**LESSON 6.
Microbiology diagnosis of zoonotic infections (brucellosis, anthrax, listeriosis, plague, tularemia)**

**LESSON PLAN:**

• Zoonotic infections and their characteristics

• Understanding of particularly dangerous infections, rules of working with their causative agents.

• Morpho-biological characteristics of Brucella. Pathogenicity factors of Brucella, pathogenesis and clinical manifestations of brucellosis. Microbiological diagnosis of brucellosis. Specific treatment and prevention of brucellosis.

• Understanding of bacteria from the genus Bacillus. Morpho-biological characteristics of black sore pathogens. Pathogenicity factors of B. anthrachis. Pathogenesis and clinical manifestations of black ulcer, microbiological diagnosis, specific treatment and prevention.

• The causative agent of listeriosis – Listeria monocytogenes, its morpho-biological characteristics, ecology. Pathogenesis and clinical manifestations of listeriosis, the role of listeria in neonatal pathology. Microbiological diagnosis of listeriosis.

• Genus Yersinia. Plague agent - Yersinia pestis, its morpho-biological characteristics, pathogenicity factors. Pathogenesis and clinical manifestations of plague. Microbiological diagnosis, specific treatment and prevention of plague. The causative agents of intestinal yersiniosis - Y. enterocolitica and Y. pseudotuberculosis, their morpho-biological characteristics, pathogenesis and clinical manifestations, microbiological diagnosis

• Morpho-biological characteristics of tularemia agents, pathogenicity factors. Pathogenesis and clinical manifestations of tularemia, microbiological diagnosis, specific treatment and prevention by serological, biological, skin-allergic and emergency methods.

***BRUCELLA***

**Trigger Words**

Small coccobacilli, slow growing, zoonotic, undulant fever

**Biology and Virulence**

Very small gram-negative coccobacilli (0.5 × 0.6 to 1.5 μm)

Strict aerobe; does not ferment carbohydrates

Requires complex media and prolonged incubation for in vitro growth

Intracellular pathogen that is resistant to killing in serum and by phagocytes

Smooth colonies associated with virulence

**Epidemiology**

Animal reservoirs are goats and sheep *(B. melitensis);* cattle and bison *(B. abortus);* swine, reindeer, and caribou *(B. suis);* and dogs, foxes, and coyotes *(B. canis)*

Infects animal tissues rich in erythritol (e.g., breast, uterus, placenta, epididymis)

Worldwide distribution, particularly in Latin America, Africa, the Mediterranean basin, the Middle East, and Western Asia

Vaccination of herds has controlled disease

Most disease in travelers

ᑏᑏ Individuals at greatest risk for diseaseare people who consume unpasteurized dairy products, people in direct contact with infected animals, and laboratory workers

**Diagnosis**

Microscopy is insensitive

Culture (blood, bone marrow, infected tissue if localized infection) is sensitive and specific if prolonged incubation is used (minimum of 3 days to 2 weeks)

Serology can be used to confirm the clinical diagnosis; fourfold increase in titer or single titer ≥1:160; high titers can persist for months to years

**Treatment, Prevention, and Control**

Recommended treatment is doxycycline combined with rifampin for a minimum of 6 weeks for nonpregnant adults; trimethoprim-sulfamethoxazole for pregnant women and for children younger than 8 years

Human disease is controlled by eradication of the disease in the animal reservoir through vaccination and serologic monitoring of the animals for evidence of disease, pasteurization of dairy products, and use of proper safety techniques in clinical laboratories working with this organism

*Brucella -* Named after Sir David Bruce, who first recognized the organism as a cause of “undulant fever”

*B. abortus - abortus,* abortion or miscarriage (this organism is responsible for abortion in infected animals)

*B. melitensis - melitensis,* pertaining to the Island of Malta (Melita), on which the first outbreak was recognized by Bruce

*B. suis - suis,* of the pig (a swine pathogen)

*B. canis - canis,* of the dog (a dog pathogen)

