia with destruction of RBC, haemoglobinuria, (***Coffee coloured urine***) mucous membrane is pale to icteric ,spleen enlarged soft dark red pulpy, diarhoea, constipation, faeces yellow except in peracute cases affected animal lose condition, emaciated, die.

Hemoglobinuria in cattle

- Appear subdued, rumination suspended, lachrymation, dripping saliva, staggering gait, death in 4 days.

- If recovered, chronic symptoms like intermittent fever and emaciation.

- Chronic case: Organism in blood smear is seen for 3-8 weeks-course extended several weeks with intermittent temperature rise upto 40-42^{0}C, animal thin and emaciated, no marked haemoglobinuria, finally animal recovers, loss of weight, icterus, hard yellow faeces.

Post mortem lesions

Sub cutaneous or Intra muscular oedema with icterus, fat yellow gelatinous, blood thin watery.

Urine dark (coffee coloured dark brown or red), Spleen enlarged with soft dark pulp, liver enlarged with yellow colour distension of gall bladder with dark bile, Plasma reddened.

Kidney congested with high concentration of parasitized RBC. Thrombosis of lung liver and Kidney.

Cerebral form: Onset is sudden- Temperature- 41.7^0C in few hours, death in 12-36 hrs.

Parasites appear to accumulate and multiply in cerebral capillaries since organisms are rarely seen in blood smears

Diagnosis

History

Clinical signs - Coffee color urine, fever, Anemia

Blood examination - Hb / PCV and detection of parasites in blood, thick and thin smear.

Cerebral forms - Examination of cerebral capillary smears.

Culture- MASP (Micro aerophilious stationary phage) first developed by Levy & Ristic 1980 for *B. bovis*, **Exoantigen**

Immuno diagnosis- LAT, IHAT, **IFAT**, **Dot- ELISA** and RIA

Molecular diagnosis

- **RAP-1** (rhoptry associated protein-1) and **AMA-1** have been widely used for B. bovis and B. bigemina, respectively.

- Multiplex nested PCR detection of B. bovis and B. bigemina based on **RAP-1** and **SpeI-AvaI**, respectively.

