# Rickettsia, Coxiella, *Ehrlichia*, Neorickettsia, *Anaplasma*

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Domain : Bacteria
Phylum : Proteobacteria
Class : 1. Alphaproteobacteria
Order : 1. Rickettsiales
Family : 1. Rickettsiaceae
Family : 2. Ehrichiaceae
Genus : Ehrlichia
Genus : Anaplasma
Genus : Cowdria
Genus : Neorickettsia
Genus : Aegyptianella
Order : 2. Rhizobiales
Family : Bartonellaceae
Genus : Bartonella
Class : 2. Gammaproteobacteria
Order : Legionellales
Family : Coxiellaceae
Genus : Coxiella
Phylum : Firmicutes
Class : Mollicutes
Order : Mycoplasmatales
Family : Mycoplasmataceae
Genus : Haemobartonella
Genus : Eprythrozoon

# *Coxiella*

Q Fever 'Query Fever'

# History

The disease was first detected in 1935 by Edward Holbrook Derrick, a renowed Australian pathologist, among abattoir workers in Brisbane, Queensland Australia.

As the etiological factor was unknown during that period, it was referred as Q**("Q" stands for "query")** fever.
**Sir Frank Macfarlane Burnett**, an Australian virologist first identified the agent as Rickettsia.

Herald Rea Cox and Gordon Davis at the Rocky Mountain Laboratory (RML) in the United States isolated the organism from tick and nomenclature was given as ***Coxiella burnetti***.

**Characteristic features:**

- They are minute obligate intra cellular parasites requiring living cells for
- multiplication.
- They were formerly considered closely related to virus.
- But based on their characters like
- cell walls similar to those of other Gram-negative bacteria.
- divide by binary fission
- possessing cell wall containing muramic acid
- metabolic enzymes independent of the host cell
- possess both DNA and RNA
- large enough to be seen under the light microscope
- held back by bacterial filters
- susceptible to antibiotics
- They are considered true bacteria, specially adapted to obligate intra cellular parasitism.

# Coxiella

**Family *Coxiellaceae*** was historically included in the order Rickettsiales together with the *Anaplasmataceae* and *Rickettsiaceae*, but the analysis of 16S and 23S rRNA gene sequences led to its re-classification into the **order Legionellales**.

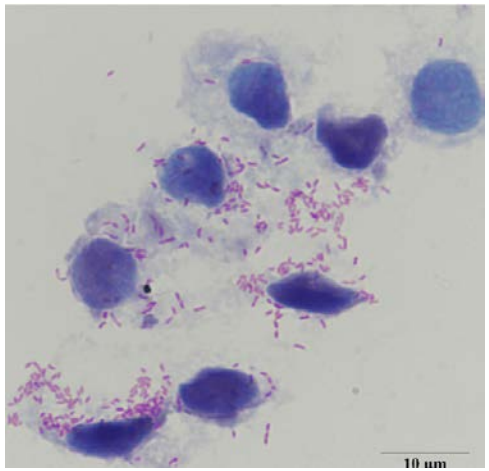
The family *Coxiellaceae* is composed of the **genera** *Coxiella, Rickettsiella* and *Aquicella*.

- ***Coxiella burnetii*** is the most significant representative of this taxonomic group as it causes **Q fever**, an emerging disease with high impact on public health, animal health, and the economy.
- Infection with *C. burnetii* is acquired by the inhalation of desiccated aerosol particles.
- **Ticks are not considered essential for the transmission of *C. burnetii* in livestock**, but they may play a role in the maintenance of the life cycle in wildlife. Indeed, there is a possibility that *C. burnetii* replicates in the midgut of ticks and appears in the feces nine days after a blood meal, and that transmission through ticks could be associated with contaminated dust from dried tick excrement.

# Coxiella

## Morphology

- Gram negative, (0.2 to 0.4mm wide, 0.4 to 1mm long)
- Pleomorphic cocco-bacillary bacterium
- Obligate intracellular
- Cell wall measuring 1.0 by 0.3 µm.
- Extremely stable in environment due to formation of endospore like form, known as "**small cell variant** (SCV)" and also have metabolically and replicatively active **large cell variant** (LCV) form.
- It is stained with **Gimenez** and other rickettsial stains.