***BACILLUS ANTHRACIS***

**Trigger Words**

Spore former, capsule, edema toxin, lethal toxin, anthrax, bioterrorism

**Biology and Virulence**

ᑏ Spore-forming, nonmotile, nonhemolytic gram positive rods

ᑏᑏ Polypeptide capsule consisting of poly-D-glutamic acid observed in clinical specimens

ᑏᑏVirulent strains produce three exotoxins that combine to form edema toxin (combination of protective antigen and edema factor) and lethal toxin (protective antigen with lethal factor)

ᑏᑏ The polypeptide capsule inhibits phagocytosis of bacteria

**Treatment, Prevention, and Control**

ᑏᑏ Inhalation or gastrointestinal anthrax or bioterrorism-associated anthrax should be treated with ciprofloxacin or doxycycline, combined with one or two additional antibiotics (e.g., rifampin, vancomycin, penicillin, imipenem, clindamycin, clarithromycin)

ᑏᑏNaturally acquired cutaneous anthrax can be treated with amoxicillin

ᑏᑏVaccination of animal herds and people in endemic areas can control disease, but spores are difficult to eliminate from contaminated soils

ᑏᑏVaccination of animal herds and at-risk humans is effective, although the development of a less toxic vaccine is desired

ᑏᑏAlternative treatments interfering with the activity of anthrax toxins are under investigation

**Epidemiology**

ᑏᑏUbiquitous in soils throughout the world

ᑏᑏ People at risk include those who consume food contaminated with the bacterium (e.g., rice, meat, vegetables, sauces), those with penetrating injuries (e.g., to eye), those who receive intravenous injections, and immunocompromised patients exposed to *B.cereus*

**Diseases**

ᑏᑏCapable of causing gastrointestinal diseases (emetic and diarrheal forms), ocular infections, and an anthrax-like disease in immunocompetent patients

 **Diagnosis**

ᑏᑏOrganism is present in high concentrations in clinical specimens (microscopy typically positive) and grows readily in culture

ᑏᑏ Preliminary identification is based on microscopic (gram-positive rods) and colonial (nonhemolytic, adherent colonies) morphology; confirmed by demonstrating capsule and either lysis with gamma phage, a positive direct fluorescent antibody test for the specific cell wall polysaccharide, or positive nucleic acid amplification assay

**Epidemiology**

ᑏᑏ *B. anthracis* primarily infects herbivores, with humans as accidental hosts

ᑏᑏ Rarely isolated in developed countries but is prevalent in impoverished areas in which vaccination of animals is not practiced

ᑏᑏ The greatest danger of anthrax in industrial countries is the use of *B. anthracis* as an agent of bioterrorism

**Diseases**

ᑏᑏ Three forms of anthrax are recognized: cutaneous (most common in humans), gastrointestinal (most common in herbivores), and inhalation (bioterrorism)

***BACILLUS CEREUS***

**Trigger Words**

Spore former, enterotoxin, gastroenteritis, eye infections

**Biology and Virulence**

ᑏᑏ Spore-forming, motile, gram-positive rods

ᑏᑏ Heat-stable and heat-labile enterotoxin

ᑏᑏ Tissue destruction is mediated by cytotoxic enzymes, including cereolysin and phospholipase C

**Treatment, Prevention, andControl**

ᑏᑏ Gastrointestinal infections are treated symptomatically

ᑏᑏ Ocular infectious or other invasive diseases require removal of foreign bodies and treatment with vancomycin, clindamycin, ciprofloxacin, or gentamicin

ᑏᑏ Gastrointestinal disease is prevented by proper preparation of food (e.g., foods should be consumed immediately after preparation or refrigerated)

 ***Important Bacillus Species***

*Bacillus-bacillum*, a small rod

*B. anthracis-anthrax*, charcoal, a carbuncle (refers to the black necrotic wound associated with cutaneous anthrax)

*B. cereus-cereus*, waxen, wax-colored (refers to colonies with a typical dull or frosted-glass surface)

