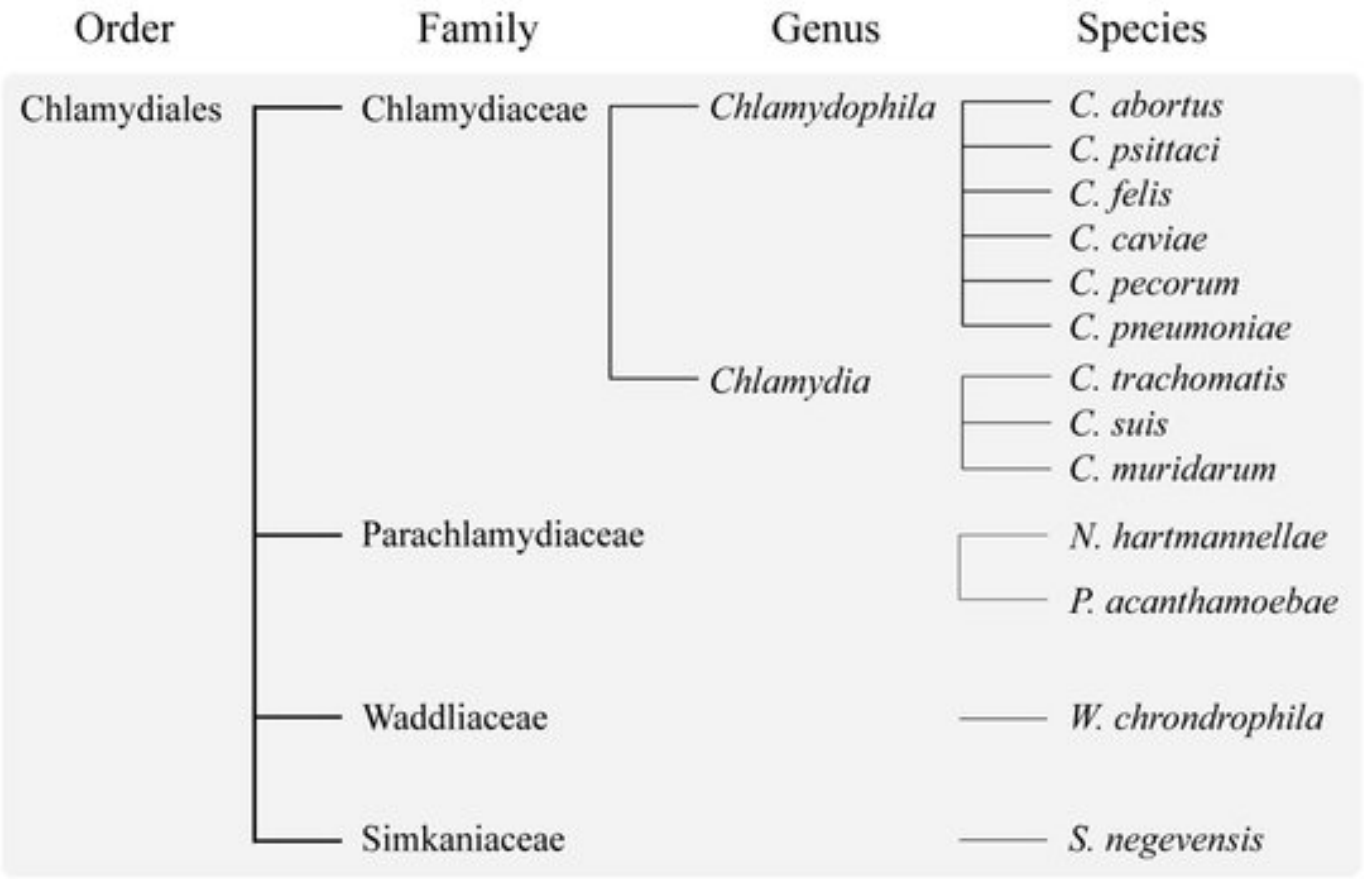
Chlamydiaceae

Chlamydia & Chlamydophila

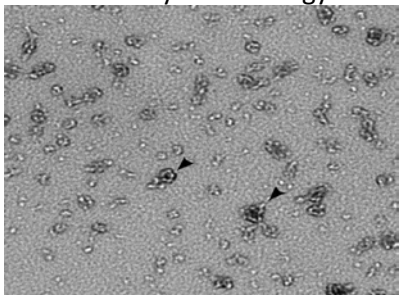
Dr. Ashok V. Bhosale
Ph.D., M.V.Sc., NET (ICAR)
Department of Veterinary Microbiology,
College of Veterinary & Animal Sciences, Udgir
Maharashtra Animal & Fishery Sciences University