# Resistance

- Unlike other Rickettsia, **Coxiella are extremely stable in the environment**.
- It is **resistant to heat and drying and can remain infectious** in milk, water, soil, dried blood and wool for months.
- They are **able to survive in phago-lysosome** at pH 5.0.
- It can withstand holding method of milk pasteurization (63$^{o}$C for 30 minutes) and is at borderline of inactivation in flash method (72$^{o}$C for 20 minutes) of milk pasteurization.
- They are also resistant to ultraviolet ray, desiccation and common disinfectants like sodium hypochlorite, quaternary ammonium and phenolic compounds.
- However, it can be inactivated by 2% formaldehyde, 1% Lysol and 5% hydrogen peroxide.

# Isolation and culture

**i) Embryonated chicken eggs**

A portion of placenta is homogenized in phosphate-buffered saline (PBS) containing antibiotics (streptomycin 100–200 µg/ml and penicillin or gentamicin 50–100 µg/ml). After low-speed centrifugation, dilutions of the supernatant fluid are inoculated into 6- to 7-day-old embryonated chicken eggs via the **yolk sac**. Eggs are preferably from specific pathogen free (SPF) hens.
Embryos that die during the first 5 days after inoculation are discarded. The yolk sacs are harvested after 10–15 days of incubation. Stained smears of the yolk sac wall are examined to ensure the absence of bacterial contamination and to determine the presence of C. burnetii.
PCR analysis can also be used to detect the presence of C. burnetii and to monitor the process of isolation. Further passages may be required to obtain an isolate in pure culture.

**ii) Cell culture**

A cell microculture system used for virus culture, the shell vial cell culture, has been adapted for isolating strict or facultative intracellular bacteria, including C. burnetii. Suspensions of clinical samples are inoculated into **human embryonic lung (HEL) fibroblasts** grown on a 1 $cm^2$ cover-slip within a shell vial. Various cell lines may be used to allow the observation of characteristic vacuoles of *C. burnetii* multiplication. Centrifugation for 1 hour at 700 g enhances the attachment and penetration of bacteria into the cells. By day 3, 10 and 21, the cytopathic effect (CPE) – ***C. burnetii*** characteristic **vacuoles in cells** – are examined using an inverted microscope.

# Isolation and culture

iii) Laboratory animals

Heavily multi-contaminated clinical samples, such as placentas, vaginal discharges, faeces, or milk, the inoculation of laboratory animals may be necessary as a filtration system.

**Mice and guinea-pigs** are the **most appropriate laboratory animals**, must be housed in appropriate biosafety and containment conditions, determined by biorisk analysis for this purpose .

Following intraperitoneal inoculation with a dose of 0.5 ml per animal, body temperature and antibody status can be monitored. This method should be performed in conjunction with serological tests on other guinea-pigs or mice that have been inoculated with the same samples.

