



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

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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		

*Candidatus Neoehrlichia mikurensis*<sup>a</sup>, previously known as *Candidatus Ehrlichia walkerii*

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Dumler et al. 2001; Andersson and Raberg 2011

<sup>a</sup> This organism still needs to be classified in appropriate genus (Jahfari et al. 2012)

- *E. canis* (Canine Monocytic Ehrlichiosis ) primarily in **monocytes** and lymphocytes
- *E. chaffeensis* (Human monocytic ehrlichiosis) primarily in **monocytes** and macrophages
- *E. ewingii* (Canine granulocytic ehrlichiosis) primarily in **neutrophil** and Eosinophil
- Today, *E. canis*, *E. chaffeensis*, and *E. ewingii* are all known to cause ehrlichiosis in humans
- *Anaplasma platys* (former *Ehrlichia platys*)(Dog) (Granulocyte)
- *Anaplasma phagocytophila* (former *Ehrlichia phagocytophila*) (cattle, sheep and goat) (Neutrophil & Eosinophil)
- *Anaplasma equi* (former *Ehrlichia equi*) (Horse) (Neutrophil & Eosinophil)
- *Anaplasma ovina* (former *Ehrlichia ovina*)(Sheep) (Neutrophil & Eosinophil)
- *Anaplasma bovis* (formerly *Ehrlichia bovis*) (cattle) (**Monocytes**) (**Disease Nopi or Nofel**)

## Canine Ehrlichiosis

- ❖ Tropical Canine Pancytopenia (TCP), Canine Monocytic Ehrlichiosis (CME), canine hemorrhagic fever, idiopathic hemorrhagic syndrome, and tracker dog disease is caused by *Ehrlichia canis*
- ❖ The organism was first recognized and described by Donatien and Lestoquard (1935) in tick infested dogs in Algeria
- ❖ Ehrlichia: named after **Paul Ehrlich**, a German bacteriologist
- ❖ Dogs can become naturally infected with several species of *Ehrlichia* including *E. canis*, *E. chaffeensis*, and *E. ewingii*
- ❖ To date, infections with *E. chaffeensis* and *E. ewingii* have only been diagnosed in dogs in the United States
- ❖ *E. canis* is the most common and form microcolonies within a membrane lined intracellular vacuole (so-called **morula**), primarily in monocytes and macrophages of mammalian hosts
- ❖ Concurrent infections are frequently seen with species of *Babesia*, *Hepatozoon*, *Leishmania* and *Bartonella* etc.
- ❖ **German shepherd dog** most susceptible breed compared to other breeds

## Definitive host

- ❖ Dogs and other Wild canids including wolves, coyotes, and foxes

## Transmission

- ❖ The organism is transmitted by transstadial (but not transovarian) (from larva to nymph to adult) of the tick (*Rhipicephalus sanguineus*)
- ❖ *Rhipicephalus sanguineus* can survive for up to 500 days as unfed adults remaining infected for at least 155 days
- ❖ Ticks are more abundant and acute infections more common during the warm season
- ❖ Also transmitted by blood transfusion
- ❖ No evidence of direct transmission of *Ehrlichia* spp. from dogs to humans

## Distribution

- ❖ *E. canis* organisms are found on all continents through out the world but are more prevalent in tropical and subtropical climates except **Australia**

# Life cycle

Host get infection through tick bite

*E. canis* enters into host

*E. canis* adhere to membrane of Monocytes

Through endocytosis enters into the cell

The organisms form pleomorphic 'elementary bodies' in phagosomes within canine mononuclear cells

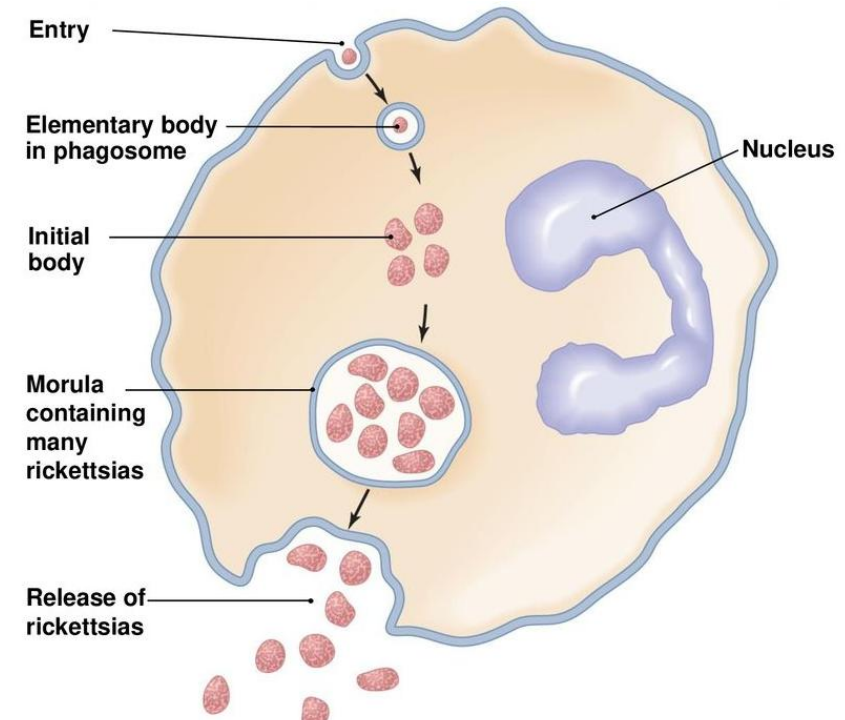
Elementary bodies grow and divide by binary fission within the phagosome for 2-3 days