New taxonomy of the order Chlamydiales

Chlamydia

'energy parasites'



- The word chlamydia comes from the Greek word '**chlamys**' means, "cloak draped around the shoulder". The bacterium's intracytoplasmic inclusions are "draped" around the nucleus of the infected cell.

- *Chlamydia* was first discovered by a Czech zoologist and parasitologist, **Stanislaus von Prowazek** in 1907 in Berlin, but scientists believe it was actively infecting people for centuries before its discovery.

- In 1963 it was recognized as bacteria rather than a virus.

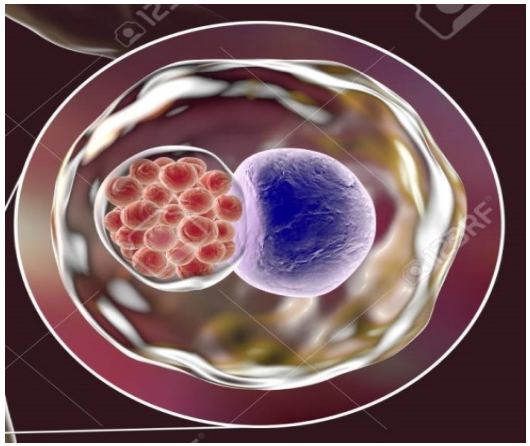


Fig. *Chlamydia trachomatis* bacteria showing reticulate bodies of Chlamydia forming intracellular intracytoplasmic inclusions (small red) near the cell nucleus (purple)

Morphology:

- Gram negative, non-motile, pleomorphic and obligate intracellular bacteria.
- They also encode **major outer membrane protein (MOMP),** a key antigenic determinant used for serological differentiation of Chlamydiae.

- Poorly stained by Gram's stain. Chlamydiae are readily stained by **Castaneda** (blue in appearance), **Giemnez and Macchiavello** (red in appearance) methods. However, Giemsa staining is preferable to stain the inclusions (Chlamydial micro colony) in the cell culture.
- Inclusion bodies of the Chlamydiae are basophilic in nature. Again, the staining of the inclusion bodies may differ depending on the species.

- In ***C. trachomatis***, the inclusion bodies contain glycogen matrix and can be easily stained with iodine solution.
- They possess both RNA and DNA and some bacterial enzymes. So they can hydrolyze glucose, pyruvate and glutamate. However, **due to restricted metabolism they are unable to generate ATPs**. Therefore, **to meet the energy requirements they need to utilize host cell derived ATPs**. For these reasons, they are also designated as '**energy parasites**'.

- Their **life cycle** is quite unique consisting of two stages-
  infectious forms as "elementary bodies" (EB) &
  non-infectious intracytoplasmic forms are called 'reticulate bodies'(RB)

- The family Chlamydiaceae consist two genera i.e. *Chlamydia* and *Chlamydophila.*

- Pathogenic species of both of the genera are *Chlamydia trachomatis, C. suis, C. muridarum* and *Chlamydophila abortus, Cph. caviae, Cph. felis, Cph. pecorum, Cph. psittaci* and *Cph. pneumoniae.*

- **Resistance:** It is a delicate organism and is easily inactivated at **56°C or at 60°C** for 10 min. It is susceptible to wide range of disinfectants like alcohol, sodium hypochlorite, silver nitrate, iodine and potassium permanganate. Because of **lipid rich membrane**, they are susceptible to chloroform and ether. However, Chlamydiae can be preserved at -70°C and liquid nitrogen for months. They are susceptible to broad range of chemotherapeutic agents like sulfonamides, tetracycline, macrolide, rifampicin, chloramphenicol and enrofloxacin.