- DNA probe showed that a **278-bp** fragment could be detected

- **18sr DNA, ITS-1** and **ITS-2** Gene also used

B. bovis

Primary

BoF (5’–3’) CAC-GAG-GAA-GGA-ACT-ACC-GAT-GTT-GA

BoR CCA-AGG-AGC-TTC-AAC-GTA-CGA-GGT-CA

Nested

BoFN TCA-ACA-AGG-TAC-TCT-ATA-TGG-CTA-CC

BoRN CTA-CCG-AGC-AGA-ACC-TTC-TTC-ACC-AT

B. bigemina

Primary

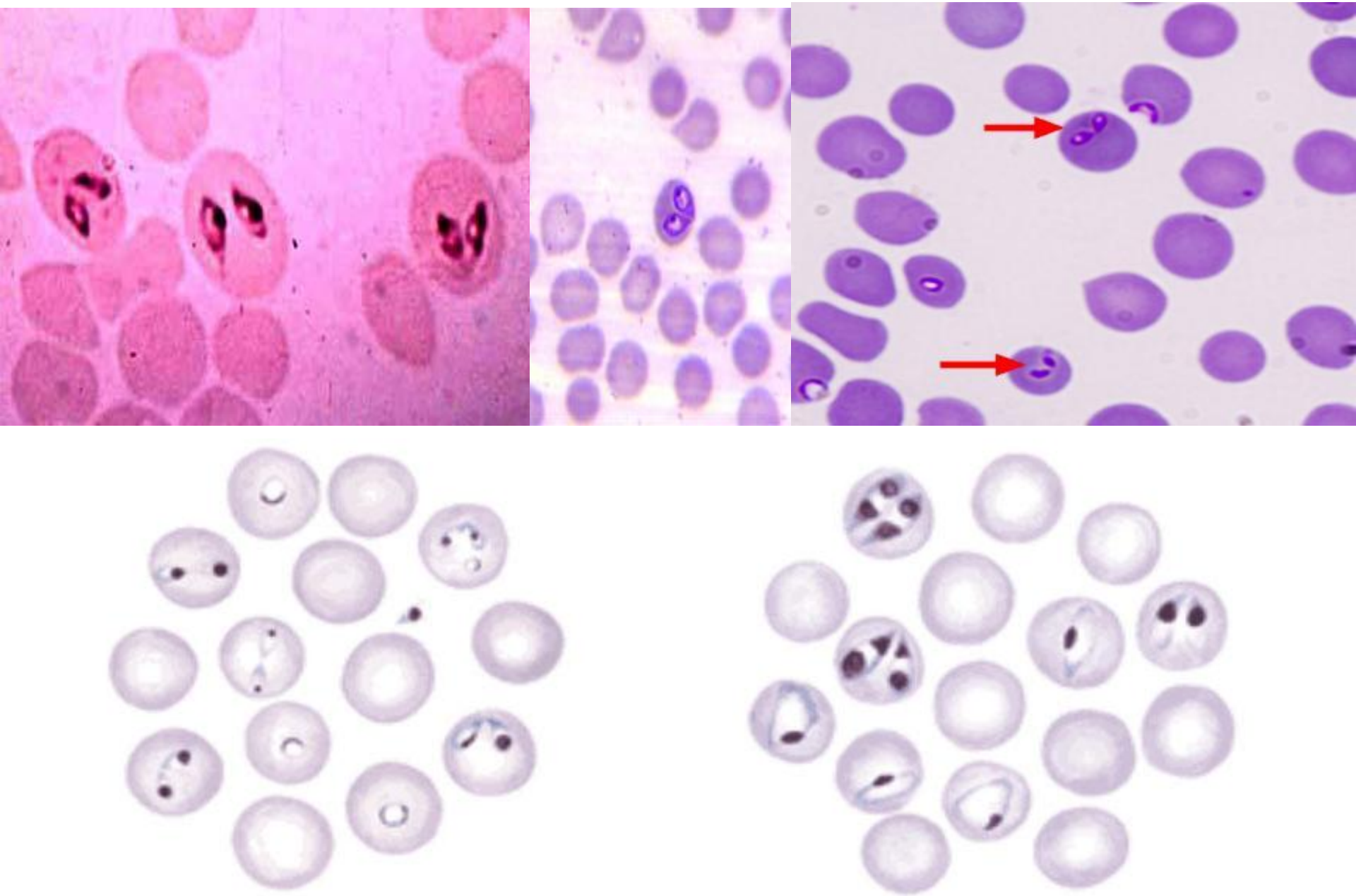
BiIA CAT-CTA-ATT-TCT-CTC-CAT-ACC-CCT-CC

BiIB CCT-CGG-CTT-CAA-CTC-TGA-TGC-CAA-AG

Nested

BiIAN CGC-AAG-CCC-AGC-ACG-CCC-CGG-TGC

BiIBN CCG-ACC-TGG-ATA-GGC-TGT-GTG-ATG



Glóbulos rojos infestados por babesias de 2 tipos.

Fuente: Queensland government.animal and plant health.tick fever.www.dpi.qld.gov.au./tickfever

