



UNIT-5 (PROTOZOA OF VETERINARY IMPORTANCE)

Topic

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



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- ❖ Certain Rickettsia have previously been considered to be protozoa these include genera Anaplasma, Haemobartonella, Aegyptianella etc.

Rickettsia	Protozoa
Unicellular prokaryotic organism (Nuclear membrane absent)	Unicellular eukaryotic organism (Well-developed nuclear membrane)
Obligatory Intra cellular organism	Intra or extra cellular
Cytoplasm absent	present
Transmission of infection only by arthropods	Transmission of infection by arthropod as well as contaminated food and water
Cannot grow into culture media but cultivable only in live tissue	Can be grow into culture media
E.g. Anaplasma and Ehrlichia	E.g. Babesia and Trypanosoma

Kingdom: Bacteria
Phylum: Proteobacteria
Class: Alphaproteobacteria
Order: Rickettsiales
Family: Anaplasmataceae

Genus:	<i>Anaplasma</i>	<i>Host</i>	<i>Ehrlichia</i>	<i>Neorickettsia</i>
Species	<i>A. marginale</i>	Cattle	<i>E. canis</i>	<i>N. risticii</i>
	<i>A. centrale</i>	Cattle	<i>E. chaffeensis</i>	<i>N. sennetsu</i>
	<i>A. bovis</i>	Cattle	<i>E. ewingii</i>	
	<i>A. ovis</i>	Sheep, goat	<i>E. muris</i>	
	<i>A. platys</i>	Dogs	<i>E. ruminantium</i>	
	<i>Aegyptianella pullorum</i>	Birds		
	<i>A. phagocytophilum</i>	Wide host range: ruminants, small mammals, horses, birds, and humans		
<i>Candidatus Neoehrlichia mikurensis</i> ^a , previously known as <i>Candidatus Ehrlichia walkerii</i>				

Dumler et al. 2001; Andersson and Raberg 2011

^a This organism still needs to be classified in appropriate genus (Jahfari et al. 2012)

Aetiological agent		Disease	Vector	Infected organism or host	Infected cell
before 2001	after 2001				
<i>Ehrlichia bovis</i>	<i>Anaplasma bovis</i>	bovine anaplasmosis	<i>Haemaphysalis</i> sp. <i>Rhipicephalus</i> sp. <i>Amblyomma</i> sp.	ruminants farming, small mammals	monocytes
<i>Anaplasma ovis</i>	<i>Anaplasma ovis</i>	bovine anaplasmosis	<i>Dermacentor</i> sp.	small ruminants (sheep, goats)	erythrocytes
<i>Anaplasma marginale</i>	<i>Anaplasma marginale</i>	bovine anaplasmosis	<i>Ixodes</i> sp. <i>Dermacentor</i> sp.	ruminants farming	erythrocytes
<i>Anaplasma centrale</i>	<i>Anaplasma centrale</i>	bovine anaplasmosis	<i>Ixodes</i> sp. <i>Haemaphysalis</i> sp.	ruminants farming	erythrocytes
<i>E. equi</i> <i>E. phagocytophila</i> Czynn timer HGE	<i>Anaplasma phagocytophilum</i> (HGA agent)	human and animal granulocytic anaplasmosis	<i>Ixodes</i> sp. <i>Dermacentor</i> sp.	small ruminants forming and wild, horses, dogs, humans	granulocyte
<i>E. platys</i>	<i>Anaplasma platys</i>	canine cyclic thrombocytopenia	<i>Rhipicephalus sanguineus</i>	dogs	platelets

Anaplasmosis in Bovines

- ❖ Anaplasmosis, also called Gall sickness, is a tick-borne disease of ruminants caused by obligate intraerythrocytic rickettsia infection. It is characterized by initial high fever, weakness, anemia, emaciation and Jaundice
- ❖ *Anaplasma marginale* (most pathogenic) primarily in **cattle** but also reported in buffalo, sheep, goat and some wild ruminants
- ❖ *Anaplasma centrale* Ruminants
- ❖ *Anaplasma bovis* Ruminants
- ❖ *Anaplasma ovis* (Sheep and goats)
- ❖ *Anaplasma centrale* causes mild anaplasmosis in cattle and was originally isolated in Africa, but is now widely used as an immunizing agent for cattle
- ❖ At least 20 different species of ticks have been reported to transmit *A. marginale* worldwide
- ❖ In general, tick vectors of *A. marginale* include *Boophilus* spp., *Dermacentor* spp., *Ixodes ricinus* and *Rhipicephalus* spp., while *Amblyomma* spp. do not appear to transmit *A. marginale*

Epidemiology

Distribution

- ❖ Bovine anaplasmosis is widely distributed throughout the tropics and sub-tropics, particularly in exotic and crossbred cattle. The geographic distribution of the disease is dependent on the density and distribution of tick vectors and reservoir host.

