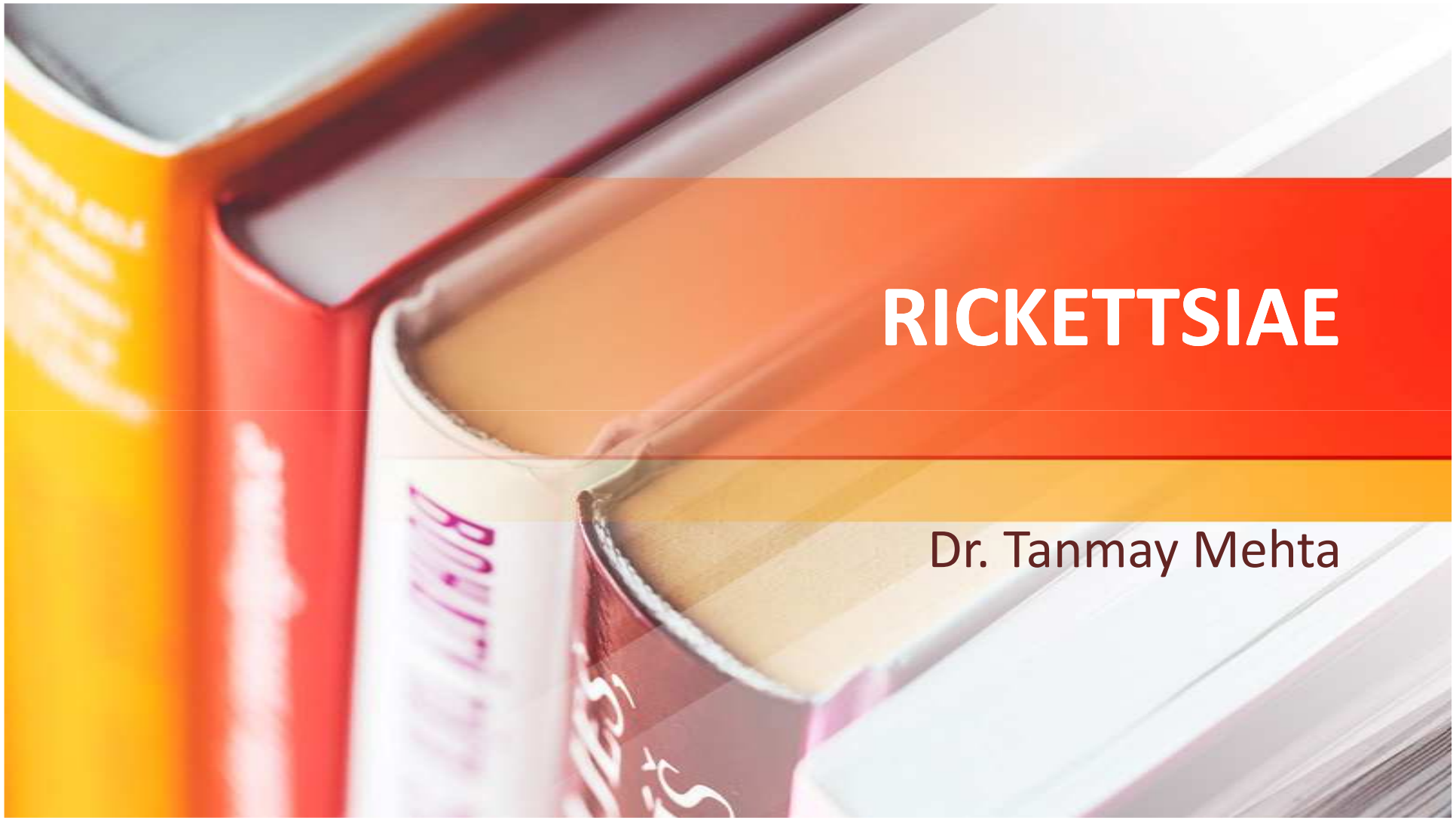


RICKETTSIAE

Dr. Tanmay Mehta

Learning objectives

At the end of the session, the students will be able to

- Describe morphology and antigens
- Describe Pathogenesis & Clinical features
- Choose appropriate lab diagnosis and interpret the results
- Describe prevention and treatment

Rickettsiae

- Gram negative coccobacilli non-motile
- Obligate intracellular organisms
- Not cultivable in artificial media
- Can grow in cell lines, animal and egg inoculation
- Transmitted by arthropod vectors, such as tick, mite, flea or louse