Treatment

Trypan blue (oldest) @100ml of 1-2% solution in normal saline given I/V only for large form of Babesia effective

Acridine derivatives (Acriflavin, Flavin, Eufalvin) @20 ml 5% solution I/V

Pirevan (Acaprin, Babesan, Piroparv, Piroplasmin, Quinuronium Sulphate) @1 ml 5% solution S/C for 50 kg Body wt.

Phenamidine @12 mg kg S/C in 40% aqueous solution.

Diminazine aceturate @ 2-3.5 mg kg Body wt deep I/M

Diampron @ 10 mg kg I/M or S/C.

Imidocarb dipropionate : Therapeutic and Prophylactic.@1-2mg/kg S/C and for prophylactic @ 3mg/kg.

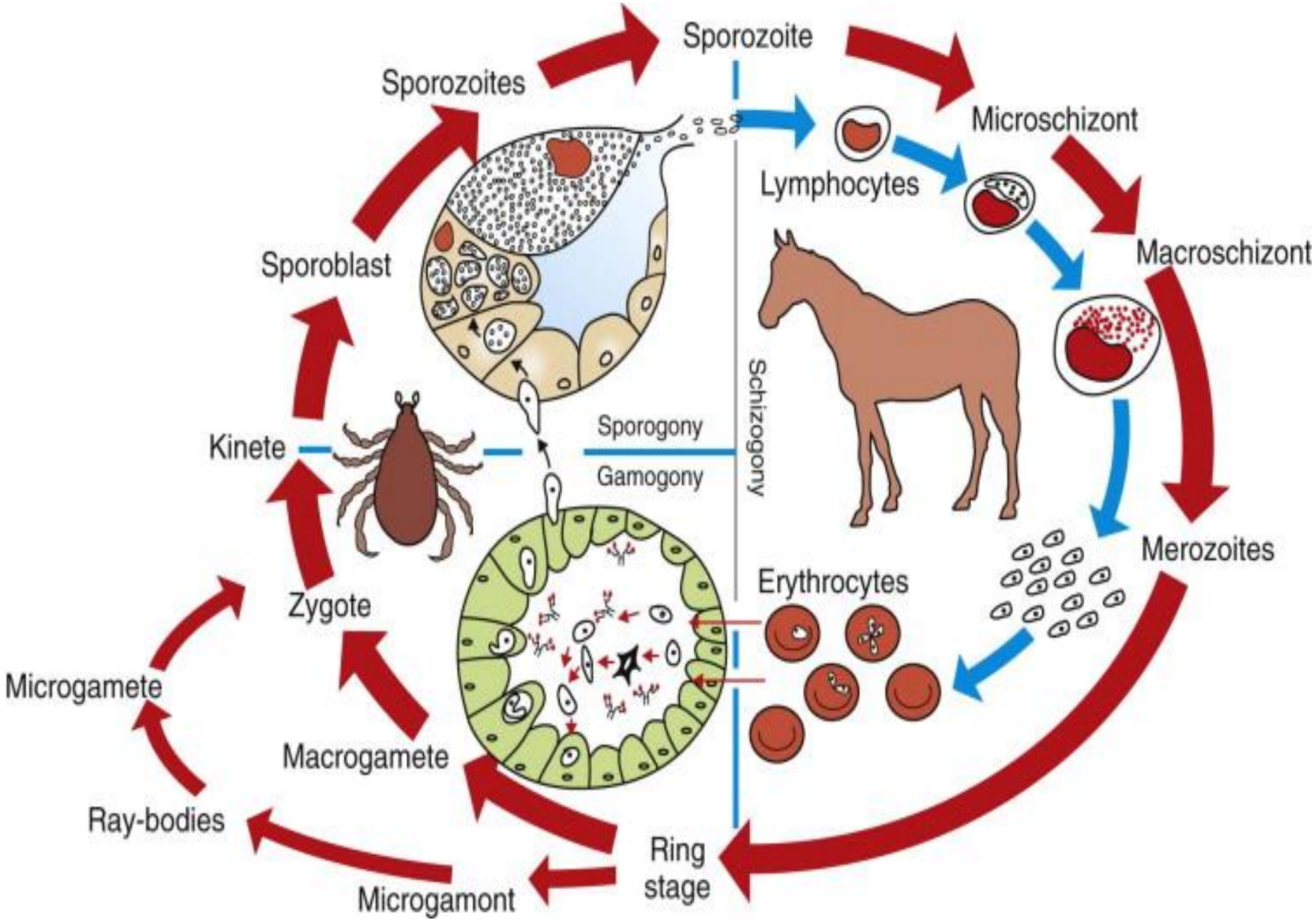
Equine Babesiosis

Babesia caballi (large form)

