RICKETTSIALES

Learning objectives

To know in detail about,

- Characters of rickettsiales
- Principal diseases, host and mode oftransmissionof rickettsial pathogens
- Q-fever and Heart water in cattle
- Weil-felix reaction
- Stains used to identify the rickettsiales
- Cultivation methods of rickettsiales
- Antigens and toxins of rickettsiales
- Pathogenesis of rickettsiales
- General approaches used to diagnose Q-fever canine ehrlichiosis, Salmon poisoning and Heart water

SYSTEMATICS

  - *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*
- They are minute obligate intra cellular parasites requiring living cells for multiplication.
- They were formerely 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 independanat of the host cell
- posess 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.

CLASSIFICATION

- Depending on the diseases they produce, the vectors that transmit them, antigenic relationships, growth properties and resistance to physical and chemical agents.

S.No	Genus	Cell parasitised in host
1	*Rickettsia*	Macrophages, leucocytes and endothelial cells
2	*Coxiella*	Circulating leucocytes
3	*Ehrlichia*	Vascular endothelial cells
4	*Cowdria*	Vascular endothelial cells
5	*Neorickettsiae*	Reticular cells of lymphoid tissue

- *Rickettsiales* are usually parasites of alimentary tract of arthropods such as fleas, lice, ticks and mites.
- Transmission is from artropod to animal.
- The principal diseases, hosts, mode of transmission of pathogens in the *Rickettsiales* are:

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	Trench fever	Human	Louse

quintana			
Coxiella burnetti	Q-fever	Human, Cattle and small ruminants	Human Contaminated dust mainly by inhalation and ingestion of contaminated milk Contact with birth fluid of ruminants Ixodes tick Animals
Ehrlichia bovis	Bovine ehrlichiosis	Cattle	*Ixodes* tick
Ehrlichia canis	Canine ehrlichiosis (Tropical canine pancytopenia)	Dog	*Rhipicephalus sanguineus* (Brown dog tick)
E.phagocytophilia	Grazing fever in cattle	Cattle &Sheep	*Ixodes* tick
E.risticii	Potomac horse fever (equine monocytic ehrlichiosis)	Horse	Vector not known
Cowdria ruminantium	Heart water	Cattle, Sheep, Goat and wild ruminants	*Ambylomma* ticks
Neorickettsia helminthoeca	Salmon poisoning	Dogs, foxes, bears, ferrets	Ingestion of salmon fish contain the infected helminth fluke(*Nanophytes salmincola)*

HISTORY AND HABITAT

History

- The name *Rickettsiales* has been given in honor of American Pathologist Howard Taylor Rickets (1909) who first observed these microorganisms in Rocky Mountain spotted fever.
- Derrick (1935) investigated cases of fever occurring in abattoir workers in Brisbane, Australia.
- As the etiology of the disease was unknown, it was referred to as Querry or Q fever.
- The causative agent was identified by Burnert (Australian) and Cox (American)- so, it named as *Coxiella burnetti*.

Habitat

- The *Rickettsiales* are essentially parasites of arthropods, replicating in the cells of gut.
- Some can be passed transovarially, in ticks and mites but others such as *Cowdria* and *Ehrlichia*, are passed transtadially.
- They do not survive the outside the living cells (host or vector) with the exception of *Coxiella burnetii*, which produce endospore like forms, that can survive in dust particles for 50 days or more.
- Several *Rickettsiales* may persist in the host in a latent form.

MORPHOLOGY AND CULTIVATION

Morphology

- Rickettsiae are small, non-motile, non-capsulated pleomorphic, coccobacillary (0.3 – 0.6 x 0.8-2µm in size) forms existing as obligate intra cellular parasite.
- Under the EM, the rickettsiae are seen to have a 3 layered cell wall and trilaminar plasma membrane, thus resembling Gram –ve bacteria.
- They stain reasonably well with Giemsa, Castaneda, (bluish purple), Gimenez, Machiavello (deep red), and Leishman stains, but poorly with Gram‘s stain.

Cultivation

- Rickettsiae multiply by simple binary fission. They have cytochromes and their metabolic reactions are aerobic.
- They possess many of the metabolic functions of bacteria but require exogenous co-factors from animal cells.
- Rickettsiae can genrate 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, HeLa and HEp2.
- Growth generally occurs in the cytoplasm of the infected cells or in some cases (spotted fever) in the nucleus.
- *E.canis* can be propagated very well in dog monocytes culture.
- *Rochalimae quintana* –the only rickettsiae which have the ability to grow on blood agar.
- Guinea pig and Mice are useful for primary isolation.

RESISTANCE, ANTIGENS AND TOXINS

Resistance

- Rickettsiae are readily inactivated by physical and chemical agents.
- 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.
- *Coxiella burnetti* is relatively resistant to physical and chemical agents.
- In dried tick faeces and in wool, it survives for a year or more at 4°C and in meat for atleast a month.
- Holding method of pasteurisaton is not effective, but the flash method is effetive.
- Rickettsiae are susceptible to tetracycline and chloramphenicol.
- Penicillin and sulphonamides are ineffective. Sulphonamides may actuallyenhance the growth of rickettsiae.

