

CHLAMYDIAE

CHAPTER-27: CHLAMYDIAE

Learning objectives

To know in detail about,

- Basophilic virus, energy parasites, psittacosis, omithosis and Bedsonian agents
- Difference between elementary bodies and reticulate bodies
- Development cycle of chlamydiae
- Stain used to identify chlamydiae
- Different cultivation methods of chlamydiae
- Antigens, toxin and pathogenesis of chlamydiae organisms
- General approaches used to diagnose chlamydiae

SYSTEMATICS

Domain	Bacteria
Phylum	<i>Chlamydiae</i>
Class	<i>Chlamydiae</i>
Order	<i>Chlamydiales</i>
Family	<i>Chlamydiaceae</i>
Genus	<i>Chlamydia</i> <i>Chlamydophila</i>

- *Chlamydia* are derived from the characteristic appearance of the inclusion bodies produced by these agents, which are seen close to the nuclei of infected cells as a cloak or mantle (chlamys meaning mantle).
- *Chlamydiae* are obligate intra cellular parasites, filterable, requiring living system for multiplication.
- They differ from viruses in
 - They possess cell wall, resembling Gram-negative bacteria but lacking muramic acid
 - Do not have an eclipse phase following an infection.
 - Produce basophilic intracytoplasmic inclusion bodies in infected cells (Hence, referred as basophilic virus).
 - Possesses both DNA and RNA
 - Multiply by binary fission.
 - Sensitive to antibiotics.
- Therefore, they occupy a position intermediate between rickettsia and viruses.
 - *Chlamydiae* are more dependant on host cell for high energy compounds such as ATP. Hence, they are called as energy parsites.

- *C.psittaci* causes variety of infections in animals such as gastritis, diarrhoea, pneumonia, enzootic abortion in ewes, abortion, orchitis, epididymitis, seminal vesiculitis, feline pneumonitis, sporadic bovine encephalomyelitis (BUSS disease), conjunctivitis, rhinitis, hepatitis, and polyserositis and poly arthritis.
- *Chlamydia* infection in psittacine birds such as parrots called as psittacosis and this infection in non-psittacine birds such as pigeons, sparrows, turkey and domestic poultry called as ornithosis.

HISTORY AND HABITAT

History

- The preliminary study in psittacosis was carried out by Sir Samuel Bedson.
- Hence, the psittacosis agents are also termed as Bedsoniae.

Natural habitat

- In both birds and mammals the G.I.tract appears to be natural inhabitant of *C.psittaci*.
- The infection with prolonged faecal shedding is characteristic.
- *C.psittaci* is commonly carried in the spleen and kidney of normal appearing birds.

MORPHOLOGY

- Chlamydiae occur in two forms, elementary bodies and reticulate bodies.

Developmental cycle of Chlamydiae

- They have a unique developmental cycle with alternating, morphologically distinct, infectious and reproductive forms.
- The elementary bodies (EB) are small, spherical 200-300nm in d.m., infectious and represents the extracellular form of the organism.
- The elementary bodies enters a cell by endocytosis and differentiate into the large (500 –1000nm size), non infectious, but metabolically active reticulate body (RB) inside an expanding vacuole.
- The RB multiplies by binary fission producing further RB's.
- At about 20hrs following infection, some of the RB's start to condense and mature within the inclusion to form EB's.
- In general, release of infectious EB's begins at about 40hrs post infection due to lysis of the cell.
- Chlamydiae are Gram negative; the EBs can be demonstrated by the use of either chemical stains or FAT and IPT.
- In modified ZN stain the EBs tend to occur in clumps and stain red against a blue background.
- When the modified ZN stained smears examined under darkfield microscopy, the EBs appears as bright green, coccial structures.
- In methylene blue stain under dark field illumination the EBs show autofluorescence, they are revealed as refractile, yellow green bodies surrounded by a halo.
- In machiavello stain, the EBs stain red against a blue background. In castanedas stain, the EBs are stained blue with a reddish background.
- Giemsa stain: Particularly useful in smears from conjunctival swab of feline pneumonitis cases.

- Infected conjunctival epithelial cells contain basophilic intracytoplasmic aggregates of *C.psittaci*.