- CNS form- common , persistent fever , anemia with icterus commonly occurs but haemoglobinuria is rare. The rare cases of acute death from *B. caballi*
- *Babesia* spp. can be found in various organs of tick vectors and do transmit transovarially from egg to larva

Theileria equi (formerly ***Babesia equi***)

- More pathogenic, acute *T. equi* infection, clinical signs are usually related to marked hemolysis and resulting anemia.
- **Exoerythrocytic schizogony** has been found.
- *Theileria equi* develop in salivary glands of tick vector and not found in other tick organs; **not transmitted transovarially** from egg to larva
- Approximately 30 species of ticks in the genera *Dermacentor*, *Hyalomma*, *Haemaphysalis*, *Ixodes*, *Rhipicephalus* and *Amblyomma* have been implicated as natural or experimental vectors,


Sporozoite
Sporozoites
Microschizont
Lymphocytes
Macroschizont
Sporoblast
Schizogony
Sporogony
Gamogony
Kinete
Merozoites
Erythrocytes
Zygote
Microgamete
Macrogamete
Ray-bodies
Ring
stage
Microgamont

- Horses with acute infection initially develop nonspecific signs such as high fevers, sometimes in excess of 104°F, lethargy, anorexia, weight loss, and peripheral edema.

- Petechiations caused by thrombocytopenia are often observed on mucous membranes, including the nictitating membrane.

- Signs of hemolytic anemia follow and include icteric or pale mucous membranes, tachycardia, tachypnea, weakness, and pigmenturia (because of either hemoglobinuria or bilirubinuria).

- Other less common clinical presentations include secondary development of pneumonia, pulmonary edema, cardiac arrhythmias, catarrhal enteritis, laminitis, and central nervous system disease characterized by ataxia, myalgia, and seizures

Diagnosis

❖the competitive inhibition enzyme-linked immunosorbent assay (cELISA), The cELISA for *T. equi* utilizes recombinant **EMA-1** and specific monoclonal antibodies

❖A recombinant form of **RAP-1** was also developed for the *B. caballi* cELISA.

❖In NRCE Hisar - Recombinant equine merozoite surface antigen-2 (**rEMA-2),** a **52 kDa** recombinant protein based ELISA (r-ELISA) was developed for detection of specific antibodies for diagnosis of *T. equi* infection in equine serum.

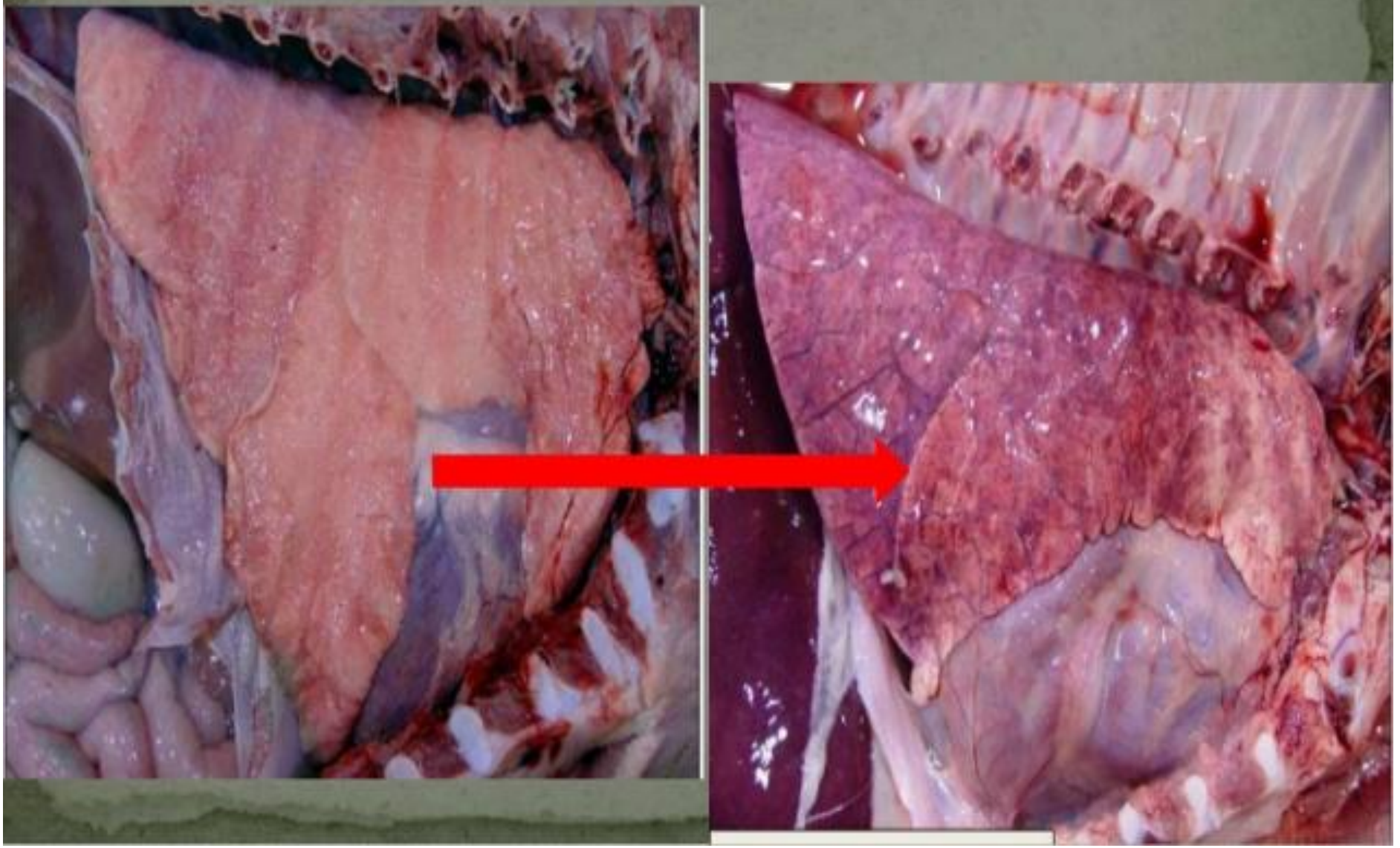
❖A total 971 serum samples were found positive for *T. equi* antibodies, indicating prevalence of specific antibodies in 37.76% Indian equine population.