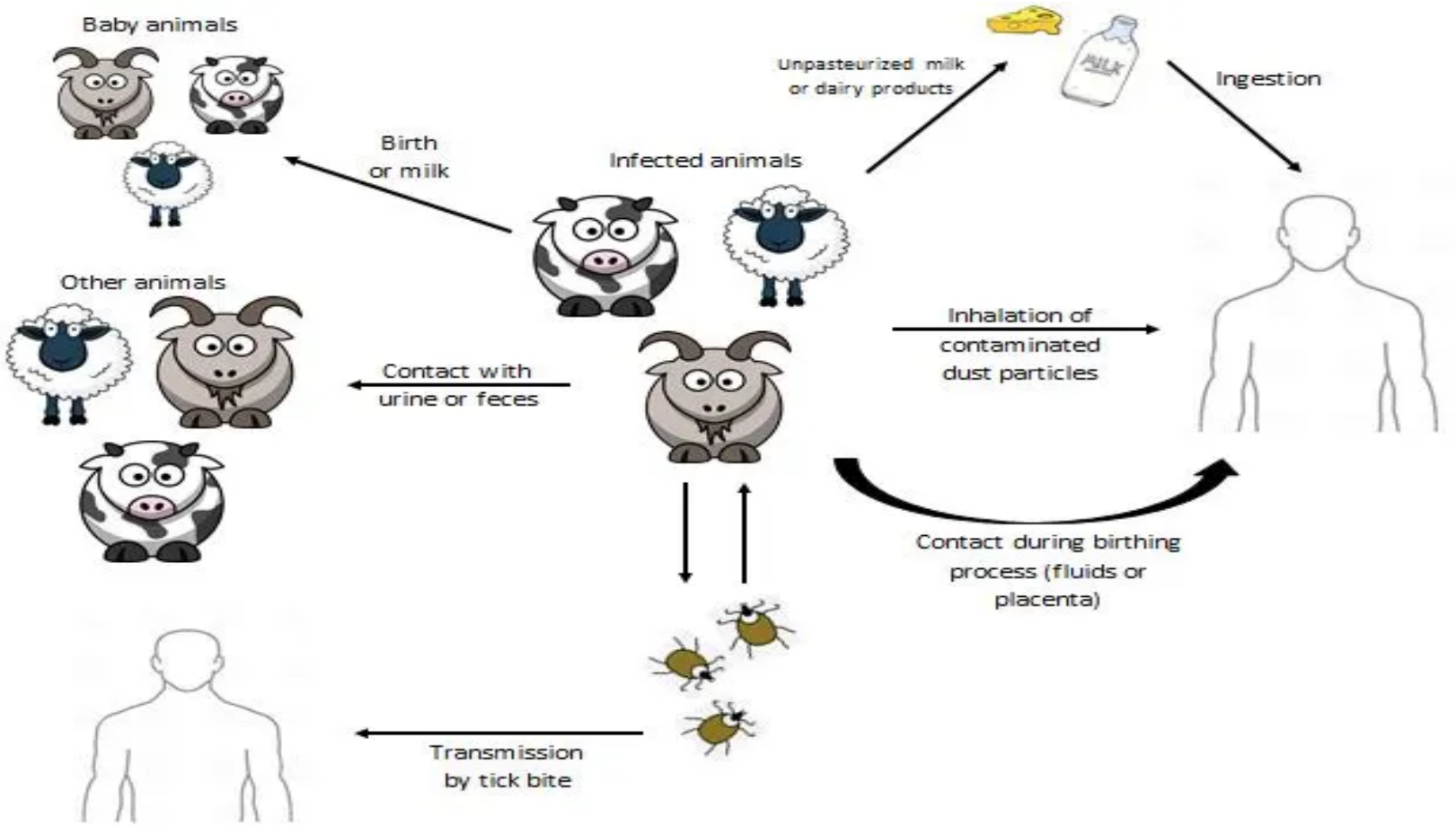
Sera are collected 21 days after inoculation. A positive result confirms a diagnosis of C. burnetii infection. If pyrexia develops, the animal is killed and the spleen is removed for isolation of the agent by inoculation into embryonated chicken eggs or in cell cultures. Microscopic examination of C. burnetii can be done using impressions and staining of the collected spleens. Alternatively, the process can be simplified by performing PCR for detection of C. burnetii DNA on spleens.

# ANTIGENS

- Exhibits antigenic variation (phase variation)
- **Phase I** - fresh isolates, powerful immunogen, cell wall associated antigen that behaves as a capsule masking Phase II antigen
- **Phase II** - recovered after repeated passage in yolk sac, lack the surface Phase I antigen, autoagglutinable

# Transmission

- **Inhalation** - soil contaminated dust, air borne dust, contaminated wool dust, contaminated bedding.
- **Ingestion** - contaminated milk, genital discharges, foetus or placenta, bedding materials or manure.
- **Contact** - persons who come in contact through their occupations eg. farm workers, veterinarians, dairy plant worker, shearers, slaughter house workers.
- **Vector** – ticks(Ixodid ticks) are the vectors of transmission.
- Sheep and goats are main reservoirs of infection.
- Nowadays ***C. burnetti* is considered as an important agent for bio-terrorism**.




Possible transmission paths and potential hosts of *C. burnetii.*

# Pathogenesis

1. Infection by inhalation of *C. burnetii* targets the **alveolar macrophages in the lungs**.
2. **Infection via the bloodstream** or the digestive tract **targets** the **Kupffer cells of the liver**.
3. *C burnetii* enters the cells of the host using a specific receptor called an integrin; either LRI (leukocyte response integrin) or IAP (integrin-associated protein).
4. After the bacteria has entered the host, a **macrophage engulfs the bacteria by a process called phagocytosis**.
5. This process forms a sack-like structure around the bacteria called a phagosome.
6. The phagosomes proceed to fuse with lysosomes to form phagolysosomes.
7. *C. burnetii is* an acidophilic bacterium, meaning metabolism and multiplication is enhanced in acidic environment.
8. The **organism may localize in the female reproductive tract and mammary glands**.