Classification

1. **Family Rickettsiaceae** - *Rickettsia and Orientia*
2. **Family Anaplasmataceae** - (1) *Ehrlichia, (2) Wolbachia, (3) Anaplasma and (4)Neorickettsia*

- Former members such as *Coxiella and Bartonella are* now excluded from the family because:

- ***Coxiella*** is not arthropod borne, transmitted by inhalational mode
- ***Bartonella*** is not an obligate intracellular parasite*;* can grow in cell-free media

Rickettsiae Versus Viruses

- Similar to viruses: smaller size and obligate intracellular
- **Actually Bacteria:**
- Gram-negative cell wall (better stained with Giemsa or Gimenez stains)
- Contain both DNA and RNA
- Possess ribosomes for protein synthesis and enzymes for the Krebs cycle

Rickettsiae Versus Viruses

- Multiply by binary fission
- Susceptible to antibacterial agents
- Large enough to be seen under the light microscope
- Not filtered by bacterial filters

Features of Rickettsiae

Broad Group	Species	Disease	Vector	Distribution
Typhus Group	R. prowazekii	Epidemic Typhus	Louse (Rubbing)	World wide (Africa & South America)
		Brill Zinsser Disease		
	R. typhi	Endemic Typhus	Flea	World wide

Features of Rickettsiae

Broad Group	Species	Disease	Vector	Distribution
Spotted Fever Group	R. rickettsii	Rocky mountain spotted fever (RMS)	Tick	America
	R.conori	Indian tick typhus (ITT)	Tick	Europe, Asia
	R.africae	African Tick bite fever	Tick	Sub-Saharan Africa
	R.akari	Rickettsial Pox	Mite (gamasid)	USA, Ukraine, Turkey, Mexico

Features of Rickettsiae

Broad Group	Disease	Rash	Eschar	LN	Weil Felix
Typhus Group	Epidemic Typhus	80% (All over the body except palm & sole)	-	-	OX 19 ++++ OX 2 +/-
	Brill Zinsser Disease				negative or weakly positive
	Endemic Typhus	80% (trunk)	-	-	OX 19 ++++ OX 2 +/-
Scrub Typhus	Scrub typhus	50%	35%	+++	OX K +++

Features of Rickettsiae

Broad Group	Disease	Rash	Eschar	LN	Weil Felix
Spotted Fever Group	Rocky mountain spotted fever (RMS)	90% (extremities & trunk)	<1%	+	OX 19 ++ OX 2++
	Indian tick typhus (ITT)	97%	50%	+	
	African Tick bite fever	50% (Vesicular)	90%	+++ +	
	Rickettsial Pox	100% (Vesicular)	90%	+++	All negative

History

- ***Rickettsia*** - Howard Taylor Ricketts (1911) discovered that Rocky Mountain spotted fever is transmitted by tick
- ***Rickettsia prowazekii*** is named by *Da Rocha Lima in* honor of von Prowazek
- Both von Prowazek and H Ricketts died of typhus which they contracted during their study
- Charles Nicolle identified the role of body lice in the transmission of epidemic typhus

GENUS RICKETTSIA

- Species of *Rickettsia* can be categorized into two groups based on the clinical manifestations

1. Typhus group
2. Spotted fever group

- **Antigenic Structure**

- Cell wall - similar to that of gram-negative bacteria

Antigenic Structure

- **Species specific outer membrane proteins (OMP):** highly immunogenic, induce the synthesis of protective antibodies- used for vaccine & for diagnosis

- OmpA - present only in spotted fever group

- OmpB - present in both spotted fever group and typhus fever group

- **Group specific alkali stable lipopolysaccharide (LPS) antigen**: found in some rickettsiae and shared by certain strains of *Proteus (OX19, OX2 and OXK strains)* → basis of Weil- Felix reaction

Pathogenesis

- **Transmission**: arthropod vectors
- **Tick and mite borne** -by biting
- **Louse and flea borne by**

- Autoinoculation following rubbing or scratching of abraded skin or mucosa contaminated by insect feces (seen in epidemic and endemic typhus) **or**

- Aerosol (by inhaling dried louse or flea feces)

