

**California Association for
Medical Laboratory Technology
Distance Learning Program**

**Chlamydiae And Their Role
In Human Disease**

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Level: Beginning to Intermediate

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Chlamydiae And Their Role In Human Disease

OBJECTIVES

Upon completion of this course the participant will be able to:

1. Discuss the principal characteristics of chlamydiae, including their intracellular life cycle and their classification.
2. Contrast the classic chlamydiae species with the newly described Parachlamydiaceae.
3. Outline the pathogenesis of chlamydial infections.
4. Discuss human diseases caused by chlamydiae, including emerging chlamydial infections.
5. Describe the possible role of chlamydia in chronic diseases.
6. Summarize current diagnostic methods and treatment options.

INTRODUCTION

Long considered a unique group of intracellular bacteria containing a few pathogenic species, the chlamydiae have recently been shown through molecular studies to represent a highly diverse group of ubiquitous organisms. In addition to well known human pathogens there is an abundance of environmental chlamydiae symbiotic in free-living amoebae and in other hosts. These symbionts are obligate intracellular parasites. Phenotypic comparison of newly described chlamydial groups suggests that all have descended from a common ancestor that replicated intracellularly within eukaryotic host cells. The minor phenotypic differences observed among chlamydial groups depend on small genomic differences.

The divergence of environmental and pathogenic chlamydiae is thought to have taken place about 700 million years ago. The common ancestor of diverse chlamydial groups was already adapted to intracellular survival in early eukaryotic cells and contained many virulence factors found in modern pathogenic chlamydiae (1).

Recent molecular studies of environmental chlamydiae have identified three families: Simkaniaceae, Parachlamydiaceae, and Waddliaceae.

CLASSIFICATION OF CHLAMYDIAE

OLD CLASSIFICATION:

Order *Chlamydiales*

Family *Chlamydiaceae*

Genus *Chlamydia*

Species: *C. psittaci*

C. trachomatis

C. pneumoniae

C. pecorum

In 1999 a paper by Everett, Bush, and Anderson introduced a reclassification of chlamydiae. The genus *Chlamydia* was replaced with the genera *Chlamydia* and *Chlamydophila*, with a total of nine species. This classification has not been accepted universally. Ongoing molecular studies have uncovered additional chlamydial groups resulting in further changes in chlamydial classification.

REVISED CLASSIFICATION OF CHLAMYDIAE

SYSTEMATICS	NATURAL HOST
Order Chlamydiales	
Family Chlamydiaceae	
Genus <i>Chlamydophila</i>	
Species:	
<i>C. abortus</i>	Ruminants
<i>C. psittaci</i>	Birds
<i>C. felis</i>	Cats
<i>C. caviae</i>	Guinea pigs
<i>C. pecorum</i>	Cattle, sheep, koalas
<i>C. pneumoniae</i>	Humans, horses, koalas
Genus <i>Chlamydia</i>	
Species:	
<i>C. trachomatis</i>	Humans
<i>C. suis</i>	Swine
<i>C. muridarum</i>	Rodents
Family Parachlamydiaceae	
Genus <i>Parachlamydia</i>	
Species: <i>P. acanthamoebae</i>	Acanthamoeba
Genus <i>Neochlamydia</i>	
Species: <i>N. hartmanellae</i>	Hartmanella
Family Waddiaceae	
Genus <i>Waddlia</i>	
Species: <i>W. chondrophila</i>	Cattle, fruit bats (?)
Family Simkaniaceae	
Genus <i>Simkania</i>	
Species: <i>S. negevenis</i>	Humans (?)
Genus <i>Fritschea</i>	
Species: <i>F. bemisiae</i>	Insects
Species: <i>F. eriococci</i>	Insects
Genus <i>Rhabdochlamydia</i>	
Species: <i>Rhabdocchlamydia</i>	Woodlice

Additional species of parachlamydiae have been described; a number of these infect various arthropods.

PRINCIPAL CHARACTERISTICS OF CHLAMYDIAE AND THEIR REPLICATION CYCLE

THE CLASSIC CHLAMYDIAE: *CHLAMYDIA* AND *CHLAMYDOPHILA* SPECIES

Role in human disease

Chlamydiae are responsible for a wide range of diseases in humans, including lymphogranuloma venereum, pelvic inflammatory disease, conjunctivitis, urethritis, cervicitis, pneumonia, psittacosis, and possibly atherosclerosis.

Genetic organization of chlamydiae

The genome of *Chlamydia trachomatis* was sequenced in 1998. It is of interest that sets of genes for peptidoglycan synthesis and for ATP biosynthetic pathways were identified in the *C. trachomatis* genome, despite the lack of peptidoglycan in chlamydial cells and their inability to generate ATP. In addition to the chromosome, chlamydiae commonly possess extrachromosomal genetic elements (plasmids). The presence of 4 to 10 plasmids per elementary body (extracellular chlamydial form) has been reported for various strains of chlamydiae. These plasmids may play a role in the virulence of chlamydiae. Studies in mice using plasmid-cured *C. muridarum* demonstrated the ability of these mutants to infect the murine genital tract, but failure to cause disease in the oviduct. If plasmid-cured strains of human *C. trachomatis* strains have similar characteristics, they have the potential to serve as vaccines to prevent human disease (2).

Metabolism

Although chlamydiae possess a number of enzymes, they have a restricted metabolic capacity. Chlamydiae lack cytochromes and therefore their metabolic reactions do not generate energy (ATP). These organisms are energy parasites that use ATP produced by their host cells for their own requirements. Energy-rich metabolic intermediates from host cells are required in order to complete the chlamydial replication cycle.

Developmental cycle and cell structure

The chlamydiae are nonmotile, Gram-negative, obligate intracellular bacteria that exhibit an intracellular and an extracellular form, and undergo a biphasic developmental cycle. All known species of chlamydiae have a common lipopolysaccharide that differs from the lipopolysaccharide of other bacteria. This molecule is present in the outer membrane of the cell envelope in both developmental forms of chlamydiae. Highly antigenic polysaccharide epitopes are present in the lipopolysaccharide layer.