- **Natural habitat:**

  - The mammals, birds and reptiles are the usual and natural hosts of the pathogens under the family Chlamydiaceae. Most of the pathogens under Chlamydiaceae family have specific hosts.

  - Chlamydiae usually spread by direct contact or through the aerosols.

RNA content is much higher in reticulate bodies. The elementary body contains a rigid cell wall and a DNA genome

Earlier due to their small structural size and intracellular existence, Chlamydiae were confused with virus. However, there are some basic differences between virus and Chlamydiae.

Difference between Virus and Chlamydiae

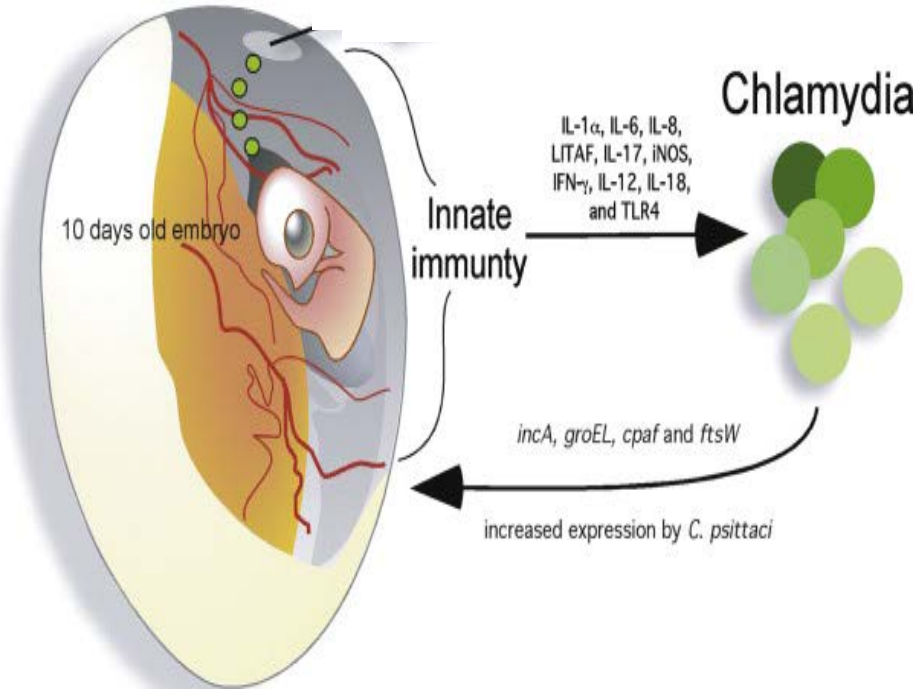
VIRUS	CHLAMYDIAE
Either DNA or RNA is present in virus.	Chlamydiae contain both DNA and RNA.
Cytoplasmic membrane and outer membrane are absent in virus	Cytoplasmic membrane and outer membrane are present
Ribosome absent	Ribosome present
Metabolically inactive	Chlamydiae have their own metabolic system.
Resistant to antibiotics	Sensitive to antibiotic

Difference between Rickettsiae and Chlamydiae

RICKETTSIAE	CHLAMYDIAE
Rickettsiae can synthesize ATP	Chlamydiae are unable to synthesize ATP
Multiply by binary fission	Follow complicated biphasic life cycle for growth
It is mainly arthropod borne infection.	Transmission can occur by direct contact, ingestion of contaminated materials or sexually transmitted

Isolation, growth and colony characteristics :

- As chlamydial metabolism is restricted and they **exploit host cell machinery** for their energy, they **require live cells for their growth**.
- Like virus, isolation can be done in the yolk sacs of embryonated hen eggs, experimental animals and cell culture.
- Fertile chicken eggs (6-8 days old) are inoculated through the yolk sac route and candled daily. Yolk sac of the embryo dying 3 or more days after incubation is examined for chlamydial inclusions.



- **Mice are ideal laboratory animal model for isolation of Chlamydiae**. The mice usually die within 10 days of **intranasal, intracerebral or intraperitoneal inoculation** and the Ebs can be isolated from viscera and peritoneal exudate.