Seasonal occurrence

- ❖ Anaplasmosis / Gal sickness outbreaks occur in warmer months (summer and autumn) when tick and biting fly challenges are high. Usually only adult animals get infected, and then only a small group or single animals

Animals susceptibility

- ❖ Cattle are the major hosts but infections also occur in zebra, water buffalo, bison, sheep, goats, deer, antelope, giraffes and camel
- ❖ *Bos indicus* type cattle are less susceptible because of their greater resistance to tick infection than *Bos Taurus* and their cross bred
- ❖ Anaplasmosis infection is higher in female than male animals due to hormonal disturbances, milk production, draught power and breeding system which pose it to weakened immune system

Age-related host susceptibility

- ❖ Calves are much more resistant to disease (although not infection) than older cattle
- ❖ Under 6 months of age, the illness is rare
- ❖ Animals between 6 months and 1 year old show only mild signs or remains asymptomatic
- ❖ Animals between 1 and 2 years of age suffer from acute but rarely fatal disease
- ❖ Cattle more than 2 years of age are most likely to have severe, acute and potentially fatal disease
- ❖ This resistance may be due to colostral antibody from immune dams, and regenerate red blood cells faster than adults

Transmission

Anaplasma marginale can be transmitted by three methods

1. Biological transmission

- ❖ Biological transmission occurs through ticks. Once a tick acquires the organism through a blood meal, the organism infects the tick's gut cells, and Malpighian tubules. Over time, other tissues within the tick, including salivary glands, become infected. When a tick feeds on cattle, it transmits the organism through its saliva.

2. Mechanical Transmission

- ❖ Mechanical Transmission by blood sucking flies and human error it can transmit the organism from infected or carrier animal to susceptible animal within five minutes

Blood sucking flies

- ❖ Long biting mouth parts penetrate through
- ❖ the skin e.g. **Horse-flies** (Family Tabanidae) **Stable fly** (*Stomoxys calcitrans*) and some time mosquitoes also
- ❖ Short mouth parts don't penetrate through the skin e.g. horn fly (*Haematobia irritans irritans*)

