Bordetella and Haemophilus

TEACHING OBJECTIVES:

1. Know the general morphology and physiology of the organisms
2. Know epidemiology and clinical symptoms
3. Understand the mechanisms pathogenesis
4. Know the diagnostic, therapeutic and preventive procedures

READING:

BORDETELLA

_Bordetella_ organisms are small, gram-negative coccobacilli which are strict aerobes. The three species of this genus vary in motility and certain biochemical characteristics. The most important human pathogen in this genus is _B. pertussis_, the organism which causes **whooping cough**. _Bordetella_ occurs worldwide and is strictly a human pathogen. The disease is spread via the respiratory route and the organism is non-invasive.

Two other species of _Bordetella_ are also clinically relevant. _B. parapertussis_ can cause a mild pharyngitis. _Bordetella bronchiseptica_ is usually an animal pathogen (i.e. kennel cough in dogs). It is rarely a cause of human disease, but can cause broncho-pulmonary symptoms in severely immunosuppressed individuals.

_Bordetella pertussis_

**Morphology and physiology:**

It is an extremely small; slow growing, strictly aerobic, Gram negative, capsulated, non-motile coccobacillus (short rod). Compared to other Bordetella species, _B. pertussis_ does not grow on common laboratory media. Selected media include _Bordet-Gengou medium_. _B. pertussis_ can be distinguished from _B. parapertussis_ in that _B. pertussis_ is oxidase positive but urease negative, while _B. parapertussis_ is oxidase negative and urease positive. _B. bronchiseptica_ is positive for both enzymes.

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<th>Growth on Blood Free Peptone</th>
<th>Urease</th>
<th>Oxidase</th>
<th>Motility</th>
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<tr>
<td><em>B. pertussis</em></td>
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<tr>
<td><em>B. parapertussis</em></td>
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<td><em>B. bronchiseptica</em></td>
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**Epidemiology:**
Most of the patients with whooping cough are less than a year old, although older children may also get the disease and the incidence among adults has increased disproportionately in recent years.

**Symptoms:**
The organism, contained in aerosol droplets, gains access via inhalation and colonizes the cilia of the mammalian respiratory epithelium. The incubation period is 7 to 10 days followed by mild symptoms of rhinitis, mild cough and sneezing occur (catarrhal stage) which last 1-2 weeks. During the catarrhal stage, the propagation of the organism increasingly compromises ciliary function resulting in the increased frequency and intensity of symptoms. At this stage the organism can be recovered in large numbers from pharyngeal cultures, and the severity and duration of the disease can be reduced by antimicrobial treatment. The patient is highly contagious and not very ill.

After 2 weeks the disease progresses to the paroxysmal stage, characterized by gradually increasing prolonged and paroxysmal coughing that often ends in a characteristic inspiratory gasp (whoop). The cough recurs at variable intervals; often every few minutes and interferes with oral intake. The swallowed mucus may induce vomiting, resulting in severe dehydration and weight loss. Hypoxia during prolonged attacks may lead to seizure, hypoxic encephalopathy or coma. During the paroxysmal stage, *B. pertussis* can rarely be recovered, and antimicrobial agents have no effect on the progress of the disease. This stage of the disease is mediated by a variety of soluble toxins.

The cough episodes gradually decrease after 2 to 4 weeks and the patient recovery can take 3-16 weeks (convalescent stage). Recovery is followed by immunity. Complications include pneumonia (due to other bacterial pathogens; rarely due to *B. pertussis*), otitis media, rectal prolapse and meningo-encephalitis.

![Figure 3: Disease Progression](image)
Pathogenesis:
The symptoms following the infection are due to many factors. In addition to the attachment to and growth on ciliated cells, the organism produces a number of exotoxins which contribute to these symptoms. Virulence is regulated by a central locus, bvs which responds to environmental stimuli.

**Colonization** is mediated by filamentous hemagglutinin, and the pertussis toxin.

1. Filamentous hemagglutinins: These molecules are not exotoxins. They are filament associated lipo-oligo-saccharides which are involved in the binding of the organism to ciliated epithelial cells.

2. Pertussis toxin (pertussigen): It is an AB-type exotoxins with 6 subunits (A: subunit 1; B: subunit 2-5 complex) which is the major cause of the abnormal cough. The toxin is both secreted into the extracellular fluid and cell bound. Subunits S2 and S3 function as adhesins, and bind the bacteria to host cells.

    The S1 subunit of pertussis toxin is an ADP-ribosyltransferase. The ADP-ribosyltransferase acts by the covalent addition of ADP-ribose to the GTP-binding Gi-protein and in so doing prevents the deactivation of adenylate cyclase. This results in the accumulation of large amounts of cAMP and consequently increased mucus secretion and the disruption of many cellular functions. Systemically it causes lymphocytosis, enhanced insulin secretions (hypoglycemia), increased IgE synthesis, increased histamine production and endotoxin sensitivity. The toxin inhibits mitogenicity for T lymphocytes and inhibits chemotaxis, phagocytosis and respiratory burst as well as impairing NK cell killing.

