



RICKETTSIACEAE

32.1 INTRODUCTION

Rickettsiae are small, pleomorphic, gram negative bacilli that multiply by binary fission. They are fastidious bacteria that are obligate intracellular parasites. They require an arthropod vector as part of their natural cycle and are transmitted to man by blood sucking arthropods. They possess both DNA and RNA. They possess a cell wall made of peptidoglycan. They are non motile and non capsulated. They reproduce by binary fission and are susceptible to antibacterial agents. However they are not visible by light microscopy. Family Rickettsiaceae contains three genera: Rickettsia, Orientia, and Ehrlichia-*Coxiella burnetti* which causes Q fever and *Rochalimaea Quintana* causing trench fever are no longer included as the former is not transmitted by arthropod vector and the latter is not an obligate intra cellular parasite. It was named after Howard Taylor Rickett who died of typhus fever contracted while working on this organism.



OBJECTIVES

After reading this lesson, you will be able to:

- enumerate members of family Rickettsiaceae.
- describe characteristics of rickettsiae.
- describe cultivation of rickettsiae.
- describe pathogenesis and disease caused by rickettsiae.

32.2 GENUS RICKETTSIAE

Genus consist of two groups on the basis of disease caused by them

Rickettsiaceae

- Typhus fever group
- Spotted fever group

Morphology

They are pleomorphic coccobacilli $0.3-0.6\mu\text{m} \times 0.8-2\mu\text{m}$ in size. They possess trilaminar cytoplasmic membrane and cell wall as seen by electron microscopy. They are gram negative though do not take stain well. They stain deep red with Machiavello and Gimenez while bluish purple with Giemsa and Castaneda stain.

Cultivation

- They are obligate intracellular parasites. They cannot be grown on cell free media. They generally grow in cytoplasm of infected cell but spotted fever rickettsiae grow in nucleus as well.
- Optimum temperature for growth is $32-35^{\circ}\text{C}$.
- They can be cultivated in yolk sac of 5-6 days old embryonated egg.
- They can grow well on HeLa, Hep-2, mouse fibroblast, Detroit 6 and other continuous cell lines.
- Mice and guinea pig can be used for primary isolation of rickettsiae from clinical samples.

Antigenic structure

Rickettsiae possess 3 types of antigens

1. Group specific soluble antigen:- It is present on surface of organism and is protein in nature.
2. Species specific antigen:- It is adherent to the cell and act as adhesin for host cell
3. An alkali stable polysaccharide:- Found in some rickettsiae and in some non motile strains of Proteus (OX 19, OX 2, OXK). This sharing of antigens forms the basis for Weil- Felix reaction used in diagnosis of rickettsial infections. In this test agglutinins are detected against these Proteus strains.

Pathogenesis

Man acquire infection by bite or faeces of an infected arthropod vector. On entry into the human body they become localised chiefly in the vascular epithelium leading to thrombus formation.

MODULE

Microbiology



Notes



Notes



INTEXT QUESTIONS 32.1

1. Rickettsiae are Gram bacilli.
2. Rickettsiae are obligate parasites.
3. Rickettsiae are transmitted to man by
4. Rickettsiae are coccobacilli.

32.2.1 Typhus fever group

This consist of-

- (a) Epidemic (classical) typhus
- (b) Brill-Zinsser disease (recrudescent infection)
- (c) Endemic typhus

Epidemic Typhus

It is reported from all parts of the world but common in Russia and eastern Europe. In India the endemic spot is Kashmir. Humans are the only natural vertebrate hosts. Its causative agent is *R. prowazekii*. Human body louse (*Pediculus humanus corporis*) and head louse (*Pediculus humanus capitis*) act as vector. Incubation period is 10-14 days. Clinical features include: high fever, chills, severe headache and vomiting. On 4-7 days a characteristic maculopapular rash appear which start from trunk and spread over limbs but sparing face, palm and soles. Patient become stuporous and delirious in 2nd and 3rd week. The case fatality is about 40%.

Brill-Zinsser disease (recrudescent infection)

Rickettsiae may remain latent in the lymphoid tissue for years. Such latent infection may be reactivated leading to recrudescent typhus. It is milder illness with shorter duration (~2weeks) and lower case fatality. Skin rashes are also mild. Disease itself is not importance but patient is infectious for louse and an outbreak can occur.

