

## **Practical lesson 17: Microbiological diagnostics of infections caused by rickettsia, chlamydia and mycoplasma**

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## **RICKETTSIAE**

Rickettsiae are obligate intracellular bacteria; that is, they can grow only within cells. They are the agents of several important diseases, namely typhus, spotted fevers such as Rocky Mountain spotted fever, Q fever, anaplasmosis, and ehrlichiosis. Other less important rickettsial diseases such as endemic and scrub typhus occur primarily in developing countries. Rickettsial pox, caused by *Rickettsia akari*, is a rare disease found in certain densely populated cities in the United States.

### **RICKETTSIA RICKETTSII & RICKETTSIA PROWAZEKII**

#### ***Diseases***

*Rickettsia rickettsii* causes Rocky Mountain spotted fever, a life-threatening disease that occurs primarily in the Southeastern states, for example, North Carolina, of the United States. *Rickettsia prowazekii* causes epidemic typhus, also a life-threatening disease that occurs mainly in crowded, unsanitary living conditions during wartime.

#### ***Important Properties***

Rickettsiae are very short rods that are barely visible in the light microscope. Structurally, their cell wall resembles that of gram-negative rods, but they stain poorly with the standard Gram stain. Rickettsiae are obligate intracellular parasites, because they are unable to produce sufficient energy to replicate extracellularly. Therefore, rickettsiae must be grown in cell culture, embryonated eggs, or experimental animals. Rickettsiae divide by binary fission within the host cell, in contrast to chlamydiae, which are also obligate intracellular parasites but replicate by a distinctive intracellular cycle. Several rickettsiae, such as *R. rickettsii*, *R. prowazekii*, and *Rickettsia tsutsugamushi* (renamed *Orientia tsutsugamushi*), possess antigens that cross-react with antigens of the OX strains of *Proteus vulgaris*. The Weil-Felix test, which detects antirickettsial antibodies in a patient's serum by agglutination of the *Proteus* organisms, is based on this cross-reaction.

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### ***Transmission***

The most striking aspect of the life cycle of the rickettsiae is that they are maintained in nature in certain arthropods such as ticks, lice, fleas, and mites and, with one exception, are transmitted to humans by the bite of the arthropod. The rickettsiae circulate widely in the bloodstream (bacteremia), infecting primarily the endothelium of the blood vessel walls. The exception to arthropod transmission is *Coxiella burnetii*, the cause of Q fever, which is transmitted by aerosol and inhaled into the lungs. Virtually all rickettsial diseases are zoonoses (i.e., they have an animal reservoir), with the prominent exception of epidemic typhus, which occurs only in humans. It occurs only in humans because the causative organism, *R. prowazekii*, is transmitted by the human body louse. The incidence of the disease depends on the geographic distribution of the arthropod vector and on the risk of exposure, which is enhanced by such things as poor hygienic conditions and camping in wooded areas. These factors are discussed later with the individual diseases.

### ***Pathogenesis***

The typical lesion caused by these rickettsiae is a vasculitis, particularly in the endothelial lining of the vessel wall where the organism is found. Damage to the vessels of the skin results in the characteristic rash and in edema and hemorrhage caused by increased capillary permeability. Vasculitis of the vessels in the brain leads to the prominent headache. The basis for pathogenesis by these organisms is unclear. There is some evidence that endotoxin is involved, which is in accord with the nature of some of the lesions such as fever, petechiae and thrombocytopenia, but its role has not been confirmed. No exotoxins or cytolytic enzymes have been found.

### ***Clinical Findings & Epidemiology***

Rocky Mountain spotted fever.  
Note widespread petechial rash.

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### ***Laboratory Diagnosis***

Laboratory diagnosis of rickettsial diseases is based on serologic analysis rather than isolation of the organism. Although rickettsiae can be grown in cell culture or embryonated eggs, this is a hazardous procedure that is not available in the standard clinical laboratory. Of the serologic tests, the indirect immunofluorescence and enzyme-linked immunosorbent assay (ELISA) tests are most often used. The Weil-Felix test is of historic interest but is no longer performed because its specificity and sensitivity are too low. A fourfold or greater rise in titer between the acute and convalescent serum samples is the most common way the laboratory diagnosis is made. This is usually a retrospective diagnosis, because the convalescent sample is obtained 2 weeks after the acute sample. If the clinical picture is typical, a single acute-phase titer of 1:128 or greater is accepted as presumptive evidence. If the test is available, a diagnosis can be made during the acute phase of the disease by immunofluorescence assay on tissue obtained from the site of the petechial rash. The Weil-Felix test is based on the cross-reaction of an antigen present in many rickettsiae with the O antigen polysaccharide found in *P. vulgaris* OX-2, OX-19, and OX-K. The test measures the presence of antirickettsial antibodies in the patient's serum by their ability to agglutinate *Proteus* bacteria. The specific rickettsial organism can be identified by the agglutination observed with one or another of these three different strains of *P. vulgaris*.