Pathogenesis

- **Transovarial transmission:** Tick and mite - can serve as both vector as well as reservoir
- **Spread**: portal of entry → lymphatics → multiply in regional lymph nodes → spread via bloodstream
- **Target sites**: endothelial cells (in addition, *R. akari & O.tsutsugamushi* attack the monocytes)
- **Phagocytosis**: Adhesion to the endothelial cells using OmpA and OmpB →phagocytosis

Pathogenesis

- **Intracellular locations**: Following phagocytosis, rickettsiae remain inside a vacuole. Later:
- *Rickettsia & Orientia* - lyses vacuoles → free in the
- *Coxiella and Ehrlichia* - continue to multiply in vacuoles
- *Coxiella* vacuole fuses with lysosome but survive inside phagolysosome
- *Ehrlichiae* - maintained inside vacuoles → killed if fused with lysosomes

Pathogenesis

- **Multiplication**: multiply slowly by binary fission (generation time 9–12 hours)
- **Cell-to-cell spread:** Spotted fever rickettsiae can spread from cell-to-cell. Other rickettsiae accumulate in the cell until lysis
- **Reason for obligate intracellular survival:** not understood

- Lack many enzymes required for basic metabolic pathways
- Prefer to use host cell ATP if available rather than produce

Pathogenesis

- **Endothelial cell injury:**
- Lipid peroxidation of host-cell membranes
- Inhibit cell apoptosis by upregulating NFkB pathway
- Endothelial cells enlarge, degenerate → thrombosis →rupture and necrosis
- **Release**: unstable and die quickly (exception *Coxiella)*

Epidemic Typhus (Louse-borne)

- Caused by *R. prowazekii.*
- **Vector**: Human body louse, *Pediculus humanus corporis*

- Acquires organism via blood meal → shed in feces

- **Mode of transmission**:

- Autoinoculation - rubbing or scratching of abraded skin or mucosa contaminated by louse feces
- Inhalation of louse feces - laboratory or bioterrorism

Epidemic Typhus (Louse-borne)

- **Clinical manifestations**:

- Acute febrile disease - headache, myalgia, eye discharge and rashes

- **Rash -** begins on the upper trunk→ generalized, involving the entire body except face, palms and soles
- **Myalgia** - usually severe (sutama “crouching” in Burundi)
- **Complications** - interstitial pneumonitis, CNS involvement (mental confusion and coma), myocarditis & acute renal failure

Epidemic Typhus (Louse-borne)

- **Risk factors**: High louse population - refugee camps, prisons and overcrowded communities
- **Zoonotic cycle**: Eastern flying squirrels & their lice and fleas maintain *R.prowazekii* in the environment
- **Geographical distribution**: Endemic in Africa (Burundi, Rwanda and Ethiopia) and South America
- **Brill–Zinsser disease**: Recrudescent illness occurring years after acute epidemic typhus

Endemic Typhus (Flea-borne)

- Endemic (murine) typhus - caused by *R. typhi infection.*
- **Vectors:** rat flea (*Xenopsylla cheopis)* or rarely cat flea *(Ctenocephalides felis)*
- **Mode of transmission**: inoculation on skin or inhalation of flea's dried feces, less frequently by the flea bite
- **Reservoir**: Rodents - *Rattus rattus and R.norvegicus*

Endemic Typhus (Flea-borne)

- **Clinical manifestations**: Incubation period - average 11 days

- Symptoms similar to epidemic typhus but milder and rarely fatal

- Fever, headache, myalgia, anorexia and rash (involving the trunk more often than the extremities)

- **Geographical distribution**: endemic worldwide, especially in warm tropics having high rat infestations

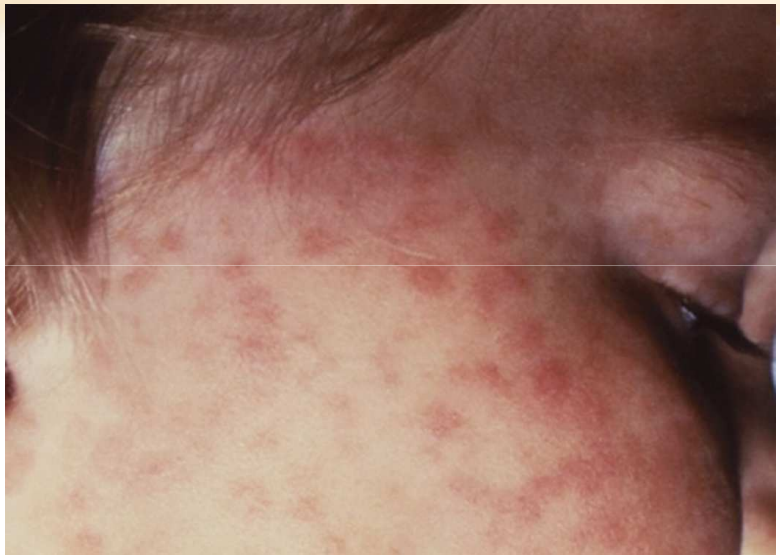
- **India**: Reported from - Shimla, Kashmir, Mumbai, Jabalpur, Lucknow and Pune.

