

4- Salmonella and Brucella

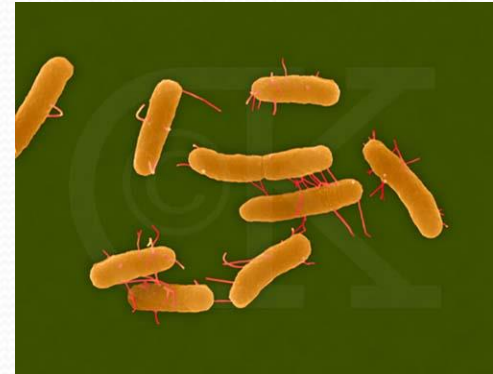
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Salmonella Typhi and Enteric Fever

General Characteristics of Salmonella

- Gram-negative
- Coliform bacilli (enteric rods)
- Motile by peritrichous flagella
- Facultative anaerobes
- Can ferment glucose but are non-lactose fermenter
- Usually produce H_2S
- Resistant to bile salts
- Contain 3 main antigens O, H and Vi antigens
- Important species:
 1. *Salmonella typhi*
 2. *Salmonella paratyphi*



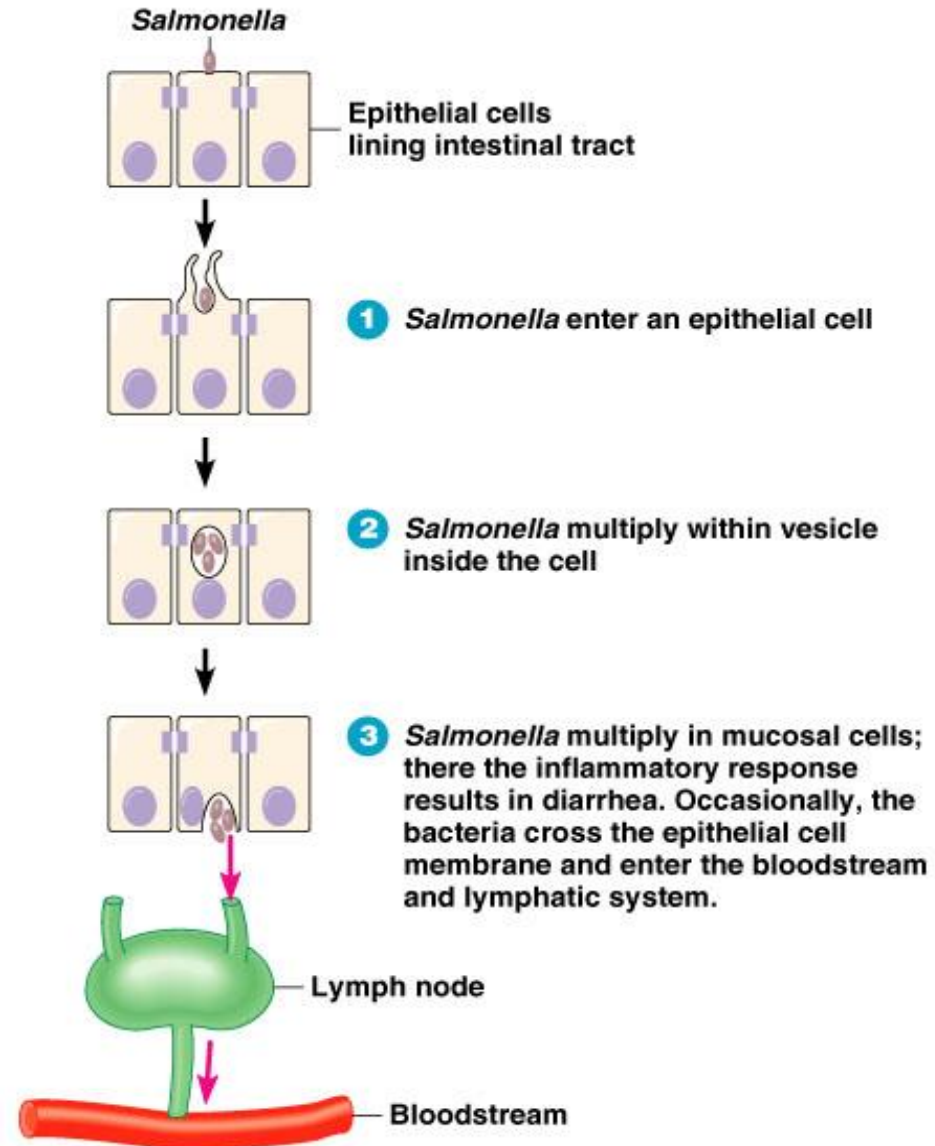
Epidemiology

- Typhoid fever is still an important cause of morbidity and mortality worldwide (16–33 million cases of typhoid fever occur annually)
- Typhoid is a strictly human disease
- Transmission:
 - Person to person spread through fecal-oral route by ingestion of contaminated food or water
 - If a patient with typhoid has not travelled to an endemic area, the source must be a visitor or someone else who prepared food
 - The pathogen can be transmitted in the water supply when sewage from carriers contaminate drinking water
 - Chronic carriers (5%) are the primary reservoir through chronic infection of the gallbladder and the biliary tract