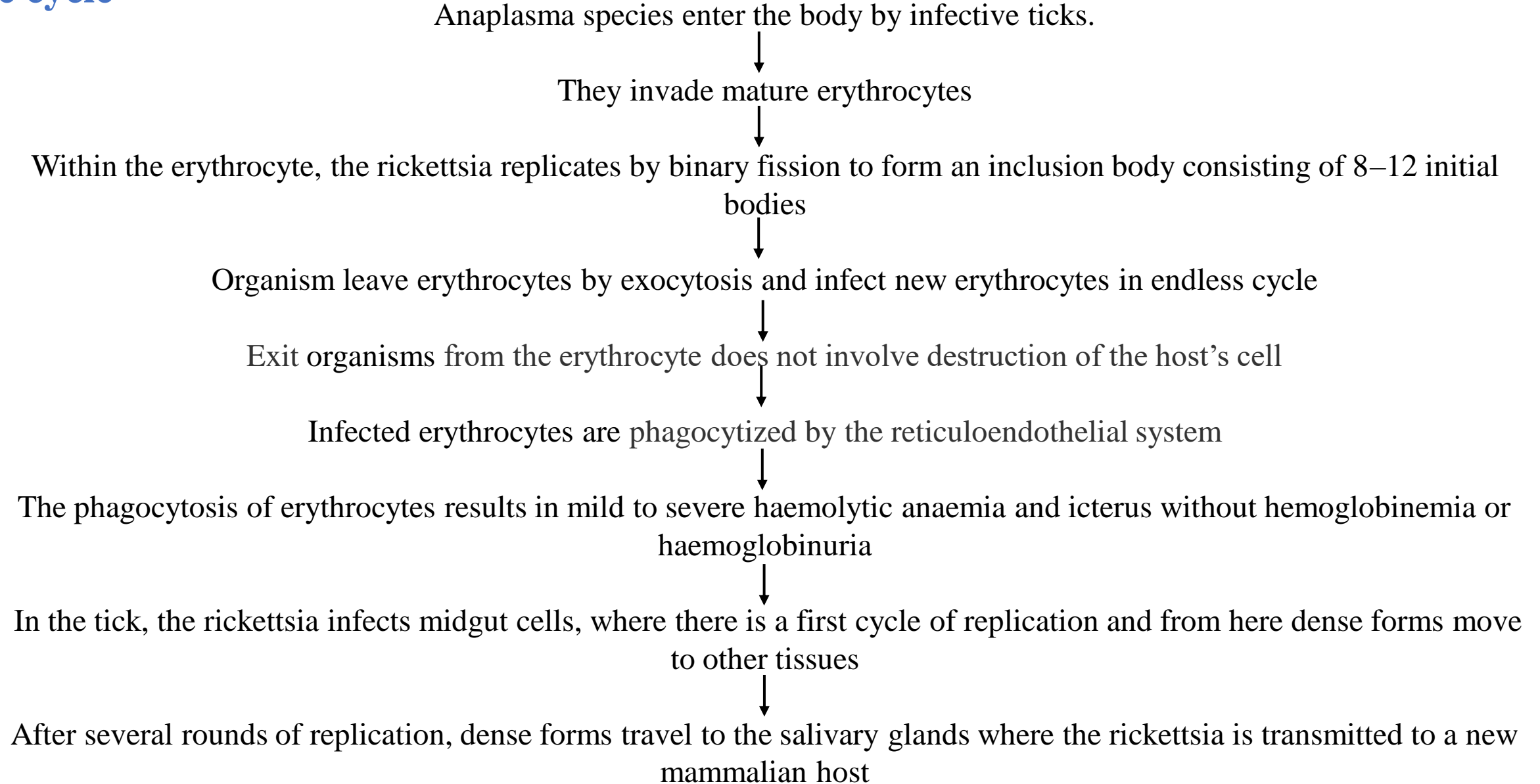
Human error

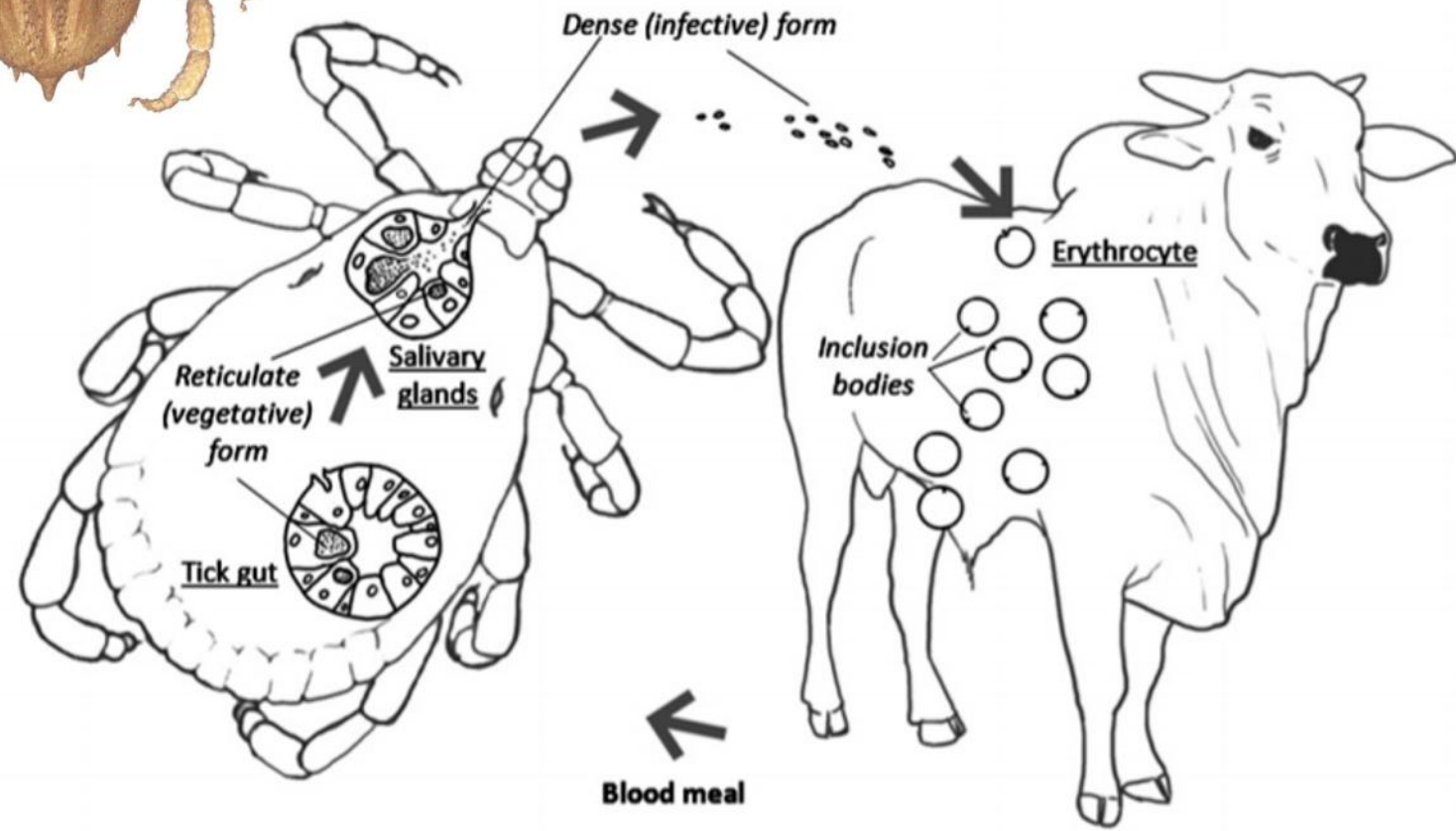
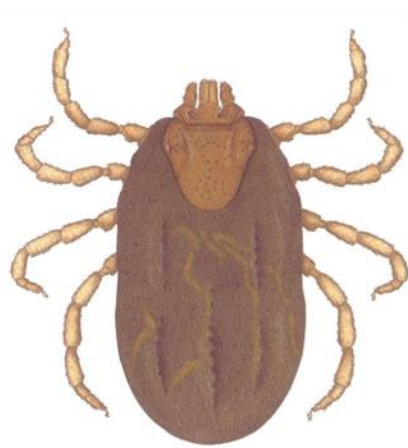
- ❖ Man is also an excellent transmitter of Anaplasmosis through reusing of needles, dehorner, ear taggers, castrating knives or other surgical instruments, tattoo instruments, and blood transfusion