Rocky Mountain Spotted Fever

- Caused by *Rickettsia rickettsii.*
- **Vector**: ticks
  - *Dermacentor andersoni* in USA
  - *Amblyomma cajennense* in Central/South America
  - *Rhipicephalus sanguineus* in Mexico, Arizona and Brazil
- **Transmission**: tick bite
- **Reservoir**: Ticks serve as vector as well as reservoir
- Other mammals - dogs, small rodents

Rocky Mountain Spotted Fever

- **Clinical manifestations**:
  Incubation period - 4 to 14 days
- Fever, headache and rash and frequently myalgia and anorexia
- Rashes on extremities (wrist and ankles) and trunk. Initially maculopapular →hemorrhagic



Rocky Mountain Spotted Fever

- **Complications**: appear late

- Vascular damage, increased permeability, edema, hemorrhage, disseminated intravascular coagulation, interstitial pneumonitis, CNS involvement, myocarditis and renal failure

- Most fatal rickettsial disease

- **Geographical distribution:**

- Endemic in high tick population areas of USA, Central and South America

- More common during tick season (summer in tropics) and among children and males

Indian Tick Typhus

- Caused by *Rickettsia conorii.*
- **Vector**: Transmitted by tick bite (*Rhipicephalus sanguineus)*
- **Clinical manifestations**: Similar to that of RMS fever
- Eschar at the site of the tick bite in 50% of cases
- **Geographical distribution**: *R. conorii* is prevalent in Southern Europe, Africa and Southern Asia
- **Other regional names** : Mediterranean spotted fever, Kenya tick typhus, Israeli spotted fever, Astrakhan spotted fever
- India - ITT is widespread (Nagpur, Jabalpur, Sagar, Pune, Lucknow, Bengaluru and Secunderabad)

Other Tick-borne Fever

- **African tick-bite fever***:* caused by *R.africae,* transmitted by tick bite and is endemic in sub-Saharan Africa
- **Maculatum disease**: Caused by *R.parkeri,* transmitted by tick bite*,* in USA and South America
- **Japanese spotted fever**: Caused by *R.japonica,* occurs in Japan and Korea
- **Queensland tick typhus**: due to *R. australis,* transmitted by Tick (*Ixodes holocyclus)*
- **Flinders Island spotted fever**: It is caused by *R. honei*

Rickettsialpox

- Caused by *Rickettsia akari*
- **Vector**: Mite bite, transovarian transmission
- **Reservoir**: Mice (*Mus musculus)*
- **Clinical manifestations**: Similar to any other rickettsial diseases, differ in:

- Vesicular rashes, Eschar at the site of mite bite, Regional lymphadenopathy

- **Geographical distribution**: endemic in USA, Ukraine, Turkey and Mexico

Laboratory Diagnosis

- **Serology**
- Antibody detection - mainstay of diagnosis -nonspecific test (Weil–Felix test) and specific tests
- **Weil–Felix Test**
- Heterophile agglutination test
- Rickettsial antibodies are detected using *Proteus antigens*
- **Procedure**: tube agglutination test

Weil Felix Test

- **Results:** Sera agglutinate with
- **OX19 and sometimes with OX2** - Epidemic and endemic typhus
- **Both OX19 and OX2** - tick-borne spotted
- **OXK** - scrub typhus
- **Negative** - rickettsialpox, Q fever, ehrlichiosis and bartonellosis
- Test should be done after 5–7 days of onset of fever
- Titre of **1:80** → possible infection

Weil Felix Test

- **False-positive titer**
- *Proteus* infection
- Fourfold rise of antibody titer in paired sera is more meaningful than a single high titer
- **False-negative**
- Prozone phenomena →obviated by testing serial dilutions sera
- Weil–Felix test being a nonspecific test should always be confirmed by specific tests