- **Cell lines** irradiated or treated with a metabolic inhibitor (like cyclohexamide at 2µg/mL) can be used for isolation of Chlamydiae. McCoy, HeLa, monkey kidney cells, mouse fibroblast cells, fish and lizard cells are generally used depending upon the species.

- Prior to inoculation into the cell culture, suspected clinical samples (faeces, placenta, urine, semen, conjunctival fluid) should be decontaminated by antibiotics like gentamicin (50 µg/mL.), vancomycin (75 µg/mL) and nystatin (500 unit/mL). The inoculated cell should be incubated at 35°C to 37°C for 48-72 hrs and stained for intracytoplasmic inclusion bodies. Fluorescein conjugated monoclonal antibody is in use nowadays. It is a more sophisticated and sensitive technique to allow earlier detection of the inclusion body.

Antigenic characteristics :

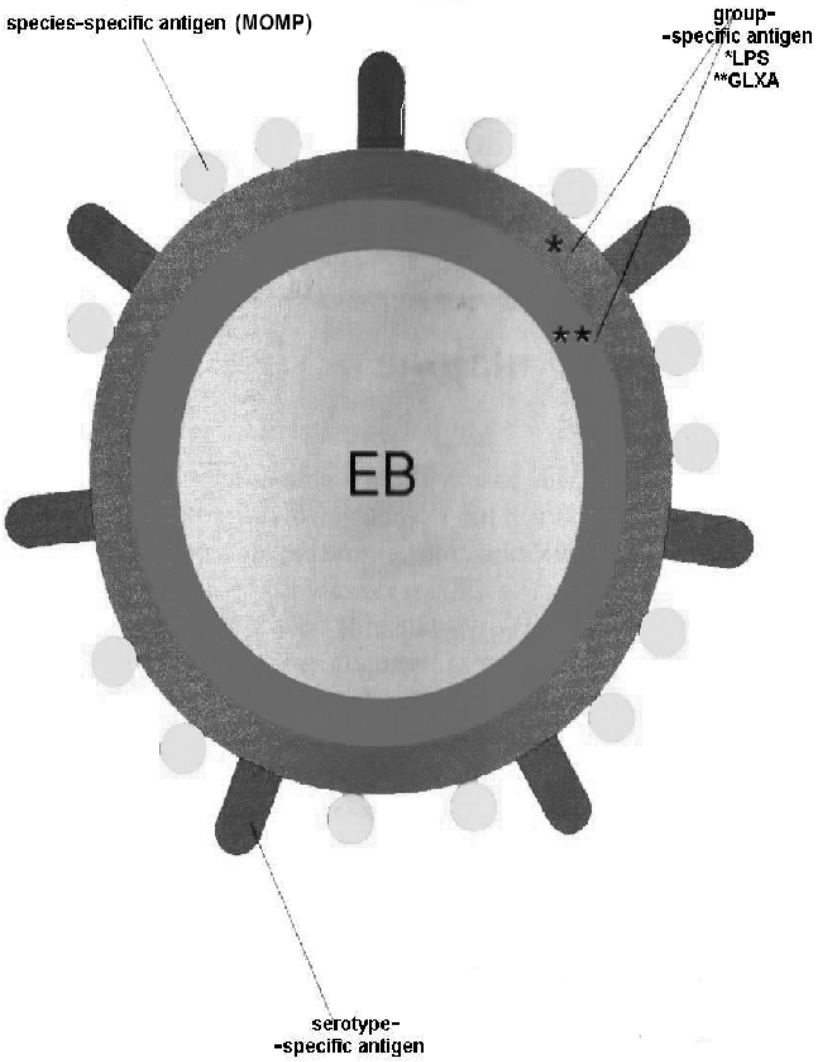
Chlamydiae possess genus, species and serotype specific antigen. The genus or group specific antigen is also called complement fixing antigen. It is heat stable and lipopolysaccharide in nature like other gram negative bacteria.

Serotype specific antigens are protein in nature (MOMP) and can be determined by micro immunofluorescence tests.

MOMP is encoded by ompA gene and there is considerable antigenic heterogeneity of this protein.

Molecular and monoclonal antibody based techniques have identified four surface-exposed variable domains that help in serotyping.