Antigens and toxins

- Atleat 3 types of antigens have been demonstrated
    - Group specific soluble antigen
    - Species specific antigen
    - Alkaline stable polysaccharide- found in 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.
- *Coxiella burnetti* is the only rickettsiae to exhibit phase variation. Fresh isolates are in Phase I.
- They become Phase II on repeated passage in yolk sac but reversion to phase I take place by passaging in guinea pigs.
- Phase II cells are autoagglutinable. Phase I activity is attributed to a surfcace CHO antigen.
- Phae I immunogen is more powerful than Phase II and elicits high titre antibodies.
- Q fever sera react with other rickettsial antigens or with *Proteus*.
- The toxins have not been isolated and identified. Haemolysins are produced by some typhus rickettsiae.
- Rickettsiae contains (endotoxin like) LPS. They are different from true endotoxins of Gram-negative bacteria.

PATHOGENESIS

    - Adherence-, which is facilitated by the surface receptors of the host cell
    - Endocytosis
    - Phagosome destruction- Rickettsiae destroy the phagosomal mambrane by phospholipase
    - Multiply within the cytoplasm or in certain cases (spotted fever) nucleus.
- Infection begins in the vascular system, organism proliferate in the endothelial and phagocytic cells and are disseminated via blood stream.
- There is obstruction of small blood vessels because of hyperplasia of infected endothelial cells and resulting thrombi.
- If capillary endothelium is affected, producing thrombi that result in haemorrhagic skin rashes.
- Q fever organism has prediliction for mammary gland and placentae in cattle and sheep. Occasionally asymptomatic infections occur.
- Q fever infection causes abortion in sheep, goats and cattle and bronchopneumonia in sheep.

- Fever, hamorrhagic rash, stupor, shock and patchy gangrene of subcutis and skin are the common signs and lesions noticed in rickettsial infections
- In case of Ehrlichiosis, the affected animals show symptoms of congested mucous membrane, purulent discharge from eyes and nose, gastritis, oedema of the hind legs and enlarged lymphnodes.
- The mortality rate is 100% in acute cases. On P.M., the lesions are pulmonary oedema, haemorrhages of lung, hydrothorax, splenomegaly and hyperplasia of lymphnodes.
- In Salmon poisoning, the mortality reaches 90%, affected dogs become weak, with vomition, depression and diarrhoea.
- Theimportant lesion is hyperplasia of lymphnodes with necrosis.

Steps involved in parasitization include

- In mammals, by direct penetration of skin as a result of feeding by an infected arthropod (tick, louse, flea or mite)
- In arthropods as the result of ingestion of blood of infected animals
- From arthropod to progeny by infected ova.
- In Q fever, wild animals such as bandicoot may constitute the primary reservoir, the infection being transmitted among them by Ixodes tick.
- Transovarial transmission has been demonstrated in Ixodes tick. The rickettsiae are abundant in tick faeces and survive in them for long periods in the dry state.
- Ticks transmit the disease to cattle, sheep and poultry. The rickettsiae are shed large numbers in the milk of infected cattle, uterine discharges, after-birth and other secretions.
- The infected material gets dried up in the atmosphere and becomes suspended in the air and transported to long distances.
- In human, the principal route of infection is mainly by inhalation of contaminated dust particle, ingestion of infected milk, and contact with contaminated material.
- In Salmon poisoning in dogs, the disease is transmitted by eating raw salmon fish, which contained the infected fluke (*Nanophytes salminicola*).
- Infected metacercariae encysted in the muscles of fish are ingested by dogs; flukes will get mature and release invasive rickettsiae.
- The fluke eggs are passed in the dog intestine. These eggs develop into meracids, which infect the snail.
- The cercaria develops within the snail and passes from snail and infects susceptible species of fish.

DIAGNOSIS

- It vary with the disease, but usually include unclotted blood for blood smears, affected tissues (such as brain in heart water), paired serum samples for serology are appropriate.
- In Q fever, besides blood, the sputum and less often the urine, may yield the causative agent.
- By Direct microscopy
  Both blood and tissue smears, stains such as Giemsa, Gimenez, Machiavello and Leishman as well as FAT are useful.
- By Isolation and cultivation
  This is often difficult and is not usually necessary for a laboratory diagnosis.
- Guineapigs and mice are useful for primary isolation. Suspected materials are inoculated i/peritoneally and the animals have to be observed for 3-4 weeks for raising their temperature. (*Rochalimae quintana* will not grow in guinea pigs and mice).

Disease	Diagnosis

Q-fever	• Detection of organisms in
	  ○ Giemsa or FAT stained smears from ruminant placentas
	  ○ Inoculation of chicken embryos, guinea pigs and hamsters
	  ○ Paired sera samples for agglutination, CFT and ELISA
	  ○ Allergic test
	• The cattle and horses are inoculated in the lower eyelids with the antigen. After3-4 days, there is acute swelling of the eyelid in the positive case with rise in temperature.

Canine ehrlichiosis	• Giemsa stained blood smears (best at 13th day post infection) - characteristic inclusion in monocytes and neutrophils • Serology – indirect FAT
Salmon poisoning	• Observation of characteristic fluke eggs in faeces • Giemsa, Gimenez or machiavello stained smears of fluid aspirated fromlymphnode
Heart water	• Giemsa or FAT stained smears from brain tissue • Inoculation of susceptible cattle or mice

Weil-felix reaction

- This test was developed by Weil and Felix (1916).
- The weil-felix reaction is a simple and specific agglutination test for the diagnosis of some rickettsial disease.
- 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. This test is usually done as either tube or rapid slide agglutination test.
- The test is of no value in diagnosis of trench fever and Q fever.

Weil- felix reaction in rickettsial disease

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

- Immunity is both cellular and humoral. Vaccines are notavailable for the preventionof the rickettsial diseases of animals.
- Tetracyclins and chloramphenicol are effective in control of Q-fever 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.