Up to 50 elementary bodies are seen in the mature inclusion (the morula)

Release of organism through rupture of morulae

The organisms are then phagocytosed by other leukocytes

Spread throughout the body



Three intracytoplasmic forms

1. Initial body
2. Elementary body
3. Morulae

## **PATHOGENESIS AND CLINICAL SIGN**

- ❖ CME is characterized by three stages, acute, subclinical and chronic

### **Acute Phase**

- ❖ Acute disease lasts between 3 to 5 weeks with clinical findings of fever, anorexia, depression, lymph adenopathy, and splenomegaly
- ❖ More variably, ocular discharge, pale mucous membranes, hemorrhagic tendencies (dermal petechiae, ecchymoses, or epistaxis), or neurological signs are seen (due to meningeal Bleeding)
- ❖ The most commonly observed hematological abnormalities are thrombocytopenia and anaemia

### **Subclinical Phase**

- ❖ A long-term subclinical phase usually follows the subsidence of clinical signs and can last for several years.
- ❖ Dogs that are unable to eliminate the infectious agent develop subclinical persistent infections and become asymptomatic carriers

## Chronic Phase

- ❖ Some infected dogs progress to a chronic phase, which can be mild or severe
- ❖ This is characterized by recurrent clinical and hematological signs including thrombocytopenia, anemia, and pancytopenia
- ❖ Dogs may have weight loss, depression, petechiae, pale mucous membranes, edema, and lymphadenopathy among other signs
- ❖ In severe cases, the response to antibiotic therapy is poor and dogs often die from **massive hemorrhage**, severe debilitation, or secondary infections
- ❖ It is very likely that *E. canis* causes immunosuppression but currently little is known about the immunobiology of this infection. A recent study in dogs was unable to demonstrate a marked immunosuppression





lethargy ,weight loss



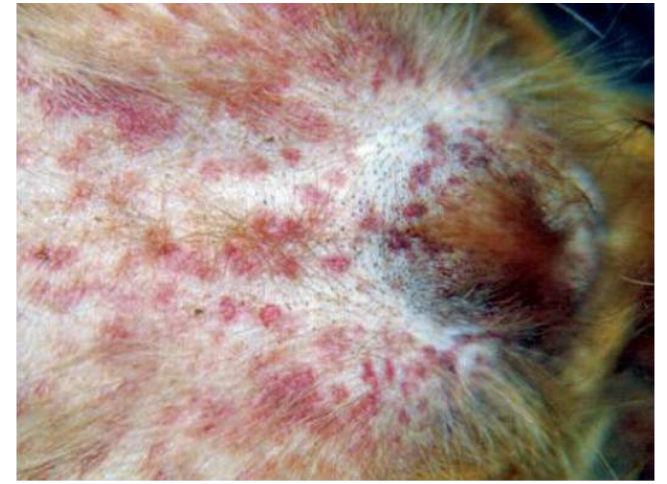
Epistaxis due to Ehrlichiosis



Ecchymoses



Petechiae hemorrhages



Ventral abdominal skin petechiation due to thrombocytopenia in a bitch



Numerous petechiae and ecchymoses on the upper lip mucosa from a dog with acute CME



Penile mucosal pallor, petechiae and ecchymoses in a dog with CME-associated aplastic pancytopenia



Scleral hemorrhage in a dog with CME



Hyphema in a dog

## POST MORTAM LESIONS

- ❖ Petechial hemorrhage seen on serosal surface of organs like lungs ,kidney ,brain, nasal cavity GI tract
- ❖ Vasculitis affecting various organs including lungs, kidneys and possibly the meninges
- ❖ Generalised Lymphadenomegaly , splenomegaly and Hepatomegaly
- ❖ Histologically, there is lymphoreticular hyperplasia and lymphocytic and plasmacytic perivascular cuffing