Serotype specific antigens are important in some species like *C. trachomatis*, However, in *Cph. pneumoniae* these variable domains are not well recognized by immune system.

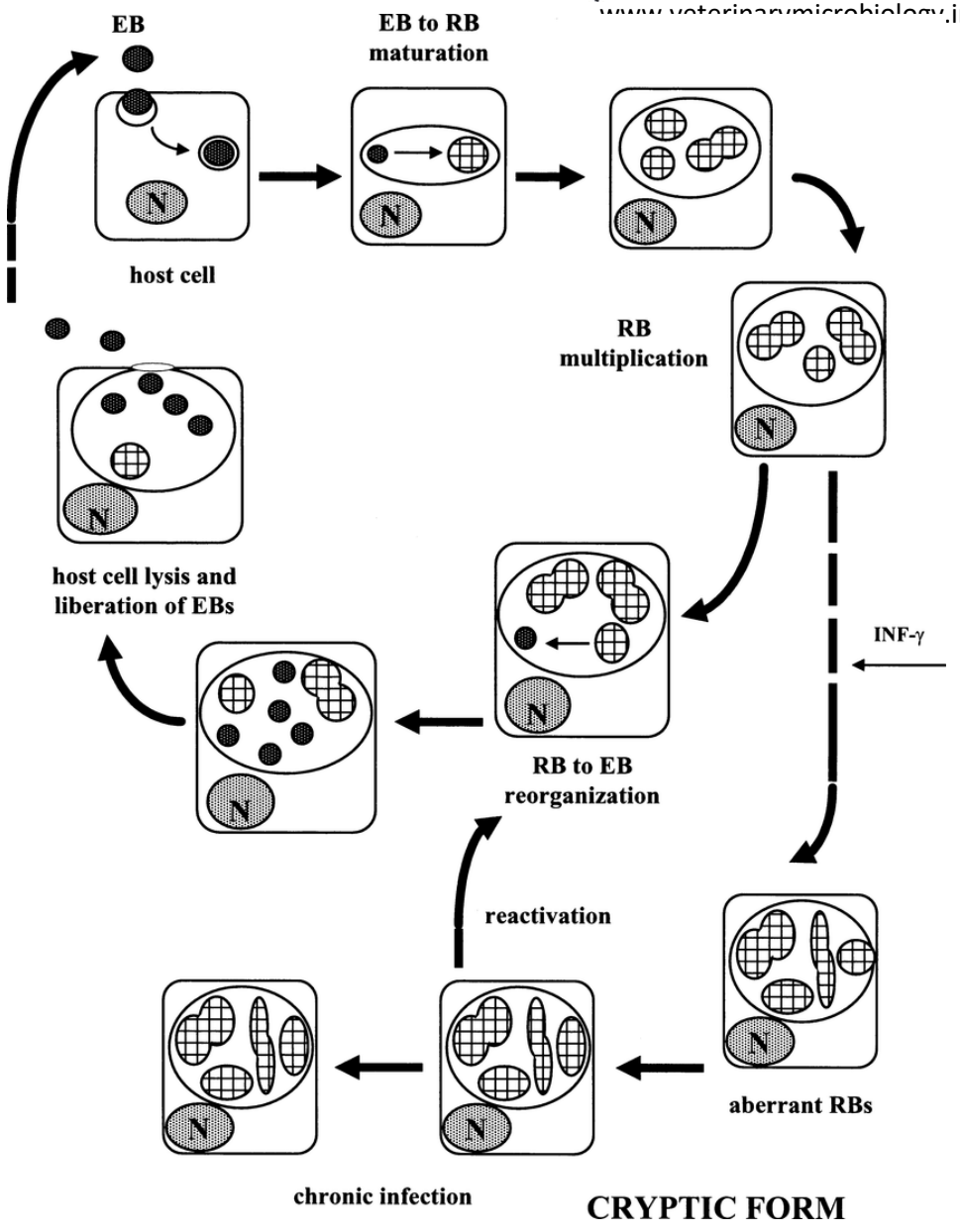


Atigenic structure of *C. trachomatis*

Antigenic Structure of *C. trachomatis*

Life cycle of chlamydiae

The life cycle is characterized by two stages pecific cell types: the small (0.3 to 0.6 µm in diameter) extracellular infectious cell, called the **Elementary Body** (EB), adheres to and invades the eukaryotic cell, and then matures within a few hours in a larger (0.6 to 1.5 µm in diameter) vegetative form, the Reticulate Body (RB), which multiplies by binary fission forming an intravacuolar microcolony, called an inclusion. Such an inclusion is detached from the phagolysosome pathway, but is dependant on the Golgi apparatus, **Finally, the RBs, reorganize into Ebs, which will be released by the host cell** and will initiate a new infection.
The cycle is generally accomplished in 36 to 96 hours, depending on the species, Eventually, under stress (e.g., interferon-gamma immune response) the microcolony may enter in a cryptic form, sustained by aberrant RBs. In such a form, chlamydiae are reputed to mantain persistent infections, with some returns to normal cycle to libere new generations of EBs.



Corsaro, Daniele & Danielle, Venditti. (2004). Emerging Chlamydial Infections. Critical reviews in microbiology. 30. 75-106. 10.1080/10408410490435106.

Recently a 60-kDa cysteine-rich outer membrane protein (OmcB) is characterized from Chlamydia which can act as genus as well as species specific antigen.

There are **three biovars of *C. trachomatis*** TRIC (trachoma and inclusion conjunctivitis), LGV (lymphogranuloma venereum) and MoPn (mouse pneumonia).

Again *C. trachomatis* biovar TRIC and biovar LGV has 15 and 3 (L1, L2 and L3) serotypes, respectively.

Whereas, ***Cph. psittaci* has eight serotypes**:

A (psittacine birds and human patients),
B (cattle, pigeon, turkey),
C (birds and human),
D (turkeys, parakeets and human),
E(human),
F (parakeets),
G (hares and muskrats) and
H (cattle).

Toxins and virulence factors