Endemic typhus

Clinical disease is similar but milder than epidemic typhus and case fatality is 1%. It is caused by *R. typhi*. Its reservoir is rat and are transmitted by flea (*Xenopsylla cheopsis*). Rat flea acquire infection by feeding on infected rat.

Rickettsiaceae

Natural cycle is rat-flea-rat and man are the dead end host. They acquire infection by the bite of infected fleas when their saliva or feces is rubbed in.

R. typhi can be differentiated from *R. prowazekii* by following test

- Neil-Mooser or tunica reaction :- when male guinea pig is inoculated intraperitoneally with blood of patient infected with *R. typhi* they develop fever and scrotal swelling. The testis cannot be pushed back into abdomen due to adhesions. This reaction is negative in case of *R. prowazekii*.
- IFA
- ELISA
- PCR based DNA tests.

32.2.2 Spotted fever group

This group include:

Table 32.1: Members of spotted fever group, their vectors and reservoir.

Members	Disease	Vector	Vertebrate reservoir
<i>R. rickettsii</i>	Rocky Mountain spotted fever	Tick	Rabbit, dog, rodents
<i>R. siberica</i>	Siberian tick typhus	Tick	Wild animals, cattle
<i>R. conori</i>	Indian tick typhus	Tick	Rodents
<i>R. japonica</i>	Oriental spotted fever	Tick	-
<i>R. akari</i>	Rickettsial pox	Gamacid mite	Mouse

Main vector of spotted fever group are ticks except for *R. akari* which is transmitted by mites. Eschar frequently develops at the site of bite except in case of rocky mountain spotted fever.

Rocky Mountain spotted fever: It is the most serious type of infection, transmitted by tick (*Dermacentor andersoni*). Incubation period is 1 week. Clinical features include fever, headache, vomiting, diarrhoea, photophobia and cough. Maculopapular rashes appears on 4th day which starts from wrist, ankles, palms and soles. Rashes may become petechial and in grave cases they may become hemorrhagic. Patient may end up into hypotensive shock, hemiplegia and die within 5 days of onset of symptoms. Mortality varies from 6- 70%.

Rickettsial Pox: Mildest form of rickettsial disease, self-limited, non-fatal, vesicular exanthema. Also known as varicelliform rickettsiosis. Vector is mite and reservoir is domestic mouse. Clinically vesicle appear at site of bite and enlargement of lymphnodes occur followed by fever, headache, malaise within 3-10 days. Illness last for 10-14 days.

MODULE

Microbiology



Notes



Notes

32.3 SCRUB TYPHUS

It is caused by *Orientia tsutsugamushi*. Migratory birds may act as reservoir and transporter of disease agent. Vector is trombiculid mites. Man are the accidental host and may acquire infection when they trespass into mite islands and aare bitten by their larvae (chiggers).

After 1- 3 weeks of bite, patient develops severe headache, fever, conjunctivitis, deafness and eschar at site of bite. After 1 week of fever, maculopapular rash appears which starts from trunk. Mortality varies from 10- 60%.

32.4 LABORATORY DIAGNOSIS OF RICKETTSIAL INFECTIONS

Samples which can be processed for diagnosis are

- Blood clots
- Serum
- Biopsy specimen of rashes
- Impression smear from organs of infected animals
- Endolymph of ticks

Diagnosis is carried out by

- (a) Isolation of rickettsiae in lab animals, fertile hen’s egg and cell cultures
- (b) Direct detection of organism and their antigen in clinical samples
- (c) Serology

32.4.1 Isolation of Rickettsiae

Blood clots ground in skimmed milk or BHI broth is inoculated intraperitoneally in male guinea pig or mice. Animal will be observed for 3-4 weeks.

Table 32.2: Reaction in guinea pig produced by different rickettsial agents.