-3- Icterus

Due to hemolytic jaundice

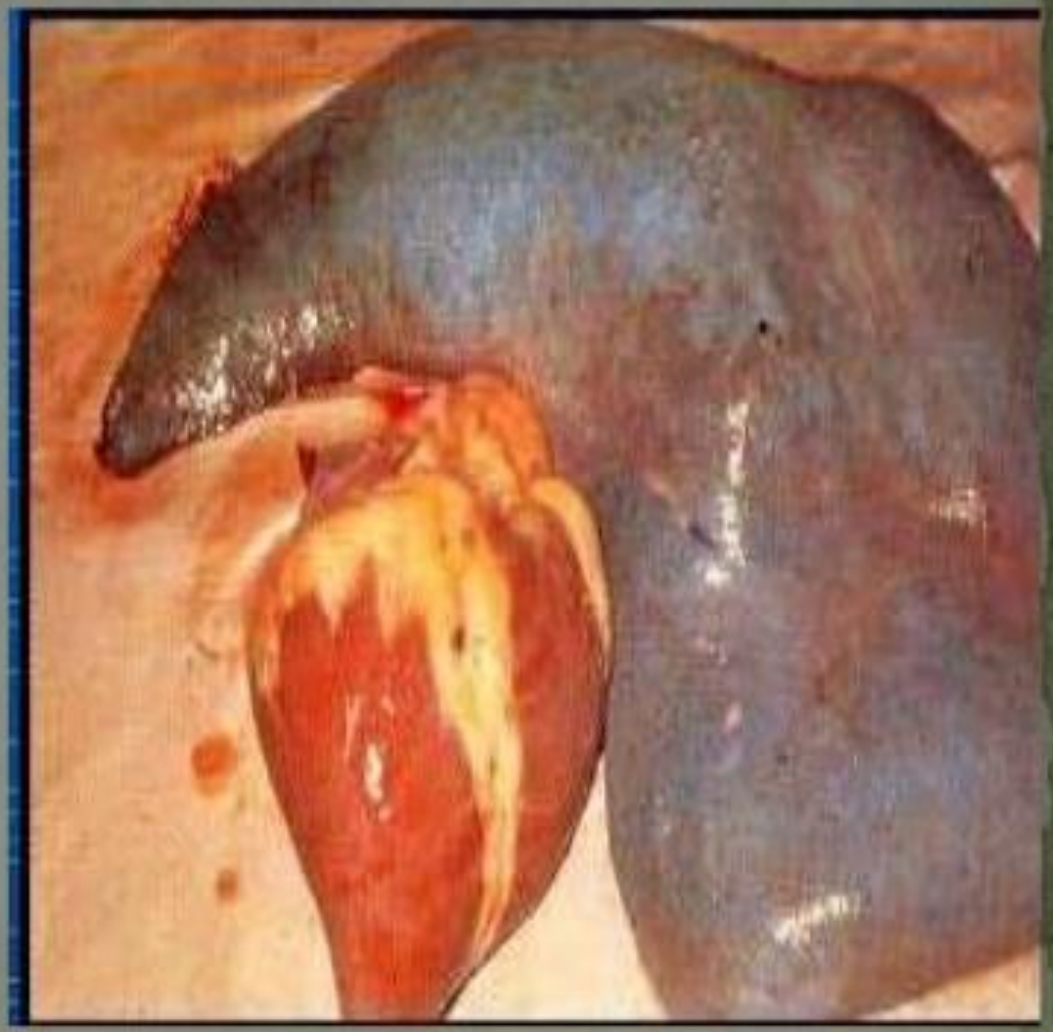
Icterus m.m of lips

-3-
Edema in lung

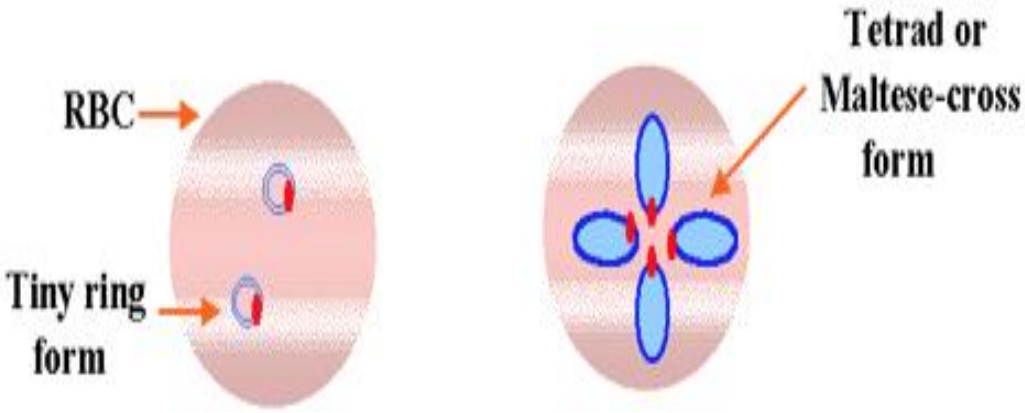


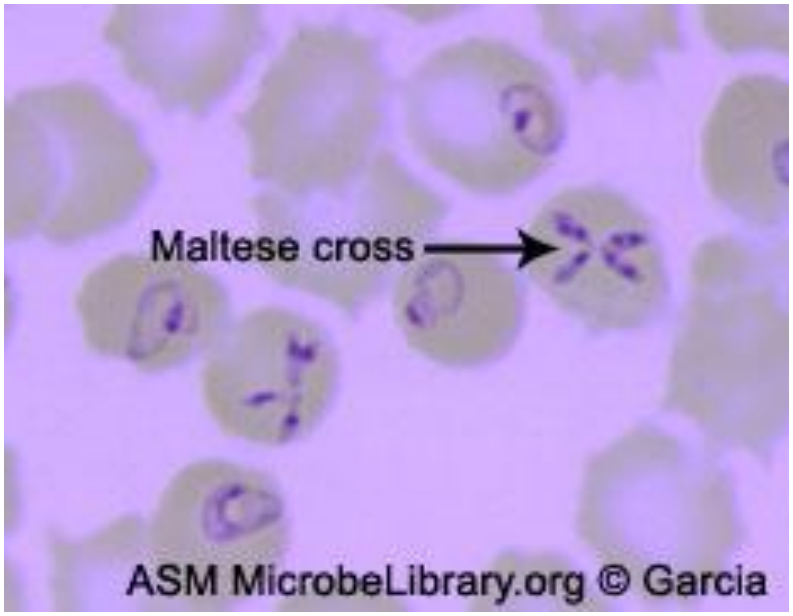
-1-

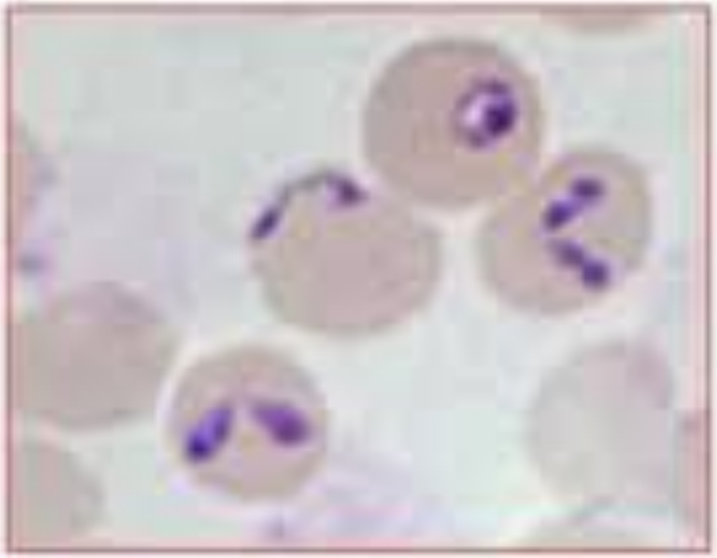
Enlargement of Spleen (splenomegaly)