# Signs and symptoms

**Clinical signs in Q-fever is usually inapparent in livestock**

Anorexia, high rise in temperature is the initial signs followed by rhinitis and coughing.
Most striking manifestation is **abortion**.
There may be **still births, dystocia** and **occasionally pneumonia**.

In cattle **metritis** is the cardinal manifestation.

## Humans

### Acute form

Sudden onset of **high fever** (104-105$^{o}$F), sweat, non productive cough, severe headache, general malaise, myalgia, confusion, sore throat, chills, nausea, vomition, diarrhoea, abdominal pain, chest pain etc.

**Fever last for 1-2 weeks, weight loss persists for some time. About 30-50% of patients suffering from pneumonia.**
**Most of the acutely infected persons recover from infection and only 1-2% die of acute Q.fever.**

### Chronic form

**Persists for more than 6 months and is a much more severe disease.**
**Endocarditis, and hepatitis are highly manifested during chronic infection.**
**About 65% of the people with chronic Q.fever may die of disease.**
**It has shorter incubation period for 2-3 weeks.**

# Diagnosis

- By Isolation and Cultivation – by experimental transmission, tissue culture or developing egg embryo. Required to be made in laboratory having Bio-safety level-3.
- By Direct microscopy – both blood and tissue smears, stains such as **Giemsa, Gimenez, Leishman** are useful.
- Modified Z-N (occasionally with Giemsa) stained smear from placental and vaginal discharge may reveal clumps of red coccobacilli.
- By Serology – CFT, micro-agglutination, indirect immunofluorescence and ELISA.
- DNA probe and Polymerase Chain Reaction (PCR) are much more sensitive techniques for early detection of organism.

# VACCINES

- Inactivated killed vaccine is used in animals.
- Formalin killed whole cell vaccine is used in man.
- Commercial vaccine (Q-vax, CSL limited, Parkville Victoria, Australia) is available for man.

# Prevention and control

- In an infected herd attempt should be made to isolate them.
- All out measures should be extended to control tick population.
- Avoid contact with fomites.
- Avoid contact with aerosols which can traverse a long distance.
- All reproduction organs like reproductive apparatus, placenta etc. should be disposed by burning or burrying.
- Cleaning should be followed by disinfection with 10% bleaching powder.
- Vectors are to be eliminated as far as possible with appropriate acaricides.
- Manure should be covered with lime.
- People who come in contact like farm people, veterinarians should keep themselves away from milk and uterine discharges.

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# Genus : Rickettsia

# Introduction

•Family: Rickettsiaceae includes a diverse group of organisms that share common features of intracellular growth and transmission by hematogenous arthropods.

•It is named after **Howard Taylor Ricketts** who discovered spotted fever rickettsia and died of typhus fever contracted during his studies.

**They are minute obligate intra cellular parasites requiring living cells for multiplication.**

**virus**

- Require living cells for multiplication.
- Can not be seen by ordinary light microscope

**Bacteria**

- Cell wall made up of peptidoglycan
- Reproduction by binary fission
- Posses both DNA and RNA
- susceptible to antibiotics
- Metabolic enzymes

# Morphology

- Rickettsiae are small, non-motile, non-capsulated pleomorphic, coccobacilli
- 0.3 – 0.6 x 0.8-2µm in size.
- Obligate intra cellular parasite.
- Gram stain not suitable- Gram negative
- Under the electron microscope, the rickettsiae are seen to have a 3 layered cell wall, trilaminar plasma membrane and outer slime layer
- G+C content of DNA is 28-33 mol. %

# Cultivation

- Optimum temperature for growth is 32-35℃ .
- Rickettsiae can generate their own energy, but they also depend on their host for some energy
- *Rickettsiales* require living cells for replication. They are readily cultivated in the yolk sac of developing chicken embryo (first shown by Cox), or in cell lines like mouse fibroblast, detroit, HeLa and HEp2.
- Growth generally occurs in the cytoplasm of the infected cells or in some cases (spotted fever) in the nucleus.
- Guinea pig and Mice are useful for primary isolation.
- *Rochalimae quintana* –the only rickettsiae which have the ability to grow on blood agar.