![Figure 4: Binding of pertussis toxin to the cell membrane](image)

**Pertussis is primarily a toxin mediated disease.** Localized damage and systemic symptoms are caused by the production of various toxins.
1. **Adenylate cyclase toxin**: This exotoxin acts locally to inhibit phagocyte and NK cell functions. It also helps the organism initiate infection. Adenylate cyclase toxin is only active in the presence of calmodulin and catalyzes the conversion of ATP to cAMP. This exotoxin was originally identified as a hemolysin since it lyases red blood cells. However, the cAMP increase caused by this exotoxin, in contrast with pertussigen, is short-lived. The hemolysin subunit causes the induction of programmed cell death in macrophages.

2. **Dermonecrotic (heat-labile) toxin**: This toxin causes inflammation and local necrosis adjacent to sites where *B. pertussis* is located. It is also a very strong vasoconstrictor and causes extravasation of leukocytes. In association with tracheal cytotoxin, it causes necrosis of the tracheal tissue.

3. **Tracheal cytotoxin**: It is a peptidoglycan-like molecule which binds to ciliated epithelial cells, preventing the ciliated cells from beating (ciliostasis). This virulence factor is not regulated by *bvg*. It also causes extrusion and destruction of ciliated epithelial cells. The destruction of these cells contributes to the symptoms of pertussis. The extrusion of ciliated cells leads to mucous plugs resulting in pulmonary obstructions and atelectasis.

4. **Lipopolysaccharide (LPS)**: Like LPS (endotoxin) of other Gram-negative bacteria, it is pyrogenic, mitogenic, and can activate and induce tumor necrosis factor production in macrophages. It also causes the induction of a number of cytokines and other inflammatory products (TNF, IL1, IL6, prostaglandins, etc.) and generates complement-activation products. The LPS of *B. pertussis* is unique in that it is heterogeneous, with two major forms differing in the phosphate content of the lipid moiety. The alternative form of Lipid A is designated Lipid X.

**Diagnosis:**
Symptoms are characteristic. Laboratory diagnosis is made by obtaining nasopharyngeal secretions. Primary cultures are obtained on *Bordet-Gengou medium* with incubation for 10-14 days. The organism grows as small transparent hemolytic colonies on blood agar. The slow growth rate makes direct fluorescent antibody testing on nasopharyngeal specimens a good diagnostic tool. PCR if available is highly sensitive and specific. Slide agglutination with specific antibodies is also used. Serologically *B. pertussis* can be distinguished from *B. parapertussis* and *B. bronchiseptica*.

**Prevention and treatment:**
Erythromycin is the antibiotic of choice. Antibiotic treatment during the catarrhal stage may ameliorate the disease. However, antibiotic treatment once the paroxysmal stage has begun may have no apparent effect on the course of the disease.

The pertussis vaccine used in the U. S. is an acellular vaccine consisting of filamentous hemagglutinins and detoxified pertussigen. These components are combined with diphtheria and tetanus toxoids in the DTaP vaccine.

**HAEMOPHILUS**
Members of the genus *Haemophilus* are small, nonmotile Gram-negative bacteria. The genus contains many species but *H. influenzae* is the most common pathogen. *Haemophilus* are present in the normal flora of the human mouth and respiratory tract. *H. influenzae type b*
polysaccharide capsule is the most virulent, although some non-encapsulated (non typeable) strains are also pathogenic.

Other species of Haemophilus that are normal flora and rarely cause disease are H. aegyptius (pink eye [purulent conjunctivitis]), H. influenzae aegyptius (Brazilian purpuric fever), H. parainfluenzae (pneumonia and endocarditis), and H. aphrophilus (pneumonia, endocarditis). H. ducreyi is the causative agent of chancroid, a sexually transmitted disease.

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<th>Organism</th>
<th>Hemolysis</th>
<th>Growth factor</th>
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<tr>
<td></td>
<td>X</td>
<td>V</td>
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<tr>
<td>H. influenzae</td>
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<tr>
<td>H. aegyptius</td>
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<td>H. ducreyi</td>
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<td>H. parainfluenzae</td>
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<td>H. aphrophilus</td>
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**Haemophilus influenzae**

**Morphology and physiology:**

The organism is a small Gram negative rod which can be grown on chocolate agar (supplemented with IsoVitalex) and requires hemin (factor X) and nicotinamide-adenine-dinucleotide (factor V) for growth. Growth with 5% CO2 is enhanced. It does not grow on normal blood agar. The factor V and factor X requirement can be used to distinguish between H. influenzae which requires both, H. parainfluenzae which requires factor V only and H. ducreyi which requires factor X only. H. influenzae will only grow on sheep blood agar around colonies of Staphylococcus aureus (satellite phenomenon).