- Chlamydiae can release **haemaglutinin and endotoxin** like other gram negative bacteria. The hemagglutinin can facilitate attachment of the organisms to the cells. On the other hand, the endotoxin is responsible for inflammatory tissue damage during infection. However, different pro-inflammatory cytokines released during chlamydial infection may also be responsible for such tissue damage.

- Chlamydia and Chlamydophila also contain several putative adhesion factors responsible for intimate attachment of the organisms to the host cells like MOMP (major outer membrane protein), a heat labile cytadhesin, OmcB and heat shock proteins (HSP).

Transmission

- The respiratory, intestinal and genital tracts constitute the major reservoir of the pathogen.
- Infection is possible by direct contact, inhalation of the infectious dust or droplet.
- Evidences are there that the chlamydiae may be transmitted by arthropods also.

Pathogenesis

- The **elementary bodies (EB) adhere to the receptors found on the microvilli** of host cells.
- Following adherence, the pathogens (EB) enter the host cells via receptor mediated endocytosis, pinocytosis and phagocytosis.
- The organisms have their own evasive mechanism for their intracellular existence.
- Therapeutic intervention or host cell immunity is not effective enough to eliminate the infection.
- During the process, chlamydial organisms utilize host cell iron and tryptophan.
- Moreover, they also modulate respiratory burst, release of nitric oxide and superoxide radicals to hide.
- Thus prolonged persistence leads to chronic inflammatory reactions in the non-immune host cells like mucosal epithelial and vascular endothelial cells and tissue damage.

Pathophysiology

Chlamydiae have the ability to establish long-term associations with host cells.

When an infected host cell is starved for various nutrients such as amino acid , Iron, or Vitamins this has a negative consequence for *Chlamydiae* since the organism is dependent on the host cell for these nutrients.

Long-term cohort studies indicate that approximately 50% of those infected clear within a year, 80% within two years, and 90% within three years

Macrophages are the principal host cells for *C. psittaci* and *C. trachomatis* LGV biovar, whereas the principal host cells for *C. trachomatis* trachoma biovar and *C. pneumoniae* strains are **columnar epithelial cells at mucosal sites**.

Host cell tropism correlates with the type of inflammation elicited by chlamydiae.