# Diagnosis

## History

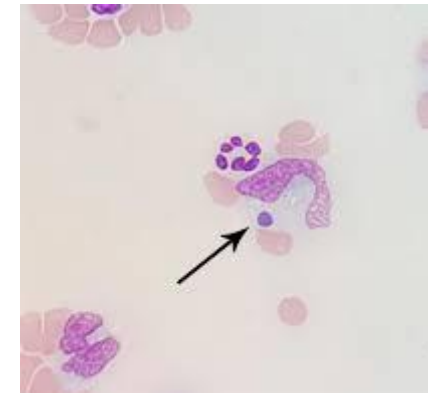
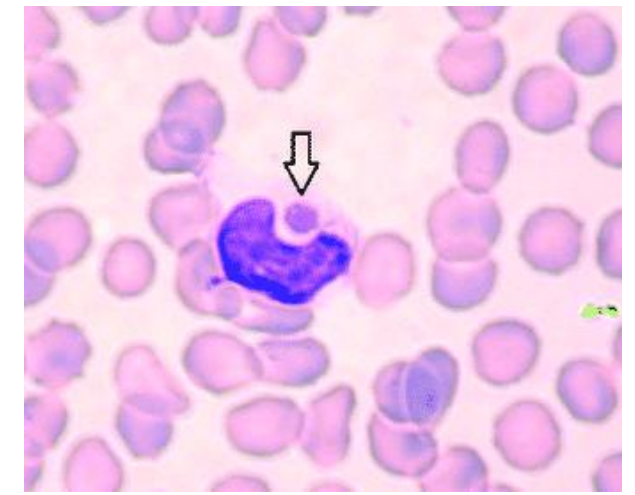
## Clinical signs

## Microscopy

- ❖ Detection of typical intracellular *E. canis* morulae on blood smear examination is highly specific for ehrlichiosis.
- ❖ Morulae are only found in low numbers in blood smears during the acute phase of infection
- ❖ Detection of morulae can be improved by evaluation of numerous buffy coat smears

## Hematology and Serum biochemistry

- ❖ A complete blood count is an important tool for the diagnosis of CME
- ❖ Moderate to severe **thrombocytopenia** is a characteristic finding of acute ehrlichiosis.
- ❖ Hypoalbuminemia, hyperglobulinemia, and hypergammaglobulinemia (mostly polyclonal, rarely monoclonal) are common in CME



- ❖ Also moderate increases in alanine aminotransferase (ALT) and alkaline phosphatase (ALP) can occur due to hepatocyte damage during the acute phase
- ❖ Dogs in the subclinical phase are clinically healthy, but variable degrees of thrombocytopenia and leukopenia may be present.
- ❖ Thrombocytopenia usually becomes severe in the chronic phase accompanied by marked anemia and leukopenia
- ❖ **Pancytopenia** due to bone marrow hypoplasia is characteristic of the chronic severe form
- ❖ A hypocellular bone marrow with varying suppression of the erythroid, myeloid, and megakaryocytic cells is seen on aspiration.
- ❖ *E. canis* can occasionally induce a protein losing nephropathy as a result of immune complex glomerulonephritis with consequent proteinuria and azotemia.

## Culture

- ❖ It is possible to culture *Ehrlichia* species in specific macrophage cell lines (canine macrophage cell line [DH82] or mouse macrophage cell line [J774.A1])

## Serology

- ❖ The indirect fluorescent antibody test (IFAT) (**‘gold standard test’**) is recommended to confirm a diagnosis of ehrlichiosis and which is widely used
- ❖ Enzyme-linked immunosorbent assays (ELISA) can also be used to confirm a diagnosis of ehrlichiosis and different **Dot-ELISA kits** for the detection of *E. canis*-IgG antibodies are commercially available
- ❖ Western immunoblot is a more specific test

## Molecular test

- ❖ PCR techniques are suggested to be the most reliable method to diagnose *Ehrlichia* infection

## Treatment

- ❖ Tetracyclines are the treatment of choice for rickettsial diseases
- ❖ For canine ehrlichiosis, tetracycline (22 mg/kg given every eight hours) or doxycycline (5 mg/kg every twelve hours) administered for four weeks is the recognized treatment (first line treatment)
- ❖ Most dogs recover from the acute and subclinical phases when treated with doxycycline or other tetracyclines at appropriate dosages for an adequate period of time
- ❖ Minocycline and Rifampicin (second line treatments)
- ❖ Imidocarb dipropionate (2 doses of 5mg/kg, IM, repeat after 2-3 weeks) drug has been successful in treating resistant *E. canis* infection
- ❖ This drug persists in the tissues for up to 1 month following one dose
- ❖ When imidocarb was given as a single IM injection, 83.9% of dogs recovered



## Supportive therapy

- ❖ Fluid therapy-for curing dehydration in infected dog
- ❖ Blood transfusions-if the dog is severely anemic
- ❖ (platelet-rich plasma)
- ❖ Glucocorticoids-(1 to 2 mg/kg prednisolone, PO)
- ❖ Livertonic medicine-effective hepatostimulante

## Monitoring of Treatment

- ❖ Clinical signs improve within 48 hours
- ❖ The platelet count should remain normal at 4 and 8 weeks
- ❖ Hyperglobulinemia should gradually resolve over 6 to 9 months
- ❖ PCR should be negative at 2 weeks after successful treatment

## Prevention

- ❖ Tick control (Manualy/medicinal)
- ❖ Recent studies have evaluated the efficacy of a spot-on formulation containing imidacloprid 10% and permethrin 50% (Advantix®) to prevent tick exposure and thus *E. canis* infection in dogs
- ❖ Use prophylactic drug Doxycycline (3mg/kg PO q24h) in highly endemic areas
- ❖ There are no vaccines currently available to protect dogs from *Ehrlichia* spp