Pathogenesis and Virulence

Virulence attributable to:

- Invasiveness
- Intracellular survival & multiplication
- Endotoxin
- Exotoxins: enterotoxins, cytotoxin



1. In the intestinal submucosa, the Vi antigen retards polymorphonuclear neutrophil (PMN) phagocytosis. This may favor uptake by macrophages. The typhoid bacteria remain within a membrane-bound vacuole and replicate, leading to macrophage death (prolonged intracellular survival in macrophages)
2. As the bacteria proliferate in macrophages, they are carried through the lymphatic circulation to the mesenteric nodes, spleen, liver and bone marrow
3. Bacteria begin to spill into the bloodstream. This seeding of Gram-negative bacteria and their LPS endotoxin starts the fever, which increases and persists with the continuing of bacteremia

Clinical Presentations

The main clinical presentation:

- **Gastroenteritis (enteritis):**

High infectious dose, transmitted by poultry and eggs
6-48h incubation period

Nausea, vomiting, diarrhea, fever, cramps, headache

- **Enteric fever**

Typhoid fever caused by *S. typhi* and less severe
paratyphoid fever caused by *S. paratyphi*

- **Septicemia**

Particularly *S. typhi*, and *S. paratyphi*
bacteraemia and systemic dissemination

- **Asymptomatic carriage**

Gall bladder is the reservoir for *S. typhi*

Enteric or Typhoid Fever

- Enteric fever is a multiorgan systemic infection characterized by prolonged fever, sustained bacteremia, and profound involvement of the RES, particularly the mesenteric lymph nodes, liver, and spleen
- The mean incubation period is 13 days
- The first sign is fever associated with a headache. The fever rises in a stepwise fashion for 72 hours. A relatively slow pulse is characteristic. In untreated patients, the elevated temperature persists for weeks.
- The fever rises to a high plateau, and the spleen and liver become enlarged. Rose spots (faint rash) on the skin of the abdomen or chest, are seen briefly in rare cases.
- Diarrhea may occur once or twice but is not a consistent feature
- The chief complications is intestinal hemorrhage and perforation rarely bacteremia may lead to dissemination to other organs

Typhoid Fever Course

- Untreated typhoid fever have 4 stages:
 1. First week: the temperature rises slowly with fluctuation, general weakness, and cough
 2. Second week: high fever in plateau around 40 °C, possible delirium, tender hepatosplenomegally, and rose spots
 3. Third week: complication start to appear, death can occur up to 30% if untreated
 4. Fourth week: fever might start to subside and the patient become a chronic carrier

Laboratory Diagnosis

- **Blood:** CBC (Leucopenia), LFT (elevated transaminases)
- **Specimens:** Blood, stool, duodenal drainage
- **Gram stain:** gram-negative rods
- **Culture:**

1. **Differential media:** MacConkey agar for rapid detection of lactose non-fermenting enterobacteria with inhibition of gram-positive bacteria
2. **Selective media:** Salmonella-shigella agar (SS agar) which favor growth of *Salmonella* and *Shigella* over other enterobacteriaceae
3. **Enrichment culture:** enriched broth media that allow growth of *Salmonella* and inhibit normal intestinal flora

Incubation for 24 hours in ambient air at 35-37 °C, produce colorless colony



- **Biochemical test:**

- Oxidase-negative

- Catalase-positive

- Glucose fermentation positive while lactose fermentation is negative

- Reduce nitrates to nitrites

• Serological tests:

1. Agglutination test: Known sera (Salmonella specific antibodies) and unknown culture are mixed on a slide and observed for clumping
2. The dilution agglutination test (Widal test):
 - To detect formation of specific anti-Salmonella antibodies in patients serum
 - Serial dilutions of serum are tested against known salmonella antigens (O and H antigens)
 - Positive with titer with O antigen $>1:320$, titer with H antigen $>1:640$, or rise in antibody titer in 2 specimens obtains with 7-10 days interval
 - The test is usually positive after 1-2 weeks of infection, and false-positive and false-negative results occur.
 - The test is not useful in diagnosis of enteric fevers caused by salmonella other than Salmonella Typhi.