Extracellular forms of chlamydiae are known as elementary bodies. This developmental form is hardy, spore-like, infectious, and metabolically inert. The DNA of elementary bodies is condensed into an eccentrically placed nucleoid. The elementary body is, generally, spherical and 0.2 to 0.3 micrometers in diameter. When studied with an electron microscope, an elementary body has granular cytoplasm reflecting the presence of 70S ribosomes. The cell envelope is double layered, resembling the cell envelope of Gram-negative bacteria. An important component of the outer cell layer is a protein, known as the major outer membrane protein (MOMP). This protein constitutes approximately 60% of the total protein mass of the elementary body cell wall. MOMP functions as a membrane channel that is permeable to ATP. Since antibodies to MOMP block cellular infection with chlamydiae, it is probable that antibody binding to MOMP prevents the uptake of host cell ATP by the intracellular pathogens. MOMP is also of major importance in the immunologic diagnosis of chlamydial infections because the MOMP layer contains strain-specific antigenic sites of chlamydial serotypes.

Intracellular developmental forms are called reticulate bodies. These are larger than elementary bodies and contain fibrillar DNA plus a high concentration of ribosomes. The cell envelope appears less complex than that of the elementary bodies. The reticulate body is the metabolically active replicating form that does not survive well outside the host cell and appears adapted to an intracellular environment.

Replication of chlamydiae

Chlamydiae are able to infect a diverse range of both nonphagocytic and phagocytic cultured cells including insect cells, epithelial cells, endothelial cells, macrophages and

monocyte-derived cell lines. The initial attachment of elementary body and host cell is mediated by electrostatic interactions with heparan sulfate molecules on the host cell surface. Specific protein receptors on the host cell surface are probably involved. Such receptors have not been identified definitively. Apparently the processes involved in attachment and uptake may differ among species of chlamydiae and even among variants of the same species. Following attachment, the elementary body enters the host cell by a process similar to endocytosis. The entry of the elementary body into the host cell is facilitated by a reorganization of the cell surface microvilli induced by the attachment of the microorganism to the host cell receptors (3). Once inside a host cell, the elementary body reorganizes into a reticulate body within a membrane-bound vacuole known as an inclusion. The inclusion membrane does not fuse with the host cell's lysosomal membrane. The reticulate body replicates by binary fission, remaining within the inclusion membrane for the duration of the intracellular growth cycle, and forming characteristic intracellular inclusions that can be observed by light microscopy. The inclusion membrane is derived from the cytoplasmic membrane of the host. After a period of exponential growth, the reticulate bodies revert to elementary bodies. This process generally takes 24 to 72 hours and takes place entirely within the cytoplasm of the infected cell. During the transformation of reticulate bodies to elementary bodies a number of late-phase proteins are synthesized, including chlamydial outer membrane complex proteins and histone-like proteins that are part of the chlamydial chromosome. Elementary bodies are released into the extracellular environment by the fusion of the membrane of the inclusion with that of the host cell or upon host cell lysis. The elementary bodies can then initiate a new cycle of infection.

Persistence of chlamydiae in host cells

In contrast to the productive replication cycle persistence of chlamydiae in host cells has been demonstrated *in vivo* and *in vitro*. Persistent phase is characterized by absence of viable organisms but the presence of chlamydial DNA and specific chlamydial proteins. Persistence of chlamydiae has been associated with a number of chlamydial diseases, such as trachoma, inclusion conjunctivitis of newborns, genital tract infections, pneumonia, arthritis, and cardiovascular disease. Chlamydial persistence may be involved in recurrence of disease when reinfection is unlikely.

Chlamydial infection of immune cells, such as monocytes, macrophages, lymphocytes, neutrophils, and dendritic cells is commonly characterized by persistence. Although immune cells are not a significant cell type for chlamydial replication they are important for dissemination of chlamydiae from the site of infection to distant tissue sites. Furthermore, the finding that chlamydiae may be demonstrated in neutrophils of healthy blood donors indicates a possible role of chlamydial persistence in transmission of disease.

THE NEWLY DESCRIBED CHLAMYDIAE: THE PARACHLAMYDIACEAE

Molecular studies have demonstrated a huge diversity of chlamydiae from environmental and clinical sources. Chlamydiae that naturally infect free-living amoebae have been placed in a separate family, the Parachlamydiaceae, based on the chlamydia-like cycle of replication and on the 80% to 90% homology of ribosomal RNA genes. These organisms are endosymbionts of amoebae and are generally not destroyed by their hosts. Because intra-amoebal growth could increase the virulence of intracellular bacteria, the parachlamydiae may be pathogenic. Furthermore, the amoebae could play an important role as reservoirs or vectors of chlamydial infections. Other parachlamydiae, such as *Neochlamydia* species and unclassified species, have been isolated from humans, cats, Australian marsupials, reptiles, fishes, as well as from various

environmental samples. New members of parachlamydiae infecting invertebrates have recently been characterized. These include *Fritschea* and *Rhabdochlamydia* that infect insects, presenting a possibility that there are insect vectors of chlamydial infections.

Replication of parachlamydiae

The life cycle of *Parachlamydia acanthamoebae* in amoebae has been studied by electron microscopy. Two stages, intracellular and extracellular, are part of the life cycle. Three morphological forms have been observed: the infective extracellular elementary bodies and crescent bodies, and the intracellular replicating reticulate bodies. Infection of amoebae takes place by phagocytosis of elementary or crescent bodies. Within 8 hours after infection, elementary and crescent bodies differentiate into the reticulate form. The reticulate bodies divide by binary fission and are able to invade the amoebal cytoplasm. Multiplication takes place mainly in the vacuoles and rarely in amoebal cytoplasm. In the vacuoles, the reticulate bodies condense into elementary and/or crescent bodies, which are released after amoebal lysis or are expelled within vesicles. A new cycle of infection can then be initiated by the elementary or crescent bodies. The presence of crescent bodies is associated with prolonged incubation time. This developmental form has been observed only in parachlamydiae and could be used as an important taxonomic feature for this group of microorganisms.