Specific Antibody Detection Tests

- **Indirect immunofluorescence assay (IFA)**
- Gold standard and reference serologic test
- Titer of ≥1:64 is considered as significant
- Sensitivity & specificity are 94–100% and 100% respectively
- **ELISA** (IgM capture ELISA)
- **Indirect immunoperoxidase assay** (IPA)
- **Rapid tests -** ICT

Other Methods of Diagnosis

- **Histological examination** of a cutaneous biopsy sample from a rash
- **Isolation**:
- Cell lines (Vero, primary chick embryo, WI-38, HeLa)
- Egg (yolk sac inoculation)
- Animal inoculation (guinea pig)
- Highly infectious - biosafety level III facilities necessary

Other Methods of Diagnosis

- **Neil-Mooser reaction:** Specimen is inoculated intraperitoneally into male guinea pigs
- *R. Rickettsii* - scrotal necrosis
- *R. Prowazekii* - fever without any testicular inflammation
- *R. typhi, R. conori and R. Akari* **-** fever and positive tunica reaction (testicular inflammation)
- **Molecular tests**: PCR

- Useful specimens: Whole blood, buffy coat fraction, skin rash biopsies, lymph node biopsies or tissue specimen

Treatment Rickettsiosis

- Doxycycline - drug of choice (100 mg bid 1–5 days)
- Alternative - Chloramphenicol
- **Prevention of Rickettsiosis**

- Vector control strategies
- Control of rodents and other animals
- Improvement of personal hygiene
- No vaccine is available

GENUS ORIENTIA

Scrub Typhus

- Caused by *Orientia tsutsugamushi*
- **Naming**: can occur in areas where scrub vegetations consisting of low lying trees and bushes
- **Affects military population**
- **Vector**: bite of infected trombiculid mites (*Leptotrombidium)*

- Chiggerosis: Among all stages of mite, larvae (chiggers) are the only stage that feed on humans → *chiggerosis*
- Mites maintain through transovarian transmission

Clinical manifestations

- **Classical triad** of an eschar, regional lymphadenopathy and maculopapular rash (40–50% of cases)
- **Non-specific manifestations** - fever, headache, myalgia, cough, and gastrointestinal symptoms
- **Complications** - encephalitis and interstitial pneumonia due to vascular injury
- **Antigenic diversity**: Five major antigenic types—Boryon, Gilliam, Karp, Kato and Kawazaki

Scrub Typhus

- **Epidemiology**: Among the rickettsial diseases, scrub typhus is most widespread
- **Zoonotic tetrad**: Four elements are essential to maintain *O. tsutsugamushi*

1. Trombiculid mites.
2. Small mammals (e.g. field mice, rats, shrews).
3. Secondary scrub vegetations or forests
4. Wet season (when mites lay eggs)

Scrub Typhus

- **Indian scenario**: re-emerging
- Most common rickettsial disease in India
- Outbreaks - sub-Himalayan belt, from Jammu to Nagaland, Puducherry, Karnataka, Tamil Nadu and Kerala
- Mainly from rural areas

Scrub Typhus

- **Treatment** : Doxycycline

- Alternatives - Chloramphenicol or azithromycin

- **Vaccine**: Effective vaccine is not yet licensed

- Several candidate vaccines under trial

LABORATORY DIAGNOSIS

- **Serology (antibody detection):**
- IgM antibodies appear by end of 1st week, and IgG by end of the 2nd week
- **Re-infection** - IgG antibodies are detectable by day 6, with IgM antibody titers being variable
- **Weil-Felix test**: Nonspecific, detects high titers of heterophile antibodies to *Proteus OXK antigens*

Scrub Typhus

- **Indirect immunofluorescence antibody (IFA):** Gold standard serological test
- **ELISA –** using 56-kDa recombinant major surface protein antigens derived from Gilliam, Karp, and Kato strains

- Cost-effective and alternative to IFA for acute diagnosis & seroprevalence

- **Rapid tests** - not recommended at present as they need further evaluation

Scrub Typhus

- **Culture**
- Egg (yolk sac), cell culture (Vero cells, MRC 5 cells, BHK21, L929 mouse fibroblast cells)
- Time consuming (4 weeks) and technically demanding
- **Molecular test**
- PCR, nested PCR, LAMP targeting genes- 56-kDa gene, 47-kDa gene, 16S rRNA gene and 60-kDa heat shock protein (*groEL) gene*