3. Transplacental

- ❖ Transplacental (intrauterine) transmission is also reported
- ❖ Infected erythrocytes move across the placenta in the uterus from infected cows to their offspring, without amplification of *A. marginale*
- ❖ This transmission appears to occur during the second or third trimester of pregnancy

Life cycle





Anaplasma marginale life cycle

- ❖ When cattle are exposed to *A. marginale*, an incubation period of 7-60 days follows depending on the infective dose
- ❖ Depending upon the strain of *Anaplasma* and the susceptibility of the host, from 10% to 90% of the erythrocytes may be parasitized in the acute stage of the infection
- ❖ At least 15% of the erythrocytes have to be parasitized before there is clinical disease
- ❖ The severity of signs depends on:
 - ❖ Age of animal
 - ❖ Previous exposure to infection.
 - ❖ Generally, older animal at first exposure, show more severe signs

Clinical Signs

- ❖ Bovine anaplasmosis have different forms of clinical phases including; Per acute, Acute, Chronic and Mild

1. Per acute form

- ❖ This usually involves **cattle over three years** old infection for the first time and is frequently fatal
- ❖ Pyrexia with rapid loss of milk production
- ❖ Anaemia with very pale mucous membranes
- ❖ Rapid breathing with excessive salivation
- ❖ Nervous signs and abnormal behaviour in some cattle

2. Acute form

- ❖ Acute anaplasmosis is **most common**
- ❖ This is seen in cattle up to **three years old** and is occasionally found in cattle between one and two
- ❖ Progressive pyrexia reaching 41°C



Anaplasmosis



- ❖ Anaemia, weakness and loss of milk yield
- ❖ Depression, inappetence, dehydration and laboured breathing
- ❖ Enlarged lymph nodes
- ❖ Jaundice or abortion in some cattle
- ❖ Temporary loss of fertility in Bulls

3. Chronic form

- ❖ The signs may follow an acute infection with gradual emaciation

4. Mild form

- ❖ This form is mainly present in cattle infected under one year old
- ❖ Signs are usually few with a mild pyrexia