PATHOGENESIS OF CHLAMYDIAL INFECTIONS

Chlamydia trachomatis infections are among the most common notifiable diseases in USA. Infection with *Chlamydia pneumoniae* is also extremely common: serological surveys indicate a nearly universal occurrence of infection with this organism. The extremely high prevalence of infections caused by *C. trachomatis* and *C. pneumoniae* reflects the successful adaptation of these bacteria to persistence in their human hosts. The infected host's immune response may fail to eliminate these intracellular bacteria, leading to clinical persistence of chlamydiae. Similarly, the immune response does not prevent re-infection with these organisms.

The initial response of the host to chlamydial infection is acute inflammation. Repeated infection by chlamydiae increases the severity of the inflammatory response and promotes chronic inflammation that may result in tissue damage and scarring. The damage may be mediated by immune cells directed against host tissues. Immune reactivity such as delayed hypersensitivity to chlamydial antigens or an autoimmune response may be involved. An alternate hypothesis is that host tissue damage is mediated by inflammation caused by the pathogen (4). According to this model of chlamydial pathogenesis, chlamydiae infect endothelial or epithelial cells. Damaged host cells secrete chemokines and growth factors, such as IL-11, IL-8, IL-12, IL-6, and GM-CSF. These factors induce the appearance of clinical signs, which include redness, edema, and a mucopurulent discharge. Secreted cytokines attract and activate neutrophils, macrophages, and immunologically reactive cells. Activated cells produce their own array of cytokines and growth factors. These factors promote the inflammatory response, cellular infiltration, and migration of activated immune cells to lymphoid follicles. Eventually, follicle necrosis, tissue damage and scarring may occur.

HUMAN DISEASES CAUSED BY CHLAMYDIAE

Infections with *Chlamydia trachomatis*

C. trachomatis strains infect the eye and the genital tract. The strains are tissue selective rather than tissue specific. Genital strains are occasionally found in the eye, while ocular strains

are sometimes isolated from the genital tract. The strains are further subdivided into serotypes or serovars on the basis of binding affinity for monoclonal antibodies.

Genital infections

Lymphogranuloma venereum; serovars L1, L2, L2a, and L3

The lymphogranuloma strains of chlamydiae are noted for their ability to invade lymphatic tissue.

Lymphogranuloma venereum is a sexually transmitted disease found more frequently in the tropical and sub-tropical parts of the world, although some cases of lymphogranuloma venereum are reported in the United States each year.

The incubation period for this disease ranges from 3 to 12 days. The primary lesion is a 5 to 8 mm soft, red, painless erosion or ulcer, which heals spontaneously in a few days. The secondary stage begins 2 to 6 weeks later and is characterized by the presence of swollen, tender inguinal lymph nodes which may drain spontaneously. These symptoms may be accompanied by fever, chills, and malaise. If this condition is not treated, genital ulcers, proctitis, and other complications may develop.

Lymphogranuloma strains of *C. trachomatis* are susceptible to antibiotics. Commonly prescribed medications include tetracycline, doxycycline, and erythromycin.

Other chlamydial genital infections: serovars D to K, Da, Ia, Ja

These chlamydial strains are the most common causes of urethritis and mucopurulent cervicitis in females and nongonococcal urethritis in males. The tissue tropism of these strains is restricted to mucosal epithelial cells. The same chlamydial strains may also infect neonates causing conjunctivitis and pneumonia.

Chlamydial genital infections are the most frequently reported infections in the United States. More than three million new infections are reported per year. The number of *C. trachomatis*-infected individuals in the United States is estimated to be in excess of six million. The age group with the highest prevalence of chlamydial infection is under 25 years of age. Although the infection is frequently subclinical and asymptomatic, several important complications may occur. Complications of chlamydial cervicitis may include pelvic inflammatory disease, ectopic pregnancy, and infertility. Chlamydial urethritis in men may lead to inflammation of the prostate gland, the seminal duct, and to Reiter's syndrome, which includes a triad of symptoms: conjunctivitis, polyarthritis, and genital inflammation.

Cervicitis is frequently asymptomatic, but some patients may complain of an abnormal vaginal discharge and occasional vaginal bleeding. Recommended antibiotic treatment for chlamydial cervicitis includes oral azithromycin or doxycycline.

Urethritis in men may result from either infectious or non-infectious causes. Symptoms, if present, include discharge of purulent material and difficult and/or painful urination. Asymptomatic infections are common. The most common microbial pathogens that cause urethritis are *Neisseria gonorrhoea* and *C. trachomatis*. Chlamydiae are responsible for approximately 15% to 55% of all cases of non-gonococcal urethritis. The same antibiotics that are used to treat chlamydial cervicitis, azithromycin and doxycycline, are highly effective in the treatment of chlamydial urethritis.

Chlamydial infections of infants

C. trachomatis infection of neonates results from perinatal exposure to the mother's infected cervix. Ocular prophylaxis with silver nitrate or antibiotic ointments does not prevent eye infection caused by perinatal transmission of *C. trachomatis* from mother to infant. The best

method for preventing neonatal chlamydial infections is diagnosis and treatment of pregnant women.