ANAPLASTACEAE

Anaplastaceae - Features

	Ehrlichia chaffeensis	Ehrlichia ewingii	Anaplasma phagocytophilum	Neorickettsia sennetsu
Causes	Human Monocytic Ehrlichiosis (HME)	Human Granulocytic Ehrlichiosis	Human Granulocytic Anaplasmosis	Human Lymphocytic Ehrlichiosis

Anaplastaceae - Features

	Ehrlichia chaffeensis	Ehrlichia ewingii	Anaplasma phagocytophilum	Neorickettsia sennetsu
Feature	Leucopenia Thrombocytopenia Elevated liver enzymes Risk factor- Immunocompromised patients	Features similar to HME but less severe Risk factor- Immunocompromised patients	Leucopenia Thrombocytopenia	Mononucleosis like illness Atypical lymphocytosis Lymphadenopathy

Anaplastaceae - Features

	Ehrlichia chaffeensis	Ehrlichia ewingii	Anaplasma phagocytophilum	Neorickettsia sennetsu
Transmitted by	Tick (Amblyomma americanum)	Tick (Amblyomma americanum)	Tick (Ixodes scapularis)	Ingestion of fish carrying infected flukes
Reservoir	White-tailed deer (rarely dogs)	White tailed deer and dogs	Mice, squirrels, and white-tailed deer	? (not known)
Distribution	USA	USA	USA	Japan and Malaysia

EHRLICHIOSIS

- They reside in vertebrate reservoirs and target vacuoles of hematopoietic cells
- Few of them are pathogenic –

- ***Ehrlichia chaffeensis-*** human monocytic ehrlichiosis
- ***Ehrlichia ewingii*** - infects neutrophils and causes human granulocytic ehrlichiosis

EHRLICHIOSIS

- ***Anaplasma phagocytophilum*** - infects neutrophils, causes human granulocytic anaplasmosis
- ***Neorickettsia sennetsu*** - infects lymphocytes & cause mononucleosis like syndrome (human lymphocytic ehrlichiosis)

Clinical feature

- **Acute febrile disease**

- Headache, myalgia, arthralgia, cough, pharyngitis, lymphadenopathy, diarrhea, nausea, vomiting, abdominal pain and changes in mental status

- **Inclusions**: reside inside phagosome, multiply to produce the following three stages of growth— elementary body, initial body, and mulberry like inclusions called Morula
- **Morulae** in neutrophil (20–75%) in peripheral blood film examination
- **Treatment**: doxycycline

COXIELLA BURNETII (Q FEVER)

- Causes **'Q fever'**
- **History**

- For long time the causative agent was unknown, hence 'Query' or Q fever
- Edward Derrick – identified *Coxiella burnetii*

- Named after the two scientists Cox and Burnet who have contributed to its discovery

COXIELLA BURNETII (Q FEVER)

- **Source of Infection**
- Primary sources - infected cattle, sheep and goats
- Reservoirs - wild animals and ticks
- **Mode of Transmission**
- Inhalation of infected dust from soil, previously contaminated by urine and feces of diseased animals
- Ingestion of contaminated milk
- Transplacental, blood transfusion or through skin abrasions/mucosa

COXIELLA BURNETII (Q FEVER)

- **Geographical Distribution**

- Endemic in most parts of the world except New Zealand and Antarctica
- India- Rajasthan, Punjab, Haryana and Delhi
- Rajasthan: overall prevalence 18.6% in humans and 24.7% in animals

COXIELLA BURNETII (Q FEVER)

- **Pathogenesis:** Escapes intracellular killing in macrophages by:
- Inhibiting the final phagosome maturation step (cathepsin fusion)
- Resistant to the acidic environment of phagolysosome by producing superoxide dismutase
- Induces autoantibodies to cardiac and smooth muscles
- Surface antigens (LPS) shows phase variation

COXIELLA BURNETII (Q FEVER)

- **Clinical Manifestations**
- **Acute Q fever**: IP - 3–30 days

- Hepatitis, interstitial pneumonia, fever, CNS involvement and pericarditis or myocarditis

- **Post Q fever fatigue syndrome**: Profound myalgia, headache, sweating, arthralgia, muscle fasciculation
- **Latency**: for 2–3 years
- **Chronic Q fever**: endocarditis usually in patients underlying valvular heart disease, or immunosuppression
- Fever is usually absent or of low grade

COXIELLA BURNETII (Q FEVER)