H. influenzae strains are divided on the basis of capsular polysaccharides (a-f) or the absence of a capsule (nontypeable). The type b capsule is composed of polyribose-ribitol phosphate (PRP).

**Epidemiology and symptoms:**

H. influenzae nonencapsulated (nontypeable) organisms are part of normal flora of the respiratory tract. The carrier rate for nontypeable strains is 50-80% of individuals. The carrier rate for H. influenzae type b is 2-4% with 95% of the invasive disease caused by this strain.

There were 2304 cases of all types of H. influenzae in the U. S. in 2005, 35 of those cases were in South Carolina. The majority of the cases were non-b and nontypeable H influenzae. The availability of the H influenzae type-b (Hib) vaccine has significantly reduced the cases of Hib meningitis in children between 5 months and 5 years of age, as well as older children, adolescents and adults. Before the availability of the vaccine H. influenzae was the most common cause of meningitis in children ages 5 months to 5 years with mortality rates of 90% in untreated cases.

Hib infection initially causes a mild upper respiratory disease (runny nose, low grade fever and headache) followed within 1-3 days by meningitis. This invasive organism enters the circulation then crosses the blood-brain barrier. The resulting meningitis ia rapidly progressing and
can result in death. Timely treatment may prevent complications and death. Complications include deafness, epilepsy and mental retardation. Hib may also cause septic arthritis, cellulitis, pneumonia, and epiglottitis, the latter results in the obstruction of the upper airway and suffocation.

Diseases caused by *H. influenzae* since the introduction of the Hib vaccine include: septic arthritis, osteomyelitis, cellulitis, pericarditis, pneumonia (most frequent is serotype f), otitis media (*S pneumoniae* and then nontypeable Hf are the most common), sinusitis, chronic bronchitis and purulent bacterial conjunctivitis

**Pathogenesis:**

The presence of a capsule, which is anti-phagocytic, is a major factor in virulence. *H. influenzae* does not produce any exotoxins. Type-b *H. influenzae* are more invasive and pathogenic than other strains. The lipopolysaccharide is responsible for the inflammatory process. All virulent strains produce neuraminidase and an IgA protease which may aid their mucosal colonization.

**Diagnosis:**

Presumptive diagnosis is based on history, physical examination and symptoms. Cultures should be obtained from CSF (meningitis symptoms), blood, pleural fluids (pneumonia), and middle ear aspirates. The organism grows well in culture on the appropriate media (chocolate agar). Blood cultures may be delayed since commercially prepared blood culture broth does not contain X and V factors. Gram staining can facilitate a presumptive diagnosis. All invasive disease should be serotyped.

**Treatment and prevention:**

Unless prompt treatment is initiated, H. influenzae-b meningitis and epiglottitis have a high mortality rate. Antibiotics of choice are cefotaxime sodium, ceftriaxone sodium or ampicillin in combination with chloramphenicol. Three Hib conjugate vaccines are available in the U. S. Each vaccine consists of capsular PRP conjugated to a protein carrier and is a part of the recommended routine vaccination schedule.

**Non *H. influenzae* species**

*H. ducreyi* is an extracellular pathogen which is the major cause of human genital ulcer disease (chancreoid) in developing countries (Asia, Africa, and Latin America) but, less commonly in the United States. A significant concern is that the genital ulcers of chancroid have been epidemiologically associated with sexual transmission of HIV virus.

Chancroid presents with single or multiple painful necrotizing ulcers at the site of infection, frequently accompanied by painful swelling and suppuration of regional lymph nodes. In males, most ulcers are found on the prepuce near the frenulum or in the coronal sulcus. The infection is generally asymptomatic in women, but most lesions are found at the entrance of the vagina. Incubation is generally 3 to 14 days after exposure followed by a tender papule that becomes pustular then ulcerates over the course of 2 days.
*H. ducreyi* is a fastidious organism and laboratory diagnosis is made by isolation of *H. ducreyi* from lesion exudates and serology. The organism can be isolated from the ulcerated chancroid exudate and a stained smear shows Gram-negative short rods in parallel rows of small rods in chains.

The incidence of chancroid in the U. S. for 2005 is 17 cases. The pathogenic factors include a peptidoglycan-associated lipoprotein, adhesive pili, and cytolethal distending toxin. Antibiotic treatment with azithromycin, ceftriaxone, ciprofloxacin, or erythromycin will cure the disease.

*H. influenzae biogroup aegyptius* is a non-encapsulated invasive organism which causes a fulminant pediatric disease *Brazilian purpuric fever*. Symptoms include high fever, hemorrhagic skin lesions, septicemia, vascular collapse, hypotensive shock and death usually within 48 hours of onset.

*H. aegyptius*, also known as the Koch-Weeks bacillus is associated with an acute purulent and contagious form of *conjunctivitis (pink eye)*.

*H. aphrophilus* is a part of the normal flora of the oral and respiratory tract. This organism can cause endocarditis and pneumonia.