Initial *C. trachomatis* perinatal infection involves the mucous membranes of the eye, oropharynx, urogenital tract, and rectum. Chlamydial infection of these areas of the body may be asymptomatic, or symptoms of disease, such as conjunctivitis, may develop 5 to 12 days after birth. In general, chlamydial etiology of infection should be considered for all infants under 30 days of age who have conjunctivitis, particularly if the mother has a history of untreated chlamydial infection. Similarly, all infants who are less than three months of age and develop pneumonia should be tested for *C. trachomatis*. Characteristic signs of chlamydial pneumonia in infants include a repetitive, staccato cough, and chest X-ray findings typical of a chlamydial infection. Generally, fever is absent and wheezing is rarely observed. When perinatal infections of the nasopharynx, the urogenital tract, and the rectum occur they may persist for as long as one year.

Treatment of chlamydial perinatal infections

Topical antibiotic therapy for chlamydial perinatal infections is generally not effective. Oral erythromycin or ethylsuccinate have been used for both perinatal chlamydial conjunctivitis and infant pneumonia. The effectiveness of a single course of erythromycin treatment is approximately 80%.

Chlamydial ocular infections: trachoma (serovars A, B, Ba, and C)

Trachoma occurs worldwide, most often in rural settings in developing countries. It is primarily a disease of poverty. Although rare in the United States, trachoma may be found in any geographic area where people live under crowded conditions, have limited water supply, poor hygiene, and deficient sanitation. Children are affected most frequently. Serious complications resulting from trachoma, such as blindness, generally don't become apparent until later in life. The disease is transmitted through direct contact with infected tissues or with secretions from infected eyes, nose, or throat, or from contaminated towels and clothes. Infection can also be spread by flies.

Trachoma has been recognized as a cause of blindness for centuries. It has been known in Egypt for more than 3,500 years. Its contagious nature was recognized, but the identity of the infecting agent was unknown. The fact that trachoma was a transmissible infectious disease was well known: numerous First World War conscripts evaded military service by infecting their own eyes with discharges from trachoma patients. The causative agent of trachoma was visualized in 1907, when Halberstaedler and von Prowazek described the presence of inclusion bodies within infected cells. In 1957 T'ang and his coworkers were able to culture the infectious agent from infected human eyes in yolk sacs of chicken embryos. In 1966 Moulder described the structure and metabolism of these disease agents, clearly demonstrating that they were intracellular bacteria with a distinctive developmental cycle.

Clinical features of trachoma

Severity of trachoma ranges from asymptomatic to mild to severe. In endemic areas repeat infections occur. Symptoms of acute disease as well as signs of a chronic infection may be present simultaneously. In an initial infection, if symptoms develop, they usually appear within 5 to 10 days. These symptoms include conjunctival infection with an irritated red eye and some mucopurulent discharge. Other symptoms include swollen eyelids and turned-in eyelashes. When the cornea is involved it appears cloudy; there is accompanying pain and photophobia. When sufficient conjunctival scarring accumulates, the upper eyelid may turn inward so that eyelashes rub against the globe. This is known as trichiasis and it is intensely irritating. In

addition to being painful, trichiasis injures the cornea. Scarring of the cornea results in impaired vision.

Prevention and treatment

The spread of trachoma can be prevented through improved sanitation and hygiene, and not sharing items such as towels and clothing.

Early treatment with antibiotics, such as erythromycin, azithromycin, or doxycycline can prevent long-term complications. In some cases eyelid surgery may be required to prevent long-term scarring.

Eradication of trachoma as a major cause of blindness

The World Health Organization (WHO) has set a target date of the year 2020 for eliminating trachoma as a major cause of blindness. In order to accomplish this goal, intervention with risk factors at individual and community level would have to take place in affected villages and neighboring communities.

In 2001 WHO published a procedure for rapid, low cost identification of communities likely to be at risk for trachoma that leads to blindness. This procedure is known as the Trachoma Rapid Assessment and it involves the following steps:

- a. Areas that have endemic trachoma are identified.
- b. Field visits are implemented to areas of highest risk within the endemic area.
- c. Field visits include the selection of at least 15 households and at least 50 children between the ages of 1 and 9. Specimens from active cases of disease are collected and submitted for laboratory testing.
- d. Areas are ranked to prioritize the need for control measures.

Infections with *Chlamydomphila pneumoniae*

Respiratory infections with *C. pneumoniae* are extremely common. It has been suggested that such infections occur at least once in the lifetime of every human being. Antibody to *C. pneumoniae* can be demonstrated in the serum of 40% to 75% of persons tested. Typically infection begins in the upper respiratory tract. A mild illness develops with fever and a nonproductive cough, or the infection may remain asymptomatic. If left untreated, or inadequately treated, the infection may progress to bronchitis, sinusitis, otitis media, and pneumonia. Untreated *C. pneumoniae* infections may become chronic. On the basis of serologic studies, these infections have been associated with a number of chronic illnesses, such as myocarditis, aseptic meningitis, asthma, chronic fatigue syndrome, multiple sclerosis, and Alzheimer's disease. Confirmation of *C. pneumoniae* infection is difficult, because the organisms are fastidious and difficult to isolate from clinical specimens. Generally, diagnosis relies on immunologic or molecular tests.

Epidemiology of infection

Transmission of infection occurs by the respiratory route, similar to viral respiratory infections. Most infections occur in schools, dormitories, military barracks, or within households. Commonly, infections occur in late childhood with peak incidence between 10 and 20 years of age. New infections or re-infections are acquired throughout life, in spite of a rising antibody titer to the pathogen. Infections appear to be more common in men than in women.

Pathogenesis and immune response

C. pneumoniae infects epithelial and endothelial cells, as well as macrophages and neutrophils. The phagocytic cells are able to serve as hosts for the pathogen and disseminate the organism throughout the body. The developing antibody response is not able to eliminate the

infecting organism. Gamma interferon and activated CD8 T cells offer some protection against the infection.

Treatment

C. pneumoniae is susceptible to a number of antibiotics, including erythromycin, tetracycline, doxycycline, azithromycin, clarithromycin, and some fluoroquinolones. It is recommended that treatment be given for at least 2 to 3 weeks.