CULTIVATION

- They require living systems for multiplication.
- They do not have cytochromes and their metabolic reactions are essentially anaerobic.
- They are highly dependant on the host cells for energy.
- **Mouse inoculation**
 - Suspected material is inoculated into mice intranasally, intra peritonally.
 - The mice die within in ten days and smears of the lung, peritoneal exudates, spleen or brain will show the elementary bodies. (Levinthal cole Lillie or LCL bodies).
- **Inoculation on 6-7 days old embryonated eggs via the yolk sac route.**
 - The LCL bodies can be demonstrated in the smears of yolk sac from infected eggs.
- **A variety of continuous cell lines are useful for cultivation of *Chlamydiae*, such as McCoy, BHk-21, L929 and vero.**
 - Chemical treatment of the cells with cycloheximide (1-2mg/ml) and 5-iodo-2-deoxy uridine (80mg/ml) will greatly enhance the cultivation.

RESISTANCE,ANTIGENSAND TOXINS

Resistance

- Chlamydiae are heat labile, being inactivated within minutes at 56°C.
- They are susceptible to ethanol, ether and low concentration of phenol and formalin.
- Infectivity is maintained for several days at 4°C.
- They can be preserved frozen at -70°C or lyophilized.
- The elementary bodies are relatively resistant and remain viable for several days under sutiable environmental condition.
- They are highly sensitive to oxytetracycline, erythromycin and tylosin.

Antigens and toxins

- All Chlamydiae share a group (genus) specific antigen.
- This antigen, also known as complement fixation antigen, is LPS in nature.
- In addition Chlamydiae possess species specific and serovar (serotype) specific antigen.
- Based on OM protein antigens, more than 10 serovars of *C.psittaci* have been described.
- Chlamydiae produce a toxin, probably protein in nature, which is lethal to mice on intra venous inoculation.
- The toxin is specifically neutralized by the antitoxin.

PATHOGENESIS

- Chlamydial organisms may be shed in faeces of carrier animals. Chlamydial elementary bodies are shed in the semen.
- Young one gets infection mainly through milk of the dam.
- Animals and humans are infected by the inhalation of infectious dust and droplets.

- In case of enzootic abortion and enteritis in ewes, the infection may take place by ingestion.
- The severity of the disease depends on
 - Strain and virulence of the agent
 - Ovine and bovine type 1 isolates are more frequently associated with abortion, genital infections and enteritis.
 - Type II isolates are associated with conjunctivitis, pneumonia, encephalomyelitis and polyarthritis.
 - Age, sex, physiological state and species of host.
 - Route of infection and degree of exposure to *Chlamydiae*
 - Environment and management practices.
- The *Chlamydiae* have a predilection for epithelial cells of the mucous membrane.
- After inhalation of infectious dust and droplets, pneumonia may develop.
- In enzootic abortion of ewes the organism localizes in cells of the placenta, abortion will occur in the last 2-3 weeks of pregnancy and this is associated with a diffuse, necrotic placentitis.
- In BUSS disease, inflammation of vascular endothelium and nervous signs are predominant.
- In avian psittacosis, as a result of certain stress conditions, the organism multiplies in the spleen and kidney of normal appearing birds and are shed in the faeces in large numbers.
- The faeces dry, producing a dust that is infectious to susceptible avian and human. In birds, respiratory, intestinal and systemic signs are seen.

DIAGNOSIS

- *Chlamydiae* can be isolated from the blood during the early stage and from the sputum on later stage.

Specimens

- In case of abortion, smears from affected cotyledons or chorion, prepared from vaginal swabs and from the wet surface of aborted fetuses.
- Uterine discharges, fetal membranes, fetal tissues are also useful.
- Aspirated synovial fluid in polyarthritis, conjunctival swab, samples of lung, liver, spleen and paired serum samples are mostly helpful.
 - Demonstration of *Chlamydia psittaci* elementary bodies on smears by using either chemical stains or Immunofluorescence staining.
In stained smears, *Brucella* species may look very similar to *Chlamydia psittaci*. But can be differentiated by serology, isolation and immunological staining methods.
 - Isolation and cultivation
By inoculation into mouse, yolk sac route of embryonated eggs, cell culture and demonstration of LCL bodies.
 - Serological tests like ELISA, CFT, IFAT, LAT
Cross reactivity between *Chlamydiae* species and other Gram negative bacteria will complicate the interpretation of serological test.
 - PAGE, Restriction endonuclease analysis and monoclonal antibody typing will help in diagnosis.

TREATMENT AND CONTROL

- Immunity is both cell mediated and humoral.

- The serotype specific antigen stimulates production of protective antibody.
- Vaccines consisting of suspension of killed organism are available for the prevention of feline pneumonitis and enzootic abortion of ewes.

Tetracycline and chloramphenicol are effective. Penicillin and sulphonamides are not useful.