Agent	Reaction in Guinea pig
<i>R. rickettsii</i>	Develop fever, scrotal necrosis and may even die
<i>R. typhi</i>	Animal develops fever and tunica reaction
<i>R. akari</i>	Animal develops fever and tunica reaction
<i>R. prowazekii</i>	Animal develops fever without tunica reaction
<i>O. tsutsugamushi</i>	Mice is preferred. Animal develops ascitis

Rickettsiaceae

Smears from peritoneum, tunica and spleen of infected animals are stained by Giemsa or Gimenez method to demonstrate rickettsiae. They can also be grown in yolk sac of embryonated hen's eggs and cell culture.

32.4.2 Direct detection of organism and their antigen

Aggregates of the organism or their antigen in biopsy specimen from rashes and liver, impression smears from organs of infected animals may be demonstrated by

- Giemsa staining
- Macciavello staining
- Gimenez staining
- Direct immunofluorescence
- Indirect immunofluorescence
- PCR
- Sequence Amplification



INTEXT QUESTIONS 32.2

1. Epidemic typhus is caused by
2. Rickettsiae may remain latent in tissue of human
3. R. akari causing spotted fever is transmitted by
4. The reservoir of Rickettsiae pox is
5. Scrub typhus is caused by

32.4.3 Serology

- (a) Non-specific reaction:- Weil- Felix Reaction (see table. 3).
- (b) Specific:- using rickettsial antigen
 - CFT with purified antigen
 - Immunofluorescence on microdots of purified rickettsial antigen
 - ELISA with particulate or extracted antigen
 - Latex agglutination

MODULE

Microbiology



Notes



Notes

Table 32.3 Weil- Felix Reaction in diagnosis of rickettsial diseases

Disease	Agglutination with <i>Proteus</i> strains		
	OX19	OX2	OXK
Epidemic typhus	++++	±	-
Brill-Zinsser disease	±	-	-
Endemic typhus	+++	±	-
Spotted fever group	++	++	-
Scrub typhus	-	-	+++
Rickettsial pox	-	-	-

Treatment: Tetracyclines and chloramphenicol can be given to treat rickettsial infections.

32.5 GENUS EHRLICHIA

They are small, gram negative, obligate intracellular bacteria. They have affinity towards blood cells and their vacuoles inside phagocytic cells are known as morula. They are tick borne. Leucopenia, thrombocytopenia and raised liver enzymes are seen in patients. Three species have been reported to infect humans.

- (a) *E. sennetsu*
- (b) *E. chaffeensis*
- (c) *E. phagocytophila*

Laboratory Diagnosis

- Demonstration of bluish intracytoplasmic inclusion (morula) in Geimsa stained blood smears.
- Demonstration of specific antibodies by immunofluorescence using cell cultures.
- PCR

Treatment: Doxycycline is recommended for treatment of ehrlichioses.

32.6 COXIELLA BURNETII (Q FEVER)- NO LONGER INCLUDED IN GENUS RICKETTSIAE

Q fever is a worldwide zoonosis and is caused by *Coxiella burnetii*. It is also an obligate intracellular pathogen. It is pleomorphic coccobacilli 0.2µm- 0.4µm × 0.4- 1.0µm. It is Gram negative but better stained with Gimenez and other rickettsial stains. It can survive holder method (63°C for 30 min) of pasteurization

Rickettsiaceae

of milk but flash method is effective. It can be inactivated by 2% formaldehyde, 1% Lysol and 5% H₂O₂.

Pathogenesis

It is transmitted among cattle, sheep and poultry by ticks. They can be shed in milk of infected animals and products of conception. They can contaminate the environment during parturition. Humans can get infection through

- Consumption of infected milk
- Handling of infected wool, meat and other animal products
- Contaminated soil, straw and clothing

Coxiella may enter through abraded skin, inhalation, ingestion. Incubation period is 2-4 weeks. Patient develop fever, chill, headache, pneumonia, endocarditis and meningoencephalitis. Spontaneous recovery is usual.

Laboratory Diagnosis

Coxiella can be isolated from blood, sputum and other clinical samples but is not recommended due to serious risk of infection to laboratory personnel. Culture can be done in guinea pig, mice and embryonated hen's eggs. Cell cultures can also be used. Diagnosis mainly relies upon the demonstration of antibodies by CFT and indirect immunofluorescence assay. PCR can be done.