# Resistance

- Rickettsiae are readily inactivated by physical and chemical agents.
- They are rapidly destroyed at 56℃
- Rickettsiae can lose their viability in storage due to loss of their intercellular ATP pool and several coenzymes.
- They can be preserved in skimmed milk or a suspending medium containing sucrose, K, Po4 and glutamate (SPG) medium.
- They can be preserved in 50% glycerol saline at 4℃ or by freeze drying
- Holding method of pasteurization is not effective, but the flash method is effective.

# Antigens and toxins

1. Group specific soluble antigen
2. Species specific antigen
3. Surface antigen- alkaline stable polysaccharide

• Some rickettsiae and in some strains of *Proteus* organism.
• The sharing of antigens between rickettsiae and *Proteus* is the basis for the Weil-Felix reaction used for the diagnosis of rickettsial infections by the demonstration of agglutinins to *Proteus* strains OX19, OX2 and OX k.

# Pathogenicity

- *Rickettsiales* are usually parasites of alimentary tract of arthropods such as fleas, lice, ticks and mites, replicating in the cells of gut.
- Transmission is from arthropod to animal.
- Targets vascular endothelium

| Species | Disease | Main host | Transmission |
|---|---|---|---|
| **R.prowazekii** | Epidemic typhus | Human | Louse |
| **R.mooseri** | Endemic typhus | Human | Rat flea |
| **R.rickettsii** | Rocky mountain spotted fever | Human, dogs, rabbit | Ticks (Dermacentor spp) |
| **R.tsutsugamushi** | Scrub typhus | Small rodents,Birds | Mite |
| **Rochalimae quintana** | Trench fever | Human | Louse |

# Rocky Mountain spotted fever in dogs



- I.P. 2-10 days
- *Rickettsia rickettsii* is maintained in the tick population by transovarial and transstadial transmission.
- An infected tick must remain attached for up to 20 hours before salivary transmission to the host occurs. The organisms, which replicate in endothelial cells of infected dogs, produce vasculitis, increased vascular permeability and haemorrhage
- **Clinical signs**- fever, depression, conjunctivitis, retinal haemorrhages, muscle and joint pain, coughing, dyspnoea and oedema of extremities.
- Neurological disturbance, which occurs in 80% of affected dogs, presents as stupor, ataxia, neck rigidity, seizures and coma
- Death may result from cardiovascular, neurological or renal damage

# Diagnosis

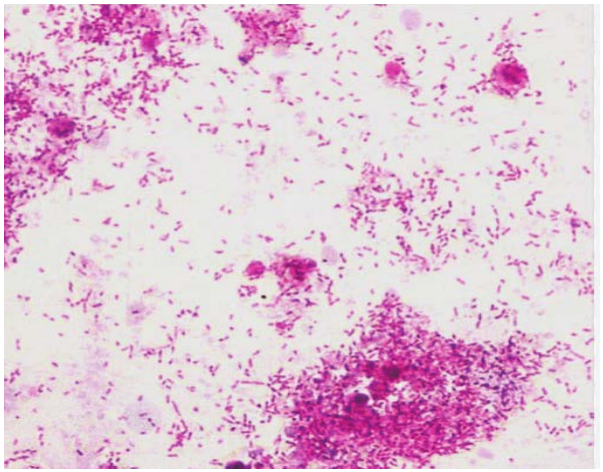
- Isolation and identification
- For diagnosis blood and tissue samples collected for culture , and serum for serological test.
- By Direct microscopy

  Both blood and tissue smears, stains such as Giemsa, Gimenez, Machiavello and Leishman as well as FAT are useful

  Giemsa, Castaneda- bluish purple

  Gimenez, Machiavello - deep red

  Leishman stain.



## Isolation and Identification:

Culture –species can grown in yolk sac of embryonated hen's egg
Guinea pigs and mice are useful for primary isolation.