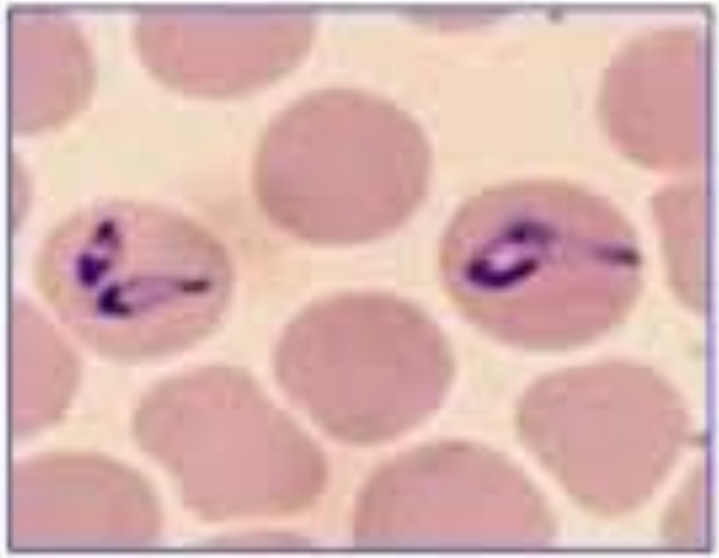
Babesia species







Babesia bovis



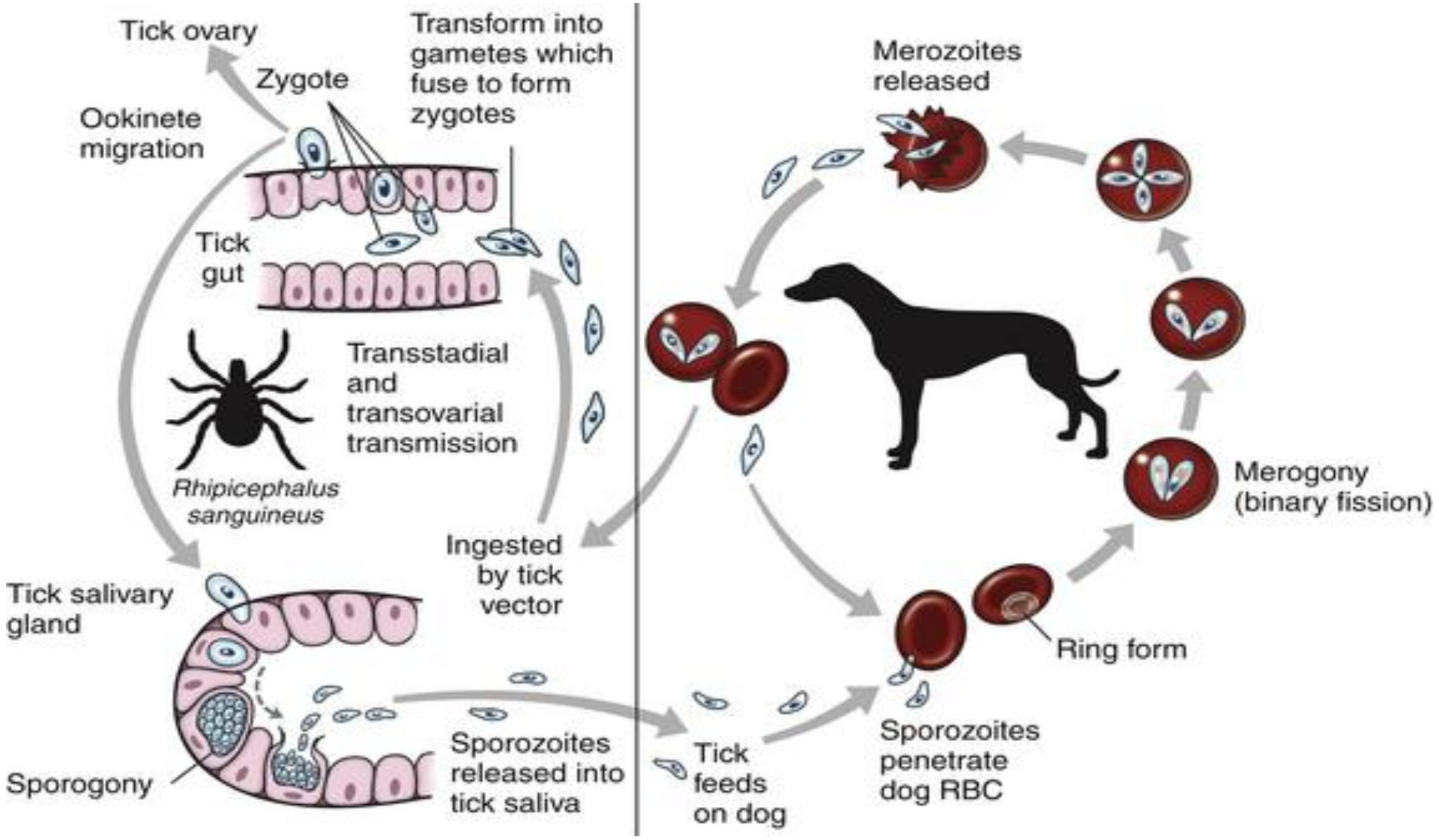
Babesia caballi

Canine Piroplasmosis/ Biliary fever / Jaundice malignant

Canine Babesidae have historically been classified as “large *Babesia*” (*Babesia canis*) and “small *Babesia*” (*Babesia gibsoni*) based on the size of their intraerythrocytic forms.

Advent of molecular phylogenetic analysis, in particular that of the 18S rRNA gene, it was recognized that the subspecies are

1. ***Babesia canis canis*** transmitted by *Dermacentor reticulatus* (in Europe)(large form , most pathogenic species)
2. ***Babesia canis rossi*** transmitted by *Haemaphysalis elliptica* (in South Africa).
3. ***Babesia canis vogelis*** transmitted by *Rhipicephalus sanguineus* (in tropical and subtropical regions),
4. ***Babesia gibsoni*** **(**chronic form*)* transmitted by *Haemaphysalis bispinosa* and *Haemaphysalis longicornis* (Asia, North America, northern and eastern Africa, and Europe)



Intra-erythorocytic piroplasms (replication) --> gametes --> fuse to form zygote --> migrates to hemocoel (undergo meiosis) --> ookinete --> sporokinete --> sporozoite

- Clinical presentations in this study ranged from peracute to subclinical and chronic forms.

- The fatal cases had exhibited less commonly observed signs like melena, local erythma of the skin and bleeding from venepuncture site.

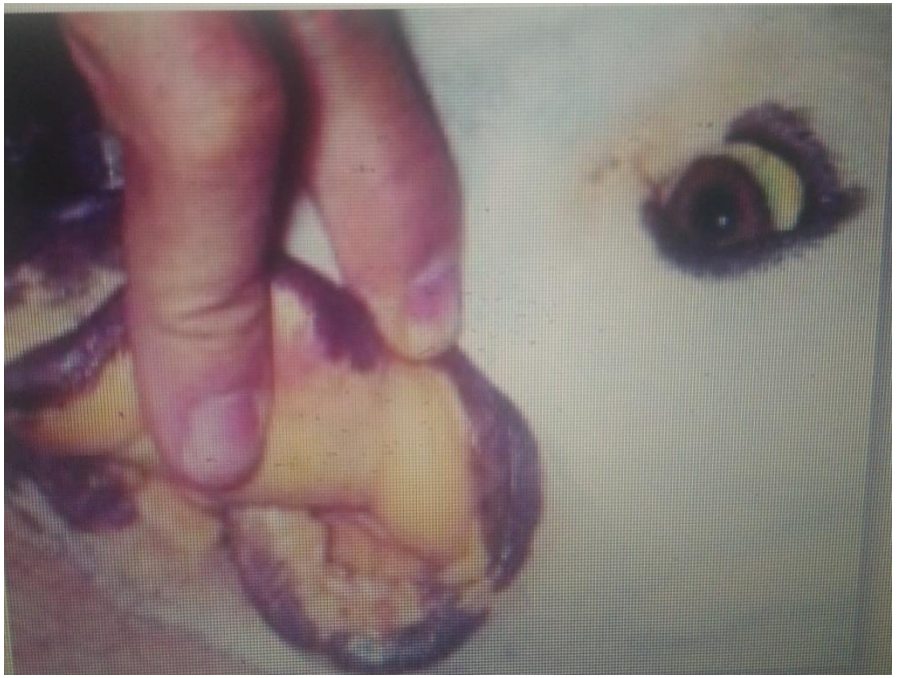
- Bleeding tendencies were the result of intravascular and extra vascular haemolyis and the cause of which could be thrombocytopenia