- **Laboratory Diagnosis**
- Pleomorphic gram-negative coccobacillus
- Extremely fastidious, highly infectious
- **Isolation**: must be done only in biosafety level 3
- Cell cultures - monkey kidney cells, Vero cells
- Shell vial cell culture - human embryonic lung fibroblasts cell line, or yolk sacs (egg) or animals such as hamsters, mice or guinea pigs

COXIELLA BURNETII (Q FEVER)

- **Antibody detection:** most commonly used diagnostic tool
- **Indirect immunofluorescence assay (IFA)** - sensitive, specific and method of choice
- IgM appears in 7–10 days of infection → IgG appears after 14–20 days of infection
- Chronic infections - IgG antibodies to phase I antigens are elevated (>1:6400 titer)
- Acute Q fever - antibodies to phase II antigens (IgG ≥1:200 and IgM ≥1:50)

COXIELLA BURNETII (Q FEVER)

- **Immunodetection of *C. burnetii in tissues:*** Immunoperoxidase staining targeting various antigens.
- **Molecular methods**:

- QpH1 plasmids - acute Q fever isolates
- QpRS plasmids - strains isolated from endocarditis
- Other target genes - 16S rRNA, 23S rRNA, superoxide dismutase & *htpAB genes*

Treatment Q fever

- **Acute Q fever**:
- Doxycycline (100 mg bd 14 days)
- Quinolones are also effective
- **Chronic Q fever**:
- Hydroxychloroquine is added to alkalinize the phagolysosome and to render doxycycline to act against organism

COXIELLA BURNETII (Q FEVER)

- **Prevention**
- Control measures include:
- **Vaccine**: Inactivated whole-cell vaccine (Q-Vax), for occupationally exposed workers
- **Good animal husbandry practices** - proper disposal of animal excreta and aborted materials, isolation of aborting animals for 14 days
- **Pasteurization of milk by Flash method** as *C.burnetii* survives Holder's method of pasteurization

BARTONELLA

- Fastidious, facultative intracellular, slow-growing, gram-negative bacteria
- Ability to invade mammalian cells and RBCs
- Pathogens—*B. bacilliformis, B. quintana, and B.Henselae*

Bartonella	Diseases	Reservoir	Transmission
B.henselae	Cat-scratch disease, Bacillary angiomatosis, Bacillary peliosis, Bacteremia, endocarditis	Cats, Other felines	Exposure to cat – by scratch or bite Cat fleas associated with cat-to-cat transmission, but not cat-to-human transmission

Bartonella	Diseases	Reservoir	Transmission
B.quintana	Trench fever Chronic bacteremia, endocarditis Bacillary angiomatosis	Humans	Louse (Pediculus humanus corporis)
B.bacilliformis	Bartonellosis (Carrion's disease) Oroya fever Verruga peruana	Humans	Sand fly (Lutzomyia verrucarum)

Bartonella henselae

- Transmitted by cat scratch or bite Cat fleas - may be responsible for cat-to-cat (but not cat-to-man) transmission
- **Cat-scratch disease (CSD)**: Also rarely caused by *Afipia felis and B.quintana*

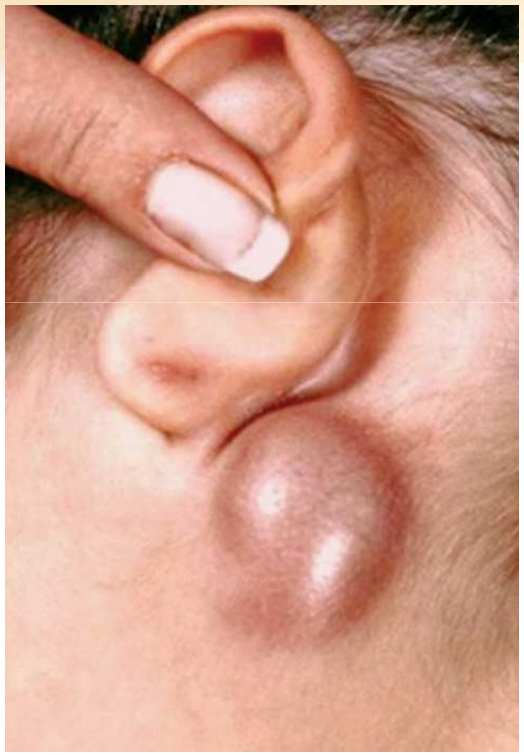
1. **Typical CSD**: More common form

- Subacute regional lymphadenopathy (most common being axillary/epitrochlear lymph nodes)
- Painless erythematous papule or pustule at the site of cat scratch