Infections with *Chlamydophila psittaci*

C. psittaci is primarily an avian pathogen, infecting psittacine birds such as parrots, cockatiels, and parakeets as well as pigeons, turkeys, ducks, ostriches, and wild birds. The infectious agent is found in droppings of sick birds and in dust contaminated by infected droppings. The bacteria can remain infectious in the environment for many months. Human infection occurs through inhalation of bacteria shed in bird feces, in dust contaminated by droppings, and in secretions from infected birds. Sheep, goats, cattle, and reptiles may also become infected but the transmission of the pathogen from these animals to human cases has not been documented.

***C. psittaci* antigenic variants**

There are several distinct antigenic variants of *C. psittaci*: A, B, C, D, E, and F. The strains can be identified by monoclonal antibodies that recognize antigenic sites on the major outer membrane protein. These strains, or serovars, are endemic in different avian species. Recently an additional serovar, E/B, isolated from turkeys, ducks, and pigeons, has been identified. Identification of *C. psittaci* variants is useful in epidemiological studies.

Human infection with *C. psittaci* (psittacosis)

Psittacosis is quite rare, with fewer than 50 confirmed cases reported in the United States each year. Persons at risk include bird owners, pet shop employees, veterinarians, and poultry workers.

Psittacosis has an incubation period of 1 to 4 weeks. The infection may be asymptomatic or there may be fever and chills, muscle ache, headache, fatigue, a dry cough, shortness of breath, blood-tinged sputum, and severe pneumonia. Occasionally complications such as endocarditis and hepatitis as well as neurological problems may occur. The case fatality rate for untreated psittacosis is 15% to 20%.

Treatment

C. psittaci is susceptible to a number of antibiotics, including tetracycline, doxycycline, erythromycin, azithromycin, and rifampin.

EMERGING CHLAMYDIAL INFECTIONS

The newly recognized Parachlamydiaceae are only distantly related to the classic *Chlamydia* and *Chlamydophila* species. Some of these new chlamydiae are possible human and animal pathogens.

There is considerable evidence supporting the role of *Parachlamydia acanthamoeba* as an emerging respiratory pathogen. A number of serological studies have demonstrated antibodies to *Parachlamydia* in patients suffering from pneumonia. In several cases these patients were immunocompromised. Although free-living amoebae are hosts for *P. acanthamoebae*, this bacterium is also able to enter and to multiply within human macrophages. The results of various epidemiological studies suggest that exposure to Parachlamydiaceae may lead to bronchitis, community-acquired pneumonia, and aspiration pneumonia.

Neochlamydia

The pathogenic potential of *Neochlamydia hartmanellae* remains to be determined. *Neochlamydia* has been recovered from free-living amoebae (*Acanthamoeba*) isolated from a contact lens of a patient with keratitis. The role of *Neochlamydia* is not clear since *Acanthamoeba* keratitis is a well-established clinical syndrome.

Simkania negevensis

Simkania is found in free-living amoebae, using the amoebae as an environmental reservoir. This bacterium has a worldwide distribution. It has been associated with bronchiolitis in infants and with lower respiratory tract infections in adults. *S. negevensis* DNA has recently been demonstrated in human arterial biopsy specimens.

Infections in other vertebrates

Waddlia chondrophila is a newly described agent of bovine abortion. *Parachlamydia salmonis* is the probable etiologic agent of gill epitheliocystis in salmon. Parachlamydiaceae have been implicated as ocular pathogens of cats.

ROLE OF CHLAMYDIAE IN CHRONIC DISEASE

Many chronic diseases are associated with inflammation. Various infectious agents have been investigated as possible causes of this inflammation. *Chlamydia* species and *C. pneumoniae* are among the infectious agents suspected of contributing to the inflammatory process associated with chronic illness.

Approximately 5% of individuals with genital *C. trachomatis* infections develop inflammatory arthritis. A recent investigation of chlamydial serovars found in synovial (joint) tissues of these patients demonstrated ocular serovars A, B, and C. Although majority of chlamydial urogenital infections are due to genital serovars, some ocular serovars may be present. Unknown selection pressures appear to favor the establishment of ocular chlamydial serovars in the synovium, to the exclusion to genital serovars.

C. pneumoniae had also been linked to a number of chronic conditions, based on serological evidence. Some of these conditions, previously listed in the section on *C. pneumoniae* infections, are asthma, chronic fatigue syndrome, multiple sclerosis, and Alzheimer's disease. In addition to these conditions, a recent study demonstrated that male patients with lung cancer have a higher antibody titer to *C. pneumoniae* than the control group.

Cardiovascular disease, including atherosclerosis, is one of the chronic diseases most thoroughly investigated for a link to *C. pneumoniae* infection. Inflammation of blood vessels plays an essential role in both initiation and progression of atherosclerosis, and chronic infection with *C. pneumoniae* may be a contributing factor to this inflammation. The presence of *C. pneumoniae* was shown in some of atherosclerotic plaques of coronary arteries that were studied with electron microscopy and immunoperoxidase staining. Attempts to isolate the organisms were negative. A number of serological studies demonstrated an association between chronic *C. pneumoniae* infection and cardiovascular disease but other studies failed to show an increased risk of an adverse outcome in cardiac patients seropositive for *C. pneumoniae*.

LABORATORY DIAGNOSIS OF CHLAMYDIAL INFECTIONS

Methods used for identification of chlamydiae in clinical specimens fall into three categories: 1) culture; 2) immunological tests including immunofluorescence; and 3) molecular techniques.

1) Cell culture

Culture of chlamydiae has long been considered the gold standard for identification of chlamydiae. This is the only method that demonstrates the presence of viable microorganisms and allows determination of antibiotic sensitivity. Cultivation of chlamydiae is highly specific but not as sensitive as some of the other diagnostic methods. The procedure is not available in all laboratories and some chlamydial species are very difficult to grow in cell culture.