Post mortem lesions

- ❖ Emaciated carcass, pale tissue and thin watery blood
- ❖ Congested kidney, enlarged liver and spleen
- ❖ Oedematous lymph nodes
- ❖ Petechial hemorrhage in epicardium and pericardium

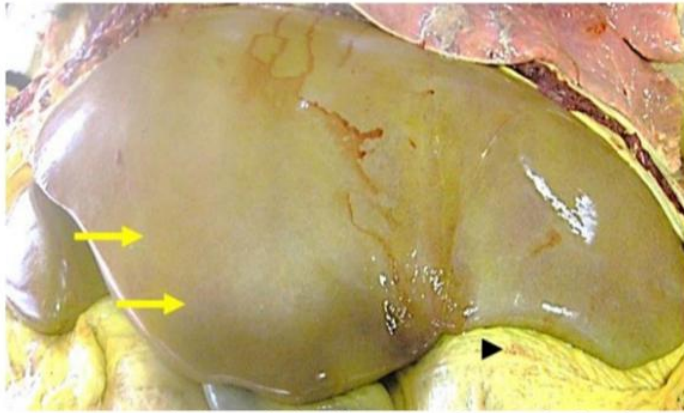


Figure 2: Gross lesion of the liver showing marked enlargement (hepatomegaly), typified by blunt edges (yellow arrow) with yellowish discoloration of the omentum (black arrow head).



Figure 3: Gross lesion of the spleen showing enlargement (splenomegaly).



Figure 4: Gross lesion of the lung showing severe congestion of the caudal lobes (arrows).

Post mortem lesions

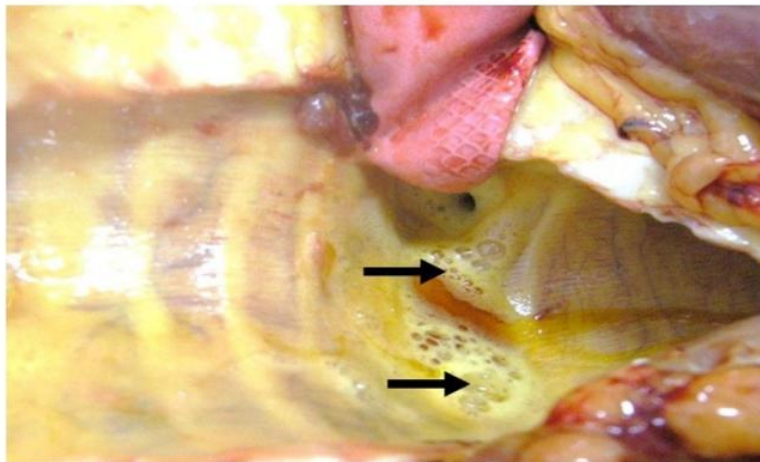


Figure 5: Gross lesion of the trachea showing frothy exudates within the lumen (arrows).



Figure 6: Gross lesion of the heart showing paint brush hemorrhages (extravasations) on the surface of the endocardium (arrows).