2. **Atypical CSD**: hepatitis, splenitis & retinitis

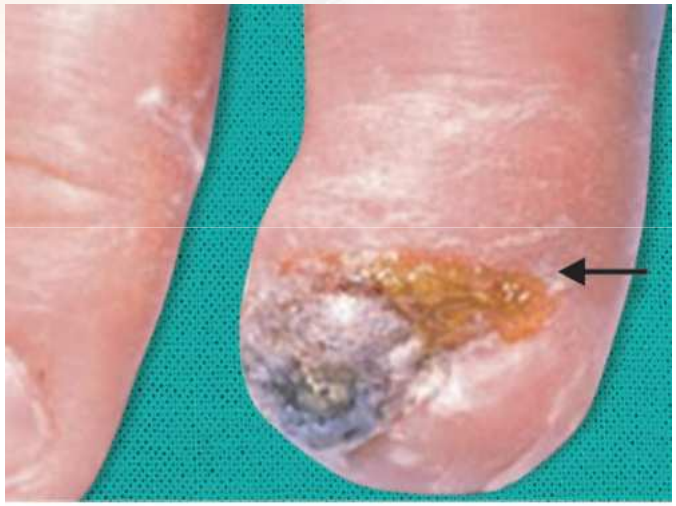
Bartonella henselae

- Enlarged lymph node of cat-scratch disease



Bartonella henselae

- **Bacillary angiomatosis (epithelioid angiomatosis)**
- Angioproliferative disorder
- Neovascular lesions involving skin and other organs
- HIV and immunocompromised conditions



Bartonella henselae

- **Bacillary Angiomatosis**
- *B. henselae and B.quintana*
- Both cause skin lesions
- *B. Henselae* - Hepatosplenic lesions subcutaneous
- *B.quintana* - lytic bone lesions
- **Bacillary peliosis**: angioproliferative disorder - involving liver (peliosis hepatitis), spleen and lymph nodes
- **Bacteremia & endocarditis**

Bartonella quintana

- Transmitted to humans by body louse feces (autoinoculated into skin due to scratching)
- **Causes**

- Trench fever
- Chronic bacteremia
- Endocarditis and
- Bacillary angiomatosis

Bartonella quintana

- **Classical trench fever**:

- Epidemics in the trenches of World War I

- Periodic mild febrile illness lasting 4–5 days with 5-day intervals between the episodes (**5 days fever)**

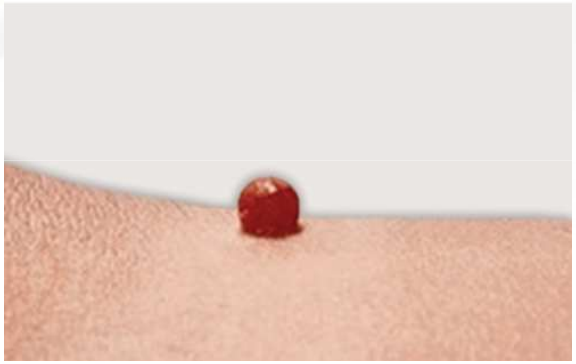
- **Silent for** Decades

- **Re-emerged trench fever**: in USA

- chronic bacteremia and endocarditis

Bartonella bacilliformis

- Transmitted by vector sandfly (Lutzomyia)
- Reservoir – only humans
- **Biphasic disease:**
- **Oroya fever or Carrion's disease**: initial, bacteremic, systemic illness presenting with or without anemia
- **Verruga peruana**: late-onset manifestation - cutaneous vascular lesions



Laboratory Diagnosis

- **Specimens** - blood, lymph node or skin biopsies
- **Microscopy**: Warthin-Starry silver nitrate staining and immunofluorescence staining
- **Culture**: Blood agar at 37^{o}C (except for B.bacilliformis at 30^{o}C) in presence of 5% CO2 and incubated for 12–15 days

- Sensitivity can be increased after cell lysis or freezing the sample

Laboratory Diagnosis

- **Antibody detection**:
- Indirect immunofluorescence assay (IFA)
- Enzyme immunoassay (EIA)
- **PCR:** Target genes - citrate synthase gene, 16S rRNA gene or heat-shock protein gene.