Treatment: Doxycycline may be given for treatment. In serious cases prolonged treatment with tetracycline, chloramphenicol may be required.

32.7 GENUS BARTONELLA

It belongs to family Bartonellaceae. They are Gram negative bacilli. This genus include

32.7.1 Bartonella bacilliformis

It is pleomorphic, Gram negative, motile coccobacillus. They are strict aerobes. They can grow on semi-solid nutrient agar containing rabbit serum and haemoglobin but growth is slow. It causes Oraya fever (Carrion's disease) which is transmitted by sandfly. Incubation period is 3 weeks to 3months. Clinical features include fever, severe headache followed by severe anaemia. Organism is found inside erythrocytes of infected patients. Hepato-splenomegaly can be present. Case fatality is 40%. A late sequel in survivors is verruga peruana.

Diagnosis is done by demonstration of organism in blood smears by Giemsa staining and by culture. Penicillin, streptomycin and tetracycline can be used for treatment of Oraya fever and verruga peruana.

MODULE

Microbiology



Notes



Notes

32.7.2 Bartonella Quintana

It is small, gram negative bacillus. It does not possess flagella. It may show twitching movement caused by fimbriae. Growth is slow on rabbit or sheep blood agar when incubated in 5% CO₂. There is no animal reservoir of this disease. It causes trench fever. Disease is spread by body louse. Infection is acquired when infected feces are scratched into the skin. Incubation period is 14- 30 days. Clinical features include headache, fever, severe pain in legs and back and roseolar rash on chest, abdomen and back. Recovery is frequent but relapses can occur.

Diagnosis is done by allowing healthy lice on patient blood and the organism may be detected in gut of lice. Culture can be done on rabbit or sheep blood agar. It can be detected in tissues by PCR.

32.7.3 Bartonella henselae

It is small, slightly curved, Gram negative bacillus. It also displays twitching motility. It can be grown on chocolate agar, Columbia agar with 5% sheep or rabbit blood and BHI agar supplemented with blood. Growth occurs in 5-15 days. It causes cat- scratch disease. Infection is acquired by contact, scratch or bite of infected cat. Clinical features include fever and lymphadenopathy. Cat scratch disease is self limiting and requires no treatment. Endocarditis can occur. In AIDS patient, it can lead to bacillary angiomatosis and bacillary peliosis.

Diagnosis is done by demonstration of organism in section of lymph node biopsies stained with Warthin- Starry silver impregnation stain. It can be grown on chocolate and Columbia agar.

**INTEXT QUESTIONS 32.3**

1. Serologically method is used in diagnosis of rickettsial disease.
2. Ehrlichia infections are transmitted by
3. Q fever is caused by
4. Bartonella bacilliform causes
5. Bartonella bacilliform is transmitted by
6. Bartonella Quintana causes fever
7. Trench fever is spread by



WHAT YOU HAVE LEARNT

- Family Rickettsiaceae contains small cocobacillary organisms. They cause typhus fever and other related diseases. They are true intracellular parasites. They cannot be grown on artificial media. They are arthropod borne. Among the typhus fever group RMSF is the most serious form of disease while the rickettsial pox is the mildest form of disease. *Ehrlichia* have affinity for blood cell of patient. *Coxiella* causes Q fever. *Bartonella* can be grown on blood and chocolate agar. They are gram negative but are better visualised by special staining.



Notes



TERMINAL QUESTIONS

1. Describe characteristics of family Rickettsiaceae?
2. Describe pathogenesis of rickettsial diseases?
3. Describe Weil- Felix reaction?
4. Describe Neil Mooser reaction?
5. Differentiate between epidemic and endemic typhus?
6. Write short note on Brill- Zinsser disease?



ANSWERS TO INTEXT QUESTIONS

32.1

1. Negative
2. Intracellular
3. Anthropods
4. pleomorphic

32.2

1. R.prowazekii
2. Lymphoid
3. Mites

MODULE

Microbiology



Notes

Rickettsiaceae

4. Domestic mouse
5. *Orientia tsutsugamushi*

32.3

1. Weilfelix reaction
2. Ticks
3. *Coxiella burnetii*
4. Oraya fever
5. Sandfly
6. Trench
7. Body louse