### ***Treatment***

The treatment of choice for all rickettsial diseases is doxycycline.

## COXIELLA BURNETII

### *Disease*

Coxiella burnetii causes Q fever. Q stands for “Query”; the cause of this disease was a question mark (i.e., was unknown) when the disease was first described in Australia in 1937.

### *Important Properties*

Coxiella burnetii has a sporelike stage that is highly resistant to drying, which enhances its ability to cause infection. It also has a very low ID50 estimated to be approximately one organism. Coxiella burnetii exists in two phases that differ in their antigenicity and their virulence: phase I organisms are isolated from the patient, are virulent, and synthesize certain surface antigens, whereas phase II organisms are produced by repeated passage in culture, are nonvirulent, and have lost the ability to synthesize certain surface antigens. The clinical importance of phase variation is that patients with chronic Q fever have a much higher antibody titer to phase I antigens than those with acute Q fever.

### *Transmission*

Coxiella burnetii, the cause of Q fever, is transmitted by aerosol and inhaled into the lungs. Q fever is the one rickettsial disease that is not transmitted to humans by the bite of an arthropod. The important reservoirs for human infection are cattle, sheep, and goats. Coxiella burnetii causes an inapparent infection in these reservoir hosts and is found in high concentrations in the urine, feces, placental tissue, and amniotic fluid of the animals. It is transmitted to humans by inhalation of aerosols of these materials.

### *Clinical Findings & Epidemiology*

Unlike other rickettsial diseases, the main organ involved in Q fever is the lungs. It begins suddenly with fever, severe headache, cough, and other influenzalike symptoms. This is all that occurs in many patients, but pneumonia ensues in about half. Hepatitis is frequent enough that the combination of pneumonia and hepatitis should suggest Q fever. A rash is rare, unlike in most of the other rickettsial diseases. In general, Q fever is an acute disease, and recovery is expected even in the absence of antibiotic therapy. Rarely, chronic Q fever characterized by life-threatening endocarditis occurs.

### *Laboratory Diagnosis*

Serologic tests, such as the indirect immunofluorescence assay, are used rather than isolation of the organism. Coxiella burnetii can be grown in cell culture or embryonated eggs but this is a hazardous procedure that is not available in the standard clinical laboratory.

### *Treatment*

The treatment of choice is doxycycline.

### *Prevention*

Persons at high risk of contracting Q fever, such as veterinarians, shepherds, abattoir workers, and laboratory personnel exposed to C. burnetii, should receive the vaccine that consists of the killed organism. Pasteurization of milk kills C. burnetii.

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## CHLAMYDIAE

Chlamydiae are obligate intracellular bacteria (i.e., they can grow only within cells). They are the agents of common sexually transmitted diseases, such as urethritis and cervicitis, as well as other infections, such as pneumonia, psittacosis, trachoma, and lymphogranuloma venereum.

### *Diseases*

Chlamydia trachomatis causes eye (conjunctivitis, trachoma), respiratory (pneumonia), and genital tract (urethritis, lymphogranuloma venereum) infections. Chlamydia trachomatis is the most common bacterial cause of sexually transmitted disease in the United States. Infection with C. trachomatis is also associated with Reiter's syndrome, an autoimmune disease. Chlamydia pneumoniae causes atypical pneumonia. Chlamydia psittaci causes psittacosis, also a disease characterized mainly by pneumonia. Chlamydia pneumoniae and C. psittaci are sufficiently different molecularly from C. trachomatis that they have been reclassified into a new genus called Chlamydophila. Taxonomically, they are now Chlamydophila pneumoniae and Chlamydophila psittaci. However, from a medical perspective, they are still known as Chlamydia pneumoniae and Chlamydia psittaci, and those are the names that are used in this book.