***Bacillus* Diseases: Clinical Summaries**

***Bacillus anthracis***

**Cutaneous anthrax:** painless papule progresses to ulceration with surrounding vesicles and then to eschar formation; painful lymphadenopathy, edema, and systemic signs may develop

**Gastrointestinal anthrax:** ulcers form at site of invasion (e.g., mouth, esophagus, intestine), leading to regional lymphadenopathy, edema, and sepsis

**Inhalation anthrax:** initial nonspecific signs followed by rapid onset of sepsis with fever, edema, and lymphadenopathy (mediastinal lymph nodes); meningeal symptoms in half the patients, and most patients with inhalation anthrax will die unless treatment is initiated immediately

***Bacillus cereus***

**Gastroenteritis:** emetic form characterized by rapid onset of vomiting and abdominal pain and a short duration; diarrheal form characterized by a longer onset and duration of diarrhea and abdominal cramps

**Ocular infections:** rapid, progressive destruction of the eye after traumatic introduction of the bacteria into the eye

**Severe pulmonary disease:** severe anthrax-like pulmonary disease in immunocompetent patients

*Bacillus cereus* Food Poisoning



**Diagnosis**

ᑏᑏ Isolation of the organism in implicated food product or nonfecal specimens (e.g., eye, wound)

***LISTERIA MONOCYTOGENES***

**Trigger Words**

Coccobacilli, \_-hemolytic, meningitis, opportunistic, foodborne illness

**Biology and Virulence**

ᑏᑏ Gram-positive coccobacilli, often arranged in pairs resembling *Streptococcus pneumoniae*

ᑏᑏ Facultative intracellular pathogen that can avoid antibody-mediated clearance

ᑏᑏ Ability to grow at 4° C, in a wide pH range, and in the presence of salt can lead to high concentrations of the bacteria in contaminated foods

ᑏᑏ Virulent strains produce cell attachment factors (internalins), hemolysins (listeriolysin O, two phospholipase C enzymes), and a protein that mediates actindirected intracellular motility (ActA)

**Epidemiology**

ᑏᑏ Isolated in soil, water, and vegetation and from a variety of animals, including humans (low-level gastrointestinal carriage)

ᑏᑏ Disease associated with consumption of contaminated food products (e.g., contaminated milk and cheese, processed meats, raw vegetables [especially cabbage]) or transplacental spread from mother to neonate; sporadic cases and epidemics occur throughout the year

ᑏᑏ Neonates, elderly, pregnant women, and patients with defects in cellular immunity are at increased risk for disease

**Diseases**

ᑏᑏ Neonatal disease can result in in utero death or multiorgan abscesses, meningitis, and septicemia

ᑏᑏ Other diseases include influenza-like symptoms, self-limited gastroenteritis, and meningitis in patients with defects in cell-mediated immunity

**Diagnosis**

ᑏᑏ Microscopy is insensitive; culture may require incubation for 2 to 3 days or enrichment at I am not sure how to make the symbol for 4 degree C as in the sentence below.

ᑏᑏ Characteristic properties include motility at room temperature, weak this should be the Greek symbol for beta-hemolysis, and growth at 4° C and at high-salt concentrations

**Treatment, Prevention, and Control**

ᑏᑏ The treatment of choice for severe disease is penicillin or ampicillin, alone or in combination with gentamicin

ᑏᑏ People at high risk should avoid eating raw or partially cooked foods of animal origin, soft cheese, and unwashed raw vegetables

***YERSINIA***

**Trigger Words**

Bubonic plague, pneumonic plague, gastroenteritis, transfusion sepsis

**Biology and Virulence**

Gram-negative, facultatively anaerobic rods

Fermenter; oxidase negative

Lipopolysaccharide consists of somatic O polysaccharide, core polysaccharide (common antigen), and lipid A (endotoxin)

*Y. pestis* is covered with a protein capsule

Some species (e.g., *Y. enterocolitica*) can grow at cold temperatures (e.g., can grow to high numbers in contaminated refrigerated food or blood products)