The LGV biovar and *C. psittaci* produce granulomatous inflammation, characteristic of **delayed hypersensitivity reactions**. The trachoma biovar produces neutrophilic exudate during acute infection and submucosal mononuclear infiltration with lymphoid follicle formation during later stages of infection.

Diseases produced

Bacteria	Host	Disease
Chlamydia trachomatis	Humans	Trachoma, sexually transmitted diseases, arthritis, conjunctivitis, pneumonia, Reiter's syndrome, lymphogranuloma venerum (LGV)
Chlamydia suis	Pigs	Conjunctivitis, rhinitis, enteritis, pericarditis, polyarthritis, pneumonia
Chlamydophila psittaci	Birds, humans Cattle and sheep	**Chlamydiosis/Ornithosis/Psittacosis In birds (Parrot Fever)**: characterized by inflamed eyes, conjunctivitis, airsacculitis, pneumonia, difficulty in breathing, watery droppings and green urates enteritis, myocarditis Encephalitis in humans. Acute pneumonia in cattle and sheep
Chlamydophila abortus	Sheep and goats Abortion & Birth of weak lambs	**Ovine Enzootic Abortion/ Enzootic abortion of ewes** (EAE) Abortion usually occurs in the last 2 to 3 weeks of pregnancy. The abortion percentage in affected flocks is low in the first year and then reaches 30% and 10% in the second and third years, respectively
Chlamydophila abortus, *Chlamydia psittaci* and *Chlamydia pecorum*	Bovine	Chlamydial abortion in bovine

Diagnosis

- Samples to be collected:
  Birds - faecal sample, conjunctival swab, nasal swab
  Arthritis patient - Synovial fluid

- Chlamydiae are relatively labile organisms and special precautions are required to avoid the chance of misdiagnosis and misinterpretation. Samples should be maintained in cold chamber and processed immediately after collection. If the samples cannot be processed within 24 hour, it should be kept at -70°C.

- For successful isolation and culture, samples should be sent to the laboratory in special **chlamydia transport medium** such as 2SP (0.2 M sucrose phosphate medium containing 10 µg/mL of aminoglycosides and fungicides are preferred.

- Broad spectrum chemotherapeutic agents like tetracycline, chloramphenicol, macrolides, sulphonamides, penicillin should not be added as they have anti-chlamydial effect.

Laboratory Examination

Direct Examination:

- Inclusion bodies may be demonstrated using appropriate stains. *C. trachomatis* infection can be detected by observing typical reinform inclusion bodies surrounding the nucleus following Giemsa, Castaneda or Macchiavello staining.

- Transmission electron microscopy (TEM) may be used for visualization of *Cph. psittaci* inclusion bodies containing reticulate bodies, condensing form and elementary bodies.

- Staining of the sample smear can also be performed with FITC labeled antibodies against species or genus specific antigen. Immunofluorescence based detection has higher sensitivity as well as specificity and can be done rapidly.

- Isolation of bacteria from clinical samples

- Immunological/Serological test: Enzyme linked immunosorbent assay (ELISA)

Frei's test

A skin test is also available using the cell mediated immune response to detect lymphogranuloma venereum (LGV) caused by *C. trachomatis*.

0.1 ml of heat-inactivated LGV grown in yolk sac of the embryonated egg is injected intradermally on the forearm. Positive reaction is indicated by the development of inflammatory nodules. The test becomes positive only 2-4 weeks of infection and remains positive for a long period. Thus the test has lower sensitivity.

Molecular Biology:

In Chemiluminescence assay acridium-ester-labeled ssDNA probe complementary to *C. trachomatis* RNA is used to detect the labeled DNA-RNA hybrid in a luminometer to measure the light emitted by the acridium ester label. Polymerase chain reaction (PCR) is also used nowadays to detect the gene *omp1* encoding for MOMP and the 16s rRNA.

Prevention and Control

Educate and protect those at risk

Clothing, gloves, cap, HEPA filters

Wet carcass with water and detergent prior to necropsy

Disinfect

1:1000 quaternary ammonium compounds

1% Lysol

70% isopropyl alcohol

1:100 bleach

THANKS

AB's Veterinary Microbiology

www.veterinarymicrobiology.in