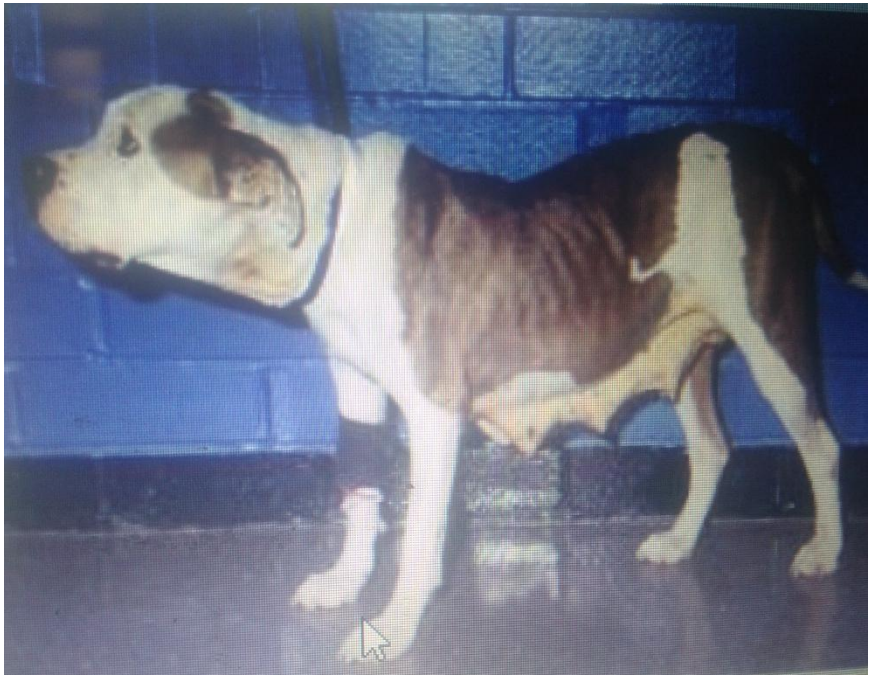
- Acute forms in this study were characterized by fever, lethargy, hemolytic anemia, lymphadenopathy and spleenomegaly,

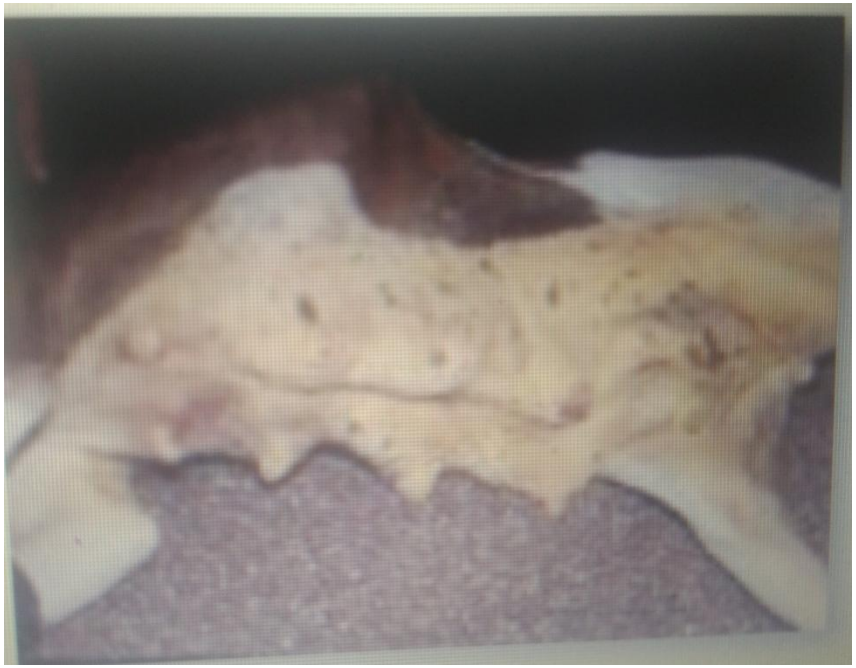
- Neutrophils were found to be significantly increased (78%) and lymphocytes were reduced (18%).

- the history of weakness, anorexia and general malaise. On clinical examination days, the animal had temperature of 104.5 F and pallor of conjunctival and oral mucus membrane.

- Dog infected with *B.gibsoni* from whole blood which revealed haemoglobin value of 3 g%, PCV 20 % and total RBC count 1.1 millions/mm3, Anemia (PCV < 25%), thrombocytopenia (Platelet count < 150000/mm3), leukopenia (WBC count < 5000/mm3).







- ✓Fever, Lack of energy, Lack of appetite
- ✓Enlarged abdomen
- ✓Weakness
- ✓Lethargy
- ✓Pale gums and tongue
- ✓Red or orange urine (Colored urine)
- ✓Jaundice (yellow tinge to skin, gums, whites of eyes, etc)
- ✓Enlarged lymph nodes
- ✓Enlarged spleen
- ✓Weight loss
- ✓Discolored stool

Diagnosis

- **Microscopic examination** (blood smear)