Virulence: refer to Box 25.2; capsule on *Y. pestis* is antiphagocytic; *Y. pestis* is resistant to serum killing; *Yersinia* with genes for adherence, cytotoxic activity, inhibition of phagocytic migration and engulfment, and inhibition of platelet aggregation

**Epidemiology**

*Y. pestis* is a zoonotic infection, with humans the accidental host; natural reservoirs include rats, squirrels, rabbits, and domestic animals

Disease is spread by flea bites or direct contact with infected tissues or person to person by inhalation of infectious aerosols from a patient with pulmonary disease

Other *Yersinia* infections are spread through exposure to contaminated food products or blood products *(Y.enterocolitica)*

Colonization with other *Yersinia* species can occur

**Diseases**

*Y. pestis* causes bubonic plague (most common) and pulmonary plague, both having a high mortality rate; other *Yersinia* species cause gastroenteritis (acute watery diarrhea or chronic diarrhea) and transfusion-related sepsis; enteric disease in children may manifest as enlarged mesenteric lymph nodes and mimic acute appendicitis

**Diagnosis**

Organisms grow on most culture media; prolonged storage at 4° C can selectively enhance isolation

**Treatment, Prevention, and Control**

*Y. pestis* infections are treated with streptomycin; tetracyclines, chloramphenicol, or trimethoprim-sulfamethoxazole can be administered as alternative therapy

Enteric infections with other *Yersinia* species are usually self-limited; if antibiotic therapy is indicated, most organisms are susceptible to broad-spectrum cephalosporins, aminoglycosides, chloramphenicol, tetracyclines, and trimethoprim-sulfamethoxazole

Plague is controlled by reduction of the rodent population and vaccination of individuals at risk

Other *Yersinia* infections are controlled by proper preparation of food products

***FRANCISELLA TULARENSIS***

**Trigger Words**

Small coccobacilli, slow growing, cysteine supplemented media, zoonotic, ulcerous-glandular, oculo-glandular, pneumonic

**Biology and Virulence**

Very small gram-negative coccobacilli (0.2 × 0.2 to 0.7 μm)

Strict aerobe; do not ferment carbohydrates

Antiphagocytic capsule

Intracellular pathogen resistant to killing in serum and by phagocytes

**Epidemiology**

Wild mammals, domestic animals, birds, and fish, and blood-sucking arthropods are reservoirs; rabbits, cats, hard ticks, and biting flies are most commonly associated with human disease; humans are accidental hosts

A total of 239 cases were seen in 2017, although the actual number may be much higher

Infectious dose is small when exposure is by arthropod, through skin, or by inhalation; large numbers of organisms must be ingested for infection by this route

**Diseases**

Clinical symptoms and prognosis determined by route of infection: ulceroglandular, oculoglandular, glandular, typhoidal, oropharyngeal, gastrointestinal, pneumonic

**Diagnosis**

Microscopy is insensitive

Culture on cysteine-supplemented media (e.g., chocolate agar, buffered charcoal yeast extract agar) is sensitive if prolonged incubation is used

fold increase in titer or single titer ≥1:160; high titers can persist for months to years

**Treatment, Prevention, and Control**

Gentamicin is the antibiotic of choice; fluoroquinolones (e.g., ciprofloxacin) and doxycycline have good activity; penicillin’s and some cephalosporins are ineffective

Disease prevented by avoiding reservoirs and vectors of infection; clothing and gloves are protective

Live attenuated vaccine available but rarely used for human disease

***Francisella tularensis***

**Ulceroglandular tularemia:** painful papule develops at the site of inoculation that progresses to ulceration; localized lymphadenopathy

**Oculoglandular tularemia:** after inoculation into the eye (e.g., rubbing eye with a contaminated finger), painful conjunctivitis

develops, with regional lymphadenopathy

**Pneumonic tularemia:** pneumonitis with signs of sepsis develops rapidly after exposure to contaminated aerosols; high mortality

unless promptly diagnosed and treated