Successful isolation of chlamydiae relies on the use of enriched sucrose phosphate transport medium and strict maintenance of cold storage of clinical specimens during transport. Various cell lines are used since each species or strain shows a relative specificity for a given cell type. Examples of cell lines that may be used are McCoy cells, HeLa 229 cells, and L434 mouse fibroblast cells. The specimen is centrifuged onto the cell monolayer to aid cellular infection and increase yield. Cultures are incubated in the presence of cycloheximide which inhibits host protein synthesis. Bacterial and fungal overgrowth is prevented by adding gentamicin, vancomycin, and amphotericin. The cultures are incubated for several days and sometimes for as long as 14 days. Determining whether the culture is positive or negative requires staining with iodine or Giemsa stain or the use of labeled polyclonal or monoclonal antibody. One or more blind passages in which apparently negative cultures are homogenized and inoculated to fresh cell cultures are sometimes required. Identification of one inclusion is sufficient to record a positive result.

2) Immunological tests

Complement fixation was the first serological test used for detecting serum antibodies to the chlamydial polysaccharide antigens in the lipopolysaccharide layer. These antigens are not strain-specific. In addition, the complement fixation test has low sensitivity for ocular infections.

Immunofluorescence tests are widely used in the diagnosis of chlamydial infections and are tests of choice for diagnosis of some of these infections.

The microimmunofluorescence technique developed by Wang and Grayston was the first method used to identify *C. trachomatis* serovars. This test can detect antichlamydial antibodies in serum or tears. Serial dilutions of the sample are placed on glass slides on which antigens of different *C. trachomatis* serovars have been fixed. Following incubation, the slides are probed with fluorescein-labeled anti-human immunoglobulin. Separate tests can detect the presence of immunoglobulin A, immunoglobulin M, and immunoglobulin G.

Other immunofluorescence assays used for diagnosis of chlamydial infections include direct and indirect fluorescent antibody tests and immunohistochemical assays.

Enzyme immunoassay

Enzyme-linked immunosorbent assays (EIA) are designed to detect antigen or antibody by producing an enzyme-triggered color change. In chlamydial infections enzyme immunoassay usually refers to an antigen detection test with antibody used to detect chlamydial antigen contained in the specimen. There are many commercial *C. trachomatis* enzyme immunoassays available. Most of these detect the chlamydial lipopolysaccharide.

3) Molecular tests

Hybridization assays

Early direct hybridization probe tests used radiolabeled *C. trachomatis* DNA and autoradiography requiring an exposure time of at least 36 hours. Commercial applications of this technique are now available. These commercial assays offer significant technical improvements in performance of hybridization probe tests.

Polymerase chain reaction (PCR) tests

PCR is a technique for amplifying DNA, and all assays based on it are part of the group of nucleic acid amplification tests. In detection of *C. trachomatis* a number of different nucleic acid sequences have been used as targets. These include the chlamydial cryptic plasmid (pCT), genes coding for MOMP, and genes coding for 16S rRNA. The PCR assay directed at plasmid genes is thought to be both sensitive and specific for the diagnosis of *C. trachomatis* infection. A commercial PCR kit, Amplicor Chlamydia, targets a sequence within the cryptic plasmid CT. Another commercial kit, the Gen-Probe APTIMA assay detects and amplifies *C. trachomatis* ribosomal RNA in cervical specimens. Recently a rapid PCR assay has been developed that detects and amplifies DNA that codes for 23S RNA. This assay is able to identify four members of the Chlamydiaceae family: *C. trachomatis*, *C. psittaci*, *C. pneumoniae* and *C. pecorum*.

Additional molecular techniques used for the diagnosis of *C. trachomatis* infections are the ligase chain reaction, the strand replacement assay, and the transcription-mediated amplification. Nucleic acid amplification tests are probably the most sensitive of all chlamydial diagnostic tests. The introduction of these tests represents a major advance in the diagnosis of chlamydial infections.

Laboratory diagnosis of infections with newly described chlamydiae

Processing of clinical samples

An adequate specimen must contain infected host cells because of the obligate intracellular nature of environmental chlamydiae. For mucosal specimens, swabbing with cotton swabs may be sufficient for the recovery of a sufficient number of infected host cells. Scraping of mucosal surfaces will increase cell yield but may also result in bleeding. Transport media developed for rickettsia, such as the 2-sucrose phosphate medium or the sucrose-glutamate phosphate medium, may be used for chlamydial clinical samples. Bacterial overgrowth may be suppressed by the addition of gentamicin and vancomycin to the transport medium. Common viral transport media should be avoided because they contain antibiotics that inhibit the growth of Parachlamydiaceae. Amphotericin B should not be added if the specimen will be tested in amoebal co-culture, since most free-living amoebae are susceptible to amphotericin B. The use of penicillin should, ideally, be avoided to prevent the induction of persistent non- multiplying aberrant bacterial forms. Microbiological specimens should be stored at 4 to 8 degrees C and processed as soon as possible. After storage for 24 hours the specimens should be frozen at -70 degrees C.

Cell culture

Historically chlamydiae had been grown in embryonated eggs but this culture method was relatively rapidly replaced by cell cultures. Embryonated eggs today are used mostly for production of large quantities of chlamydial antigens or for cultivation of fastidious strains.

Simkania negevensis and *Waddlia spp.* have been cultured in a variety of mammalian cell lines. These include Vero cells, HeLa, Hep-2, human macrophages and other cell lines. Vero cells are currently used for cultivation of *Simkania* and *Waddlia species*. Centrifugation of the clinical sample before applying it to the cell monolayer increases the growth yield of the organisms in cell culture. The culture protocol includes RPMI 1640 medium with 10% calf serum, the addition of antibiotics, and no cycloheximide. Cytopathic effect develops as soon as 36 hours after inoculation of clinical samples or after several days, depending on the species of parachlamydiae.

Parachlamydia acanthamoebae is difficult to grow in mammalian cell lines. Instead, amoebic co-culture can be used.