### *Important Properties*

Chlamydiae are obligate intracellular bacteria. They lack the ability to produce sufficient energy to grow independently and therefore can grow only inside host cells. They have a rigid cell wall but do not have a typical peptidoglycan layer. Their cell walls resemble those of gram-negative bacteria but lack muramic acid. Chlamydiae have a replicative cycle different from that of all other bacteria. The cycle begins when the extracellular, metabolically inert, "sporelike" elementary body enters the cell and reorganizes into a larger, metabolically active reticulate body. The latter undergoes repeated cycles of binary fission to form daughter reticulate bodies, which then develop into elementary bodies, which are released from the cell. Within cells, the site of replication appears as an inclusion body in the cytoplasm, which can be stained and visualized microscopically.



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### ***Transmission & Epidemiology***

Chlamydia trachomatis infects only humans and is usually transmitted by close personal contact (e.g., sexually or by passage through the birth canal). Individuals with asymptomatic genital tract infections are an important reservoir of infection for others. In trachoma, *C. trachomatis* is transmitted by finger-to-eye or fomite-to-eye contact. Chlamydia pneumoniae infects only humans and is transmitted from person to person by aerosol. Chlamydia psittaci infects birds (e.g., parrots, pigeons, and poultry, and many mammals including humans). Humans are infected primarily by inhaling organisms in airborne dry bird feces. Sexually transmitted disease caused by *C. trachomatis* occurs worldwide, but trachoma is most frequently found in developing countries in dry, hot regions such as northern Africa. Trachoma is a leading cause of blindness in those countries. Patients with a sexually transmitted disease are coinfectd with both *C. trachomatis* and *Neisseria gonorrhoeae* in approximately 10% to 30% of cases.

### ***Pathogenesis & Clinical Findings***

Chlamydiae infect primarily epithelial cells of the mucous membranes or the lungs. They rarely cause invasive, disseminated infections.



# Chlamydia symptoms



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### ***CHLAMYDIA TRACHOMATIS***

Chlamydia trachomatis has more than 15 immunotypes (A–L). Types A, B, and C cause trachoma, a chronic conjunctivitis endemic in Africa and Asia. Trachoma may recur over many years and may lead to blindness but causes no systemic illness. Types D–K cause genital tract infections. In men, it is a common cause of nongonococcal urethritis (often abbreviated NGU), which is characterized by dysuria and a watery, nonpurulent urethral discharge. The discharge may be slight, detectable only by staining of underwear overnight. This infection may progress to epididymitis, prostatitis, or proctitis. In women, cervicitis develops and may progress to salpingitis and pelvic inflammatory disease (PID). Repeated episodes of salpingitis or PID can result in infertility or ectopic pregnancy. Infants born to infected mothers often develop mucopurulent conjunctivitis (neonatal inclusion conjunctivitis) 7 to 12 days after delivery, and some develop chlamydial pneumonia 2 to 12 weeks after birth. Chlamydial conjunctivitis also occurs in adults as a result of the transfer of organisms from the genitals to the eye. Patients with genital tract infections caused by C. trachomatis have a high incidence of reactive arthritis and Reiter's syndrome, which is characterized by urethritis, arthritis, and uveitis. These are autoimmune diseases caused by antibodies formed against C. trachomatis crossreacting with antigens on the cells of the urethra, joints, and uveal tract. Chlamydia trachomatis L1–L3 immunotypes cause lymphogranuloma venereum, a sexually transmitted disease with lesions on genitalia and in lymph nodes. Infection by C. trachomatis leads to formation of antibodies and cell-mediated reactions but not to resistance to reinfection or elimination of organisms.

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### ***CHLAMYDIA PNEUMONIAE***

*Chlamydia pneumoniae* causes upper and lower respiratory tract infections, especially bronchitis and pneumonia, in young adults. Most infections are mild or asymptomatic. The clinical picture resembles other atypical pneumonias, especially that caused by *Mycoplasma pneumoniae*. It is unclear whether *C. pneumoniae* causes upper respiratory infections such as sinusitis and otitis media.

#### ***Laboratory Diagnosis***

Chlamydiae form cytoplasmic inclusions, which can be seen with special stains (e.g., Giemsa stain) or by immunofluorescence. In general, the Gram stain is not useful as the organisms are too small to visualize within the cytoplasm. However, a gram stain of a urethral discharge that shows neutrophils but no gram-negative diplococci resembling *Neisseria gonorrhoeae* is presumptive evidence for infection by *C. trachomatis*. Nucleic acid amplification tests (NAATs) using the patient's urine are widely used to diagnose chlamydial sexually transmitted disease. Tests not involving culture, such as NAAT, are now more commonly used than culture-based tests. In exudates, the organism can be identified within epithelial cells by fluorescent-antibody staining or hybridization with a DNA probe. Chlamydial antigens can also be detected in exudates or urine by enzyme-linked immunosorbent assay (ELISA). Chlamydiae can be grown in cell cultures treated with cycloheximide, which inhibits host cell but not chlamydial protein synthesis, thereby enhancing chlamydial replication.