1. Blood sample collected from patients
2. A blood clot ground in skimmed milk or any suitable suspending medium is inoculated i/peritoneally.
3. Animal observed for 3-4 weeks for raising their temperature. (*Rochalimae quintana* will not grow in guinea pigs and mice).
4. In Rocky mountain spotted fever gunia pig develop fever, scrotal necrosis may even die

➢Rickettsia grow well in 3-5 days on Vero cell MRC 5 cell cover slip cultures and can be identified by immunofluroscence using group and strain specific monoclonal antibodies

- **Weil- felix reaction in rickettsial disease**
  specific agglutination test(**either tube or rapid slide agglutination test)**
  The basis of the test is the sharing of an alkali stable carbohydrate antigen by some rickettsiae and by certain strains of *Proteus*, *P.vulgaris* OX19 and OX2 and *Proteus mirablis* OX k.

| Disease | Agglutination with | | |
|---|---|---|---|
| | OX 19 | OX 2 | OX k |
| **Epidemic typhus** | +++ | ± | - |
| **Endemic typhus** | +++ | ± | - |
| **Rocky Mountain spotted fever** | ++ | ++ | - |
| **Scrub typhus** | - | - | +++ |
| **Trench fever** | - | - | - |
| **Q-fever** | - | - | - |

# Treatment and control

- Rickettsiae are susceptible to tetracycline and chloramphenicol.
- Penicillin and sulphonamides are ineffective.
- Sulphonamides may actually enhance the growth of rickettsiae.
- Vaccines are not available for the prevention of the rickettsial diseases of animals.
- Requires adequate pasteurisation of milk and care in the handling of animals an their products.
- Eradication of ticks is very helpful in control of rickettsial infection

**Neorickettsia**

- *Neorickettsia helminthoeca* is an obligate intra-cytoplasmic bacterium that causes salmon poisoning disease (SPD), an acute, febrile, fatal disease of dogs.
- Common signs include lack of appetite, vomiting, diarrhea (which may include blood), fever, weakness, enlarged lymph nodes, weight loss, discharge from the eyes or nose, increased respiratory rate, increased heart rate, muscle tremors and seizures. If the infection is not treated, most dogs will die within 2 weeks

- **Trematode - Fluke -** *Nanophyetus salmincola* is potentially very significant, however, because the **flukes can transmit** *Neorickettsia helminthoeca*.
- Neorickettsia species can have complex cycles with infection of trematodes, the cercariae of which may infect snails and aquatic insects, which again are ingested by fish, mammals, and birds. N. helminthoeca can infect flukes and canids, causing salmon poisoning disease in dogs.

***Ehrlichia ruminatium***

- **Heartwater** is a noncontagious, tickborne disease caused by *Ehrlichia ruminantium*, an intracellular parasite in the order *Rickettsiales*.
- *E. ruminantium* is a gram-negative, pleomorphic coccus. It is unable to survive outside of its host for more than a few hours and can only be transmitted through its ***Amblyomma* tick vector**
- **Transmitted vertically and through the colostrum of carrier dams.**
- **Clinical Signs:** Acute febrile disease, with a sudden rise in body temperature, which may exceed 41°C within 1–2 days after the onset of fever. Fever is followed by inappetence, sometimes listlessness, diarrhoea, particularly in cattle, and dyspnoea indicative of lung oedema**.**
- Nervous system problems may follow, including excessive chewing motions, incoordination, circling, and a high-stepping gait. Some animals may experience convulsions.
- **Postmortem Findings of Heartwater (Cowdriosis)** Disease in Cattle: Postmortem examinations reveal the accumulation of fluid in the pericardial sac, pleural and peritoneal cavities**.**

## Anaplasma

- Anaplasmosis is a blood cell parasite of cattle with a worldwide distribution, but the disease is most common in tropical and subtropical areas. *Anaplasma marginale* is the most common organism involved in cattle, and it is transmitted through the bite of Dermacentor spp.
- **Anaplasmosis** is a form of 'tick fever' in cattle, also known as yellow bag or yellow fever. It is an infectious disease of the red blood cells caused by the rickettsial bacteria Anaplasma marginale. Most commonly transmitted by ticks, A. marginale causes disease primarily in cattle.
- **The main signs of anaplasmosis are fever, jaundice and anorexia.** Additional clinical signs may include progressive anemia (pale gums and eyes), weakness, inappetence, loss of coordination, aggression, difficulty breathing, rapid pulse, decreased milk production, brown urine, and sudden death

# THANKS

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