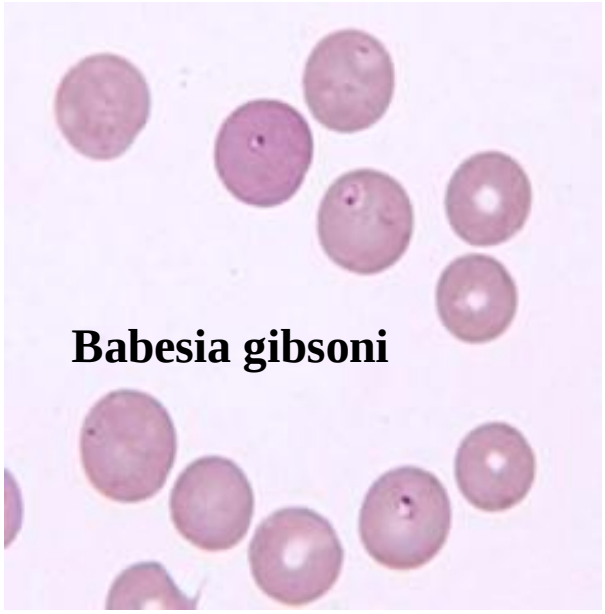
- **Blood analysis ; CBC** ◗ Thrombocytopenia is the most common feature regardless of the Babesia spp.
◗ Macrocytic anemia and autoagglutination are variable (not all animals are anemic).
◗ Leukogram is highly variable.

- **Serum biochemistry profile**: hyperglobulinemia, hyperbilirubinemia, increased liver enzyme activities, azotemia (*B canis rossi*, *B gibsoni*), and hypoalbuminemia.

- **Urinalysis:** bilirubinuria, hemoglobinuria, and proteinuria.

- **Coombs test (direct):** Can be positive in 85% of cases
◗ Direct Coombs test is used to test for autoimmune hemolytic anaemia


Babesia gibsoni


Babesia gibsoni


Babesia canis

●**Coagulation testing:** ◗ Thrombocytopenia. ◗ Disseminated intravascular coagulation has also been reported.

● **Animal inoculation**

● **Serology**
(IFAT, ELISA, Complement assay, immunoblot)
◗ Indirect fluorescent antibody (IFA) testing: Cannot differentiate among Babesia spp.

● **Molecular tools**
(PCR, LAMP…)
◗Polymerase chain reaction (PCR) testing: High specificity and sensitivity. Can determine species or subspecies with specific PCR assay or DNA sequencing

Recently a rapid simple and more sensitive technique called Loop mediated Isothermal Amplification (LAMP) has been developed and by the use of additional loop primers to increase efficiency and rapidity.

LAMP technique associated with fluorescent dyes like Syber Green allows visual detection of amplified products and measurement of turbidity.

Unlike PCR, this technique doesn’t need extraction of DNA as well as use of a thermocycler as it can be carried out at a temperature range of 60-65˚C.

LAMP (Loop mediated isothermal amplification) assay targeting hyper variable region of the 18S rRNA gene and indirect-ELISA, dot-ELISA and sandwich-ELISA using **rBgSA-1** protein were developed for diagnosing *Babesia gibsoni* infection in dogs.

Treatment

Most dogs show response to treatment in 24–72 hours; however, it can take up to 7 days before results are apparent

Imidocarb dipropionate (6.6 mg/kg IM once, repeat in 7–14 days) reduces morbidity and mortality in most cases of *Babesia* spp infection.

- Treatment of choice for *B. canis vogeli* but is ineffective for clearance of *B. gibsoni* and *B. conradae*.
- Pretreatment with atropine (0.02 mg/kg SC 30 minutes before Imidocarb) reduces cholinergic side effects (ie, salivation, lacrimation, vomiting, diarrhea, tachycardia, dyspnea).

Diminazene aceturate (3.5–7 mg/kg SC or IM q1–2wk) is effective against *B. canis* but is unavailable in the United States.

- Not capable of clearing *B. gibsoni* or *B. conradae* infection.

● **Atovaquone** (13.3 mg/kg PO q8h) and azithromycin (10 mg/kg PO q24h) combination therapy has effectively cleared *B. gibsoni* and *B. conradae* infections.

◗ Atovaquone should be given as liquid suspension with a fatty meal to ensure adequate absorption.

● **Clindamycin** (25 mg/kg PO q12h), Metronidazole (15 mg/kg PO q12h), and doxycycline (5 mg/kg PO q12h) have been associated with clearance of *B. gibsoni* after administration for ~3 months, but true treatment efficacy is unknown.

◗ Clindamycin combination protocol in this study showed a rapid recovery rate than that of Diaminazine aceturate and such cases made uneventful recovery.

● **Oxytetracycline** @ 20 mg/kg body weight intravenously daily for three days followed by Doxycycline @ 5 mg/Kg orally for seven Dimenazine aceturate @ 5 mg/Kg I/M

Precautions

- The authors do not recommend immunosuppressive drugs for treatment of babesiosis.

Patient Monitoring

- In hospital settings, hematocrit concentration and platelet count can be monitored daily until improvement is seen.

- Continue monitoring q1-2wk until hematocrit and platelet numbers have normalized.

- PCR testing at 60 and 90 days after treatment is recommended to rule out treatment failure.

Complications

- At high doses, imidocarb dipropionate and diminazene aceturate have been associated with liver and kidney failure.

Pirodog and Nobivac Piro

- **Pirodog**@ (ND) is a novel vaccine against canine babesiosis. It is commercially distributed and has been used in France since May 1988
- Pirodog@ (ND) is a first generation vaccine prepared from culture supematants of ***B. cani*s** with a saponln adjuvant (MOREAU & LAURENT, 1984).
- Pirodog (ND) is injected subcutaneously.
- Primary vaccination is done by giving 2 injections of Pirodog at 21-28 d intervals, in winter or in summer during periods when epidemiological peaks do not occur.
- A booster injection is necessary every year after primary immunization.

Nobivac Piro

- The active substance of Nobivac Piro is soluble parasite antigen (SPA) from ***Babesia cani***s and ***Babesia rossi*** cultures.
- Onset of immunity: 3 weeks after the basic vaccination course.
- Duration of immunity: 6 months after the last (re-)vaccination.