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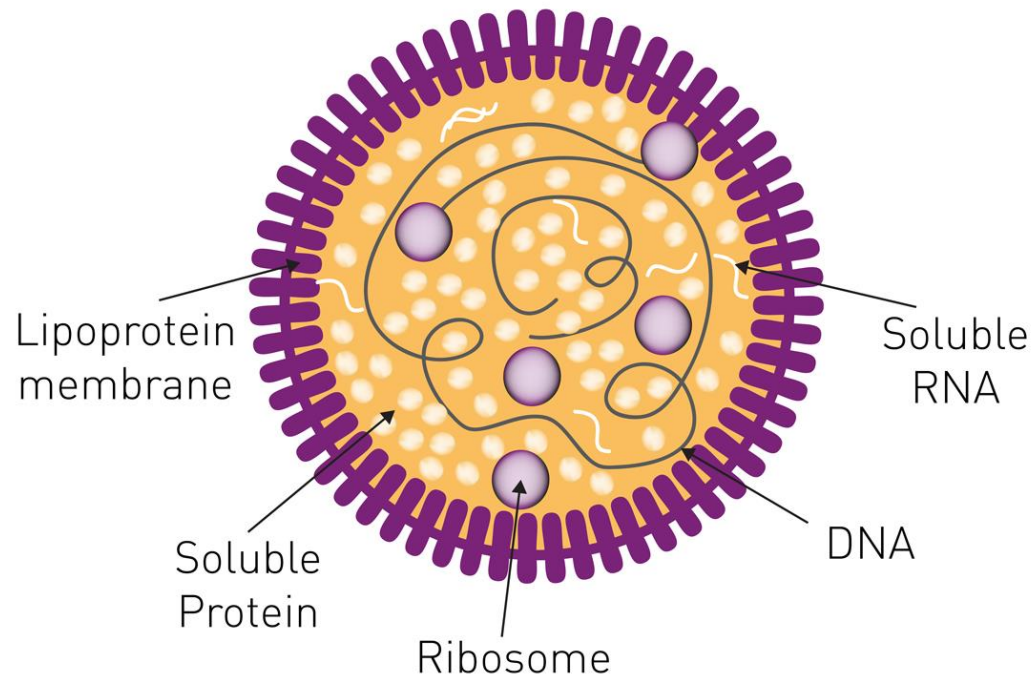
### ***Treatment***

All chlamydiae are susceptible to tetracyclines, such as doxycycline, and macrolides, such as erythromycin and azithromycin. The drug of choice for *C. trachomatis* sexually transmitted diseases is azithromycin. Because the rate of coinfection with gonococci and *C. trachomatis* is high, any patient with a diagnosis of gonorrhea should also be treated for *C. trachomatis* with azithromycin. The drug of choice for neonatal inclusion conjunctivitis and pneumonia is oral erythromycin. The drug of choice for *C. psittaci* and *C. pneumoniae* infections and for lymphogranuloma venereum is a tetracycline, such as doxycycline.

### ***Prevention***

There is no vaccine against any chlamydial disease. The best preventive measure against *C. trachomatis* sexually transmitted diseases is to limit transmission by safe sex practices and prompt treatment of both the patient and the sexual partners, including persons who are asymptomatic. Sexual contacts should be traced, and those who had contact within 60 days should be treated. Several types of sexually transmitted diseases are often present simultaneously. Thus, diagnosis of one requires a search for other causative agents. Oral erythromycin given to newborn infants of infected mothers can prevent inclusion conjunctivitis and pneumonitis caused by *C. trachomatis*. Note that erythromycin ointment used to prevent neonatal gonococcal conjunctivitis is much less effective against neonatal chlamydial conjunctivitis. Oral erythromycin should be used.

# Mycoplasma



## MYCOPLASMAS

*Mycoplasma pneumoniae* causes “atypical” pneumonia.