Amoebic co-culture

A wide variety of free-living amoebae are able to sustain the growth of new chlamydiae, but not all strains of a free-living amoeba are susceptible to infection with these agents. The use of more than one strain of amoeba or the use of several amoebic species is recommended to increase the rate of isolation of parachlamydiae from clinical or environmental samples. Amoebic co-culture may be done by inoculating the clinical sample into a cell culture system where mammalian cells are replaced by cultured free-living amoebae. An alternative method is the addition of an enrichment for amoebae to the clinical sample. If parachlamydiae are present within the amoebae, they will be liberated from their amoebic hosts by lysis. The released bacteria can then be grown with another strain of free-living amoebae.

Detection of bacteria that grow in either cell culture or amoebic culture may be done by different staining methods. Since chlamydiae give inconsistent results with Gram stain, Giemsa or Gimenez stains or immunofluorescence can be used. *In situ* fluorescent hybridization technique is also suitable for demonstration of chlamydiae. When stained by the Gimenez method, the bacteria appear reddish (fuchsin) against a green background (malachite green).

Serological methods

An enzyme-linked immunosorbent assay (ELISA) has been developed for the diagnosis of *Simkania negevensis* infection. This ELISA detects immunoglobulin A and immunoglobulin G but not immunoglobulin M.

Immunofluorescent techniques have been successfully used in identification of parachlamydiae in a number of studies. Western blotting technique has been used to confirm the results of immunofluorescence tests.

Molecular techniques

Nucleic acid amplification by the polymerase chain reaction (PCR) has been successfully applied to identification of the newly described chlamydiae. PCR techniques that target ribosomal genes have been most useful for identification of this group of organisms. For the new chlamydiae sequencing of the 16S rRNA gene appears to be the best method for strain identification. Primers have also been developed for 23S rRNA genes. Other genes of parachlamydiae have been used as targets for amplification by PCR.

Laboratory diagnosis of infections with *Chlamydia* and *Chlamydophila*

***C. trachomatis* genital and neonatal infections:**

Cell culture

Direct immunofluorescence

Enzyme immunoassays

Nucleic acid hybridization tests

Nucleic acid amplification tests: these have the highest sensitivity of all assays listed.

***C. pneumoniae* respiratory infections**

Use of culture for the detection of *C. pneumoniae* is difficult because of problems associated with growing *C. pneumoniae* in cell culture.

Nucleic acid amplification tests are used in research and, occasionally, in clinical laboratories. Serological tests may be used, but complement fixation test does not distinguish *Chlamydophila* from *Chlamydia*. The microimmunofluorescence test is useful for diagnosis of *C. pneumoniae* infections.

Psittacosis

Cell culture

Serology

Lymphogranuloma venereum

This disease is frequently diagnosed on the basis of lymph node biopsy findings and clinical symptoms. Laboratory tests that could aid in diagnosis are indirect immunofluorescence and serological tests.

Trachoma

The diagnosis is generally based on clinical symptoms. Diagnostic laboratory tests include: Direct microscopy of conjunctival scrapings: the slides are stained with Giemsa stain or with acridine orange and iodine and examined for inclusion bodies.

Cell culture

Enzyme immunoassays for detection of chlamydial antigen

Serological tests for chlamydial antibody in tears and serum

Nucleic acid hybridization tests

Nucleic acid amplification tests

SUMMARY

Chlamydiae represent a diverse group of intracellular bacteria widely distributed in the environment. Some chlamydiae are pathogenic in humans and in other vertebrates whereas others are symbiotic in free-living amoebae. These diverse chlamydial groups share a common ancestor. Chlamydial classification is currently undergoing a change. On the basis of molecular studies the chlamydiae that cause pneumonia and psittacosis are now placed in a new genus *Chlamydophila*. A new family and several new genera have been created for environmental chlamydiae. This classification is not universally accepted at this time.

Chlamydia and *Chlamydophila* are responsible for a wide range of human diseases.

These diseases include trachoma, lymphogranuloma venereum, genital infections, neonatal infections, pneumonia, and psittacosis. Chlamydial genital infections are the most frequently reported infections in the United States.

The chlamydiae are Gram-negative, obligate intracellular bacteria with a limited metabolic capacity. They are not able to generate energy and therefore depend on host cells for their energy requirements. Chlamydiae undergo a biphasic developmental cycle and have an extracellular and an intracellular phase of growth. The extracellular forms of chlamydiae are called elementary bodies. These are spherical, spore-like, infectious, and metabolically inert. The intracellular forms are called reticulate bodies. They are larger than elementary bodies, are metabolically active, and are adapted to an intracellular environment.

Chlamydiae are able to infect a wide range of host cells. Replication of chlamydiae takes place within membrane-bound vacuoles in the cytoplasm of infected cells. Within the vacuoles, the elementary bodies reorganize into reticulate bodies which multiply by binary fission. After 24 to 72 hours the reticulate bodies transform into elementary bodies, which are released from the host cells and are able to initiate a new cycle of infection.

The recently described environmental chlamydiae have been placed in a newly created family, the Parachlamydiaceae. These organisms are endosymbionts of free-living amoebae. The replication cycle of these bacteria is similar to that of classical chlamydiae with the exception that two infective extracellular forms are present. The second form is called the crescent body.

Infections with *C. trachomatis* and *C. pneumoniae* are extremely common and have a tendency toward persistence. Re-infections are also common and tend to increase the severity of the host's inflammatory response to the pathogen. Chronic inflammation frequently results in

tissue damage and scarring. *C. trachomatis* infects the eye and the genital tract depending on the tissue preference of the infecting strain. Genital infections include cervicitis, urethritis, and lymphogranuloma venereum. Complications that may develop as a consequence of the initial infection include pelvic inflammatory disease, ectopic pregnancy, infertility, and prostatitis. The same strains that cause genital infections may be involved in neonatal infections. Other strains of *C. trachomatis* cause trachoma, an ocular infection that is a major public health problem in developing countries.