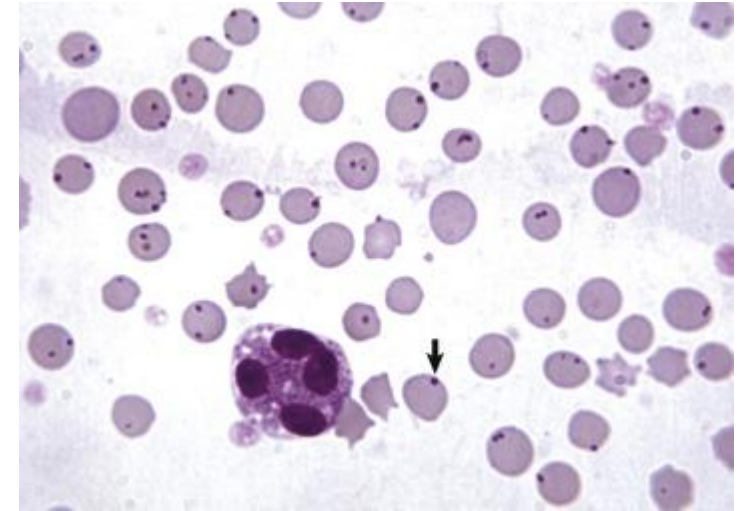
Diagnosis

History

Clinical signs

Post mortem lesions

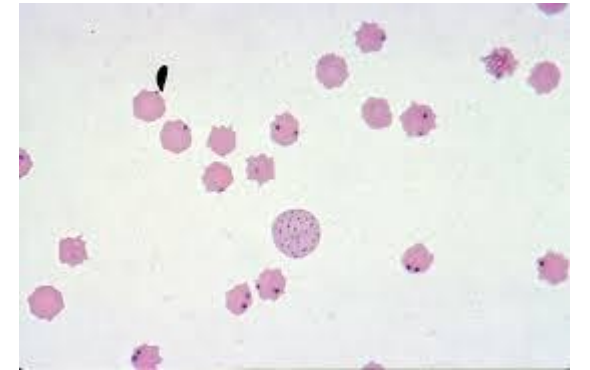
Microscopic



❖ If anaplasmosis is suspected, a blood smear should be taken from the tip of either the ear or the tail of the sick animals

Hematological changes

❖ Decrease erythrocyte count, hematocrit and hemoglobin concentration



Animal inoculation test

❖ The gold standard for the demonstration of *A. marginale* free blood is the subinoculation of blood from the suspect animal into a splenectomized calf that is highly susceptible to infection (Coetzee et al., 2006)

Serological tests

- ❖ Complement fixation (CF) test, Capillary agglutination assay, Card agglutination test (CAT)
- ❖ Indirect fluorescent antibody (IFA) test
- ❖ Enzyme linked immunosorbent assays (ELISA) such as a cELISA, indirect ELISA and dot ELISA
- ❖ The two serological tests currently preferred for identifying infected animals are the **cELISA** and the **CAT** (OIE, 2008a)

Molecular Test

- ❖ Nucleic-acid-based tests [polymerase chain reaction (PCR)]
- ❖ Polymerase chain reaction assays targeted at the *Anaplasma* **msp4** and/or $\text{msp1}\alpha$ genes, **msp5** and **16S rRNA** genes
- ❖ Recently, real-time PCR (RT-PCR) was successfully applied to the detection and quantification of *A. marginale* DNA (**msp1b gene**) in the blood of naturally infected cattle

Treatment

- ❖ Tetracycline (tetracycline, chlortetracycline, oxytetracycline, rolitetracycline, doxycycline, minocycline)) is an excellent antibiotic for treating an acute case of anaplasmosis when you treat in time
- ❖ Oxytetracycline (OTC) (30 mg/kg of a 300 mg/ml solution IM once; 30 mg/kg of a 300 mg/ml solution IM twice at a 5-day interval; and 22 mg/kg of a 200 mg/ml solution IV q24h for 5 days, which is the former OIE recommendation mentioned earlier)
- ❖ Imidocarb is also highly efficacious against *A. marginale* as a single injection (as the dihydrochloride salt at 1.5 mg/kg, SC, or as imidocarb dipropionate at 3 mg/kg)
- ❖ Enrofloxacin is less effective

Control and Prevention

- ❖ Maintenance of *Anaplasma*-free herds through import and movement control, testing, and elimination of carrier cattle
- ❖ Vector control
- ❖ Prevention of iatrogenic transmission
- ❖ Administration of antibiotics and
- ❖ Preimmunization with live vaccines and immunization with killed vaccines
- ❖ The **Anaplaz** vaccine (Fort Dodge) and the **Plazvax** vaccine (Schering-Plough) previously both vaccines are used in the USA but now removed from the market
- ❖ Recent proteomic and genomic research approaches have permitted to identify 21 new proteins within the outer membrane immunogen in addition to the well-characterized **MSP** (MSP1–MSP5)

Kill vaccine

- ❖ A killed vaccine (Anaplasmosis Vaccine, University Products, LLC, Baton Rouge, LA) is approved by the USDA as an experimental vaccine and is available in a number of states on approval of state animal health
- ❖ This vaccine purportedly uses the same antigens and purification procedures used in the discontinued Plazvac product
- ❖ Protective immunity requires two doses (28 days apart) and booster doses are recommended every 1-2 years depending on herd history



Live vaccine

- ❖ A live vaccine (Anavac, PHL Laboratories, Davis, CA) is available for use in California and is preserved in liquid nitrogen
- ❖ This vaccine is safe for use in cattle 11 months of age and younger; however, clinical disease severity increases with the age of vaccinated cattle. This vaccine is used as a method for controlled infection.
- ❖ Vaccination typically produces a lifelong immune carrier status.