### *Important Properties*

Mycoplasmas are the smallest free-living organisms; many are as small as 0.3  $\mu\text{m}$  in diameter. Their most striking feature is the absence of a cell wall.<sup>1</sup>

Consequently, mycoplasmas stain poorly with Gram stain, and antibiotics that inhibit cell wall (peptidoglycan) synthesis (e.g., penicillins and cephalosporins) are ineffective. Their outer surface is a flexible cell membrane; hence these organisms can assume a variety of shapes. It is the only bacterial membrane that contains cholesterol, a sterol usually found in eukaryotic cell membranes.

Mycoplasmas can be grown in the laboratory on artificial media, but they have complex nutritional requirements, including several lipids. They grow slowly and require at least 1 week to form a visible colony. The colony frequently has a characteristic “fried-egg” shape, with a raised center and a thinner outer edge.

### *Pathogenesis & Epidemiology*

*Mycoplasma pneumoniae*, a pathogen only for humans, is transmitted by respiratory droplets. In the lungs, the organism is rod-shaped, with a tapered tip that contains specific proteins that serve as the point of attachment to the respiratory epithelium. The respiratory mucosa is not invaded, but ciliary motion is inhibited and necrosis of the epithelium occurs. The mechanism by which *M. pneumoniae* causes inflammation is uncertain. It does produce hydrogen peroxide, which contributes to the damage to the respiratory tract cells. *Mycoplasma pneumoniae* has only one serotype and is antigenically distinct from other species of *Mycoplasma*. Immunity is incomplete, and second episodes of disease can occur. During *M. pneumoniae* infection, autoantibodies are produced against red cells (cold agglutinins) and brain, lung, and liver cells. These antibodies may be involved in some of the extrapulmonary manifestations of infection. *Mycoplasma pneumoniae* infections occur worldwide, with an increased incidence in the winter. This organism is the most common cause of pneumonia in young adults and is responsible for outbreaks in groups with close contacts such as families, military personnel, and college students. It is estimated that only 10% of infected individuals actually get pneumonia. *Mycoplasma*

### ***Clinical Findings***

Mycoplasma pneumonia is the most common type of atypical pneumonia. It was formerly called primary atypical pneumonia. (Atypical pneumonia is also caused by Legionella pneumophila [Legionnaires' disease], Chlamydia pneumoniae, Chlamydia psittaci [psittacosis], Coxiella burnetii [Q fever], and viruses such as influenza virus and adenovirus. The term atypical means that a causative bacterium cannot be isolated on routine media in the diagnostic laboratory or that the disease does not resemble pneumococcal pneumonia.) The onset of Mycoplasma pneumonia is gradual, usually beginning with a nonproductive cough, sore throat, or earache. Small amounts of whitish, nonbloody sputum are produced. Constitutional symptoms of fever, headache, malaise, and myalgias are pronounced. The paucity of findings on chest examination is in marked contrast to the prominence of the infiltrates seen on the patient's chest X-ray. The disease resolves spontaneously in 10 to 14 days. In addition to pneumonia, M. pneumoniae also causes bronchitis. The extrapulmonary manifestations include Stevens-Johnson syndrome, erythema multiforme, Raynaud's phenomenon, cardiac arrhythmias, arthralgias, hemolytic anemia, and neurologic manifestations such as Guillain-Barré syndrome.

### ***Laboratory Diagnosis***

Diagnosis is usually not made by culturing sputum samples; it takes at least 1 week for colonies to appear on special media. Culture on regular media reveals only normal flora. Currently, a polymerase chain reaction (PCR) assay that detects M. pneumoniae specific nucleic acids in sputum or in respiratory secretions is the best diagnostic procedure. Serologic testing for the presence of antibodies in the patient's serum may also be useful.

### ***Treatment***

The treatment of choice is either a macrolide, such as erythromycin or azithromycin, or a tetracycline, such as doxycycline. The fluoroquinolone levofloxacin is also effective. These drugs can shorten the duration of symptoms, although, as mentioned earlier, the disease resolves spontaneously. Penicillins and cephalosporins are inactive because the organism has no cell wall.

### ***Prevention***

There is no vaccine or other specific preventive measure.

### ***Other Mycoplasmas***

Mycoplasma hominis has been implicated as an infrequent cause of pelvic inflammatory disease. Ureaplasma urealyticum may cause approximately 20% of cases of nongonococcal urethritis. Ureaplasmas can be distinguished from mycoplasmas by their ability to produce the enzyme urease, which degrades urea to ammonia and carbon dioxide.