Members of the genus *Chlamydophila* infect the respiratory tract. *C. pneumoniae* causes pneumonia and *C. psittaci* is the agent of psittacosis. Some of the newly discovered environmental chlamydiae are also implicated in human disease. Chronic infections with chlamydiae have been linked to other diseases such as atherosclerosis.

Chlamydial infections can be treated with antibiotics such as erythromycin, azithromycin, or doxycycline.

Diagnosis of chlamydial infections is based on clinical symptoms and on laboratory diagnostic assays. These assays include cell culture, serological tests, various immunofluorescent assays, and a number of molecular techniques that involve amplification of chlamydial DNA.

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REVIEW QUESTIONS

Course #DL-982

Choose the **one** best answer.

1. Organisms known as chlamydiae are
 - a. viruses that are symbiotic in free-living amoebae
 - b. intracellular bacteria
 - c. always pathogenic
 - d. free-living protozoa

2. The new classification of chlamydiae
 - a. places all chlamydiae in one genus
 - b. recognizes several genera of environmental chlamydiae
 - c. reclassifies chlamydiae as viruses
 - d. separates the genus *Chlamydia* into 5 new genera

3. *Chlamydophila* species include
 - a. *C. psittaci* and *C. trachomatis*
 - b. *C. pneumoniae* and *C. suis*
 - c. *C. psittaci* and *C. pneumoniae*
 - d. *C. trachomatis* and *C. pneumoniae*

4. Chlamydiae are
 - a. able to grow on nutrient agar
 - b. able to replicate extracellularly as well as within cells
 - c. energy parasites that depend on the energy metabolism of host cells
 - d. strictly anaerobic and easily destroyed by atmospheric oxygen

5. Reticulate bodies are
 - a. intracellular replicating forms of chlamydiae
 - b. chlamydial spores
 - c. hardy extracellular forms of chlamydiae
 - d. able to grow on blood agar

6. Elementary bodies are
 - a. Gram-positive bacteria related to *Micrococcus*
 - b. replicative forms that multiply in the nucleus of infected cells
 - c. extracellular, infectious forms of chlamydiae
 - d. intracellular, non-infectious forms of chlamydiae

7. Replication cycle of chlamydiae
 - a. takes place in the nucleus of host cells
 - b. takes place extracellularly
 - c. takes place on the outer surface of the host cell membrane
 - d. takes place in the cytoplasm of host cells

8. The crescent body is
 - a. an artifact caused by staining
 - b. found in classical chlamydiae
 - c. an infective extracellular form found in parachlamydiae
 - d. a polysaccharide capsule present on chlamydiae

9. *C. trachomatis* serovars A, B, and C infections
 - a. may cause blindness
 - b. are transmitted at birth
 - c. induce permanent immunity to reinfection
 - d. are not associated with inflammation

10. Lymphogranuloma venereum is a
 - a. disease caused by *C. trachomatis*
 - b. disease caused by environmental chlamydiae
 - c. lung infection
 - d. gastrointestinal infection

11. *C. trachomatis* serovar D is isolated from a patient. The patient's diagnosis is most probably
 - a. trachoma
 - b. non-gonococcal urethritis
 - c. psittacosis
 - d. lymphogranuloma venereum

12. Trichiasis is
 - a. an intestinal infection
 - b. caused by rubbing of eyelashes against the eye globe
 - c. a protozoan infection
 - d. a fungal infection

13. Psittacosis in the U.S. is most commonly found in
 - a. pet store workers
 - b. promiscuous males
 - c. newborn infants
 - d. households with poor hygiene

14. Respiratory infections with *C. pneumoniae*
 - a. are usually identified by culture
 - b. cannot be treated with antibiotics
 - c. may become associated with several chronic diseases
 - d. are unable to induce an antibody response

15. Newly described environmental chlamydiae include
 - a. *Chlamydophila pneumoniae*
 - b. *Chlamydophila psittaci*
 - c. *Chlamydia trachomatis*
 - d. *Parachlamydia acanthamoebae*

16. Newly described environmental chlamydiae are
 - a. found as symbionts in free-living amoebae
 - b. classified as viruses
 - c. never involved in human disease
 - d. classified as protozoa

17. Laboratory cultivation of chlamydiae is
 - a. possible in a nutrient broth medium
 - b. not possible under any conditions
 - c. possible in mammalian cell cultures
 - d. possible on chocolate agar

18. Nucleic acid amplification tests include
 - a. complement fixation test
 - b. polymerase chain reaction assays
 - c. enzyme immunoassays
 - d. direct fluorescent antibody tests

19. Parachlamydiae can be cultured in the laboratory
 - a. in amoebic co-culture
 - b. in brain heart broth
 - c. on blood agar
 - d. on nutrient agar

20. Direct microscopy of conjunctival scrapings may be useful
 - a. in the diagnosis of pneumonia
 - b. in the diagnosis of psittacosis
 - c. in the diagnosis of lymphogranuloma venereum
 - d. in the diagnosis of trachoma

**Course #DL-982 - Chlamydiae And Their Role In Human Disease
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Please circle the one best answer for each question.

- | | | | | | | | | | |
|-----|---|---|---|---|----|---|---|---|---|
| 1. | a | b | c | d | 11 | a | b | c | d |
| 2. | a | b | c | d | 12 | a | b | c | d |
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| 4. | a | b | c | d | 14 | a | b | c | d |
| 5. | a | b | c | d | 15 | a | b | c | d |
| 6. | a | b | c | d | 16 | a | b | c | d |
| 7. | a | b | c | d | 17 | a | b | c | d |
| 8. | a | b | c | d | 18 | a | b | c | d |
| 9. | a | b | c | d | 19 | a | b | c | d |
| 10. | a | b | c | d | 20 | a | b | c | d |

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(strongly agree) 5 4 3 2 1 (strongly disagree)

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