Treatment

Enteritis:

- Fluid and electrolyte replacement
- Control of nausea and vomiting
- Antibiotics not recommended for enteritis because it prolong disease duration

Enteric fever:

- Antibiotics (chloramphenicol, ceftriaxone, ciprofloxacin)
- With proper antimicrobial therapy, patients feel better in 24 to 48 hours, their temperature returns to normal in 3 to 5 days, and they are generally well in 10 to 14 days

Prevention

- Control by proper preparation of food "**Boil it, cook it, peel it, or forget it**"
- The provision of clean water supplies
- Hygiene and sanitation with emphasis on proper hand washing
- Vaccination can reduce risk of disease for travelers in endemic areas (vaccination is available and is 50-70% effective)
- Identify & treat carriers of *S. typhi* & *S. paratyphi*



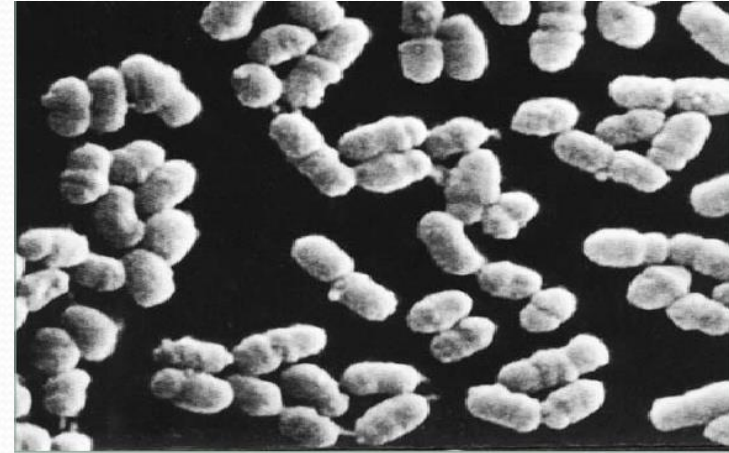
Brucella and Brucellosis

Introduction

- Brucellosis = Malta fever = Mediterranean Fever = Undulant fever
- David Bruce (1855-1931) sent to Malta to provide medical care to the troops. 1887 isolated “micrococcus” from spleens of 4 soldiers died of the disease
- Zoonotic disease
- Six species
 1. *B. abortus* - mainly cattle
 2. *B. melitensis* - sheeps & goats
 3. *B. suis* - pigs
 4. *B. canis* - dogs
 5. *B. ovis* - sheep (not human pathogen)
 6. *B. neotomae* - desert wood rat (not human pathogen)

General Characteristics

- Gram - ve cocci, coccobacilli, bacilli
- Very small
- Non fermenters
- Strict aerobic
- Non motile
- Non spore forming
- Grow in regular media -prolonged incubation > 4 weeks
- Two major antigenic variants (A and M)
- True pathogens: isolation always associated with disease, always clinically significant



Epidemiology

- 500,000 human cases per year worldwide
- The disease is common in Mediterranean and Arabic area
- Animals are natural reservoir mainly domestic animals
- Brucellosis is a genitourinary infection of sheep, cattle, pigs, and other animals
- Concentrated in animal milk, urine, genital organs
- Rout of transmission:
 1. Oral : unpasteurised milk & products of raw milk or meet
 2. Skin: accidental penetration or abrasion; at risk farmers & veterinarians
 3. Other routes: conjunctival, blood transfusion, and transplacental

- People at risk:
 1. Farmers
 2. Abattoir employees, government meat inspectors, and others who handle livestock or meat products
 3. Veterinarians
 4. Laboratory workers
- An outbreak of *B. melitensis* in Texas was traced to unpasteurized goat cheese brought in from Mexico



Pathology and Virulence

- Facultative intracellular pathogens of mononuclear-phagocyte system
 1. Bacteria are phagocytosed by macrophage or polymorphonuclear leukocyte
 2. Survive intracellularly by inhibiting killing
 3. Carried to spleen, liver, bone marrow, lymph nodes
 4. Form granulomas (mass of granulation tissue produced in response to chronic infections, inflammation, or foreign bodies) and cause destructive tissue damage
 5. Release of bacteria from granuloma into the systemic circulation responsible for the recurrent chills and fever of the clinical illness

Clinical Presentation

- **Acute disease** often develops with initial nonspecific symptoms of malaise, chills, fatigue, weakness, myalgias (muscles), weight loss, arthralgias, and cough
- **Chronic disease** and recurrence are common because it can survive in phagocytic cells and multiply to high concentrations
 1. Fever with sweating in the evening (periodic fever)
 2. Headache, anorexia, body aches and weight loss
 3. Lymphadenopathy, hepatomegaly, and splenomegaly
- **Complications:**
arthritis, epididymo-orchitis, spondylitis, neurobrucellosis, liver abscess, and **endocarditis** (the latter potentially fatal)

- Brucellosis starts with malaise, chills, and fever 7 to 21 days after infection. Drenching sweats in the late afternoon or evening are common, as are temperatures in the range of 39.4 to 40° C. The pattern of periodic nocturnal fever (undulant fever) typically continues for weeks, months, or even 1 to 2 years
- Patients become chronically ill with associated body aches, headache, and anorexia. Weight loss of up to 20 kg may occur during prolonged illness
- Less than 25% of patients show detectable enlargement of the reticuloendothelial organs, the primary site of infection. Of such findings, splenomegaly is most common, followed by lymphadenopathy and hepatomegaly

Laboratory Diagnosis

1. **Specimen:** blood, biopsy tissue from lymph nodes, bone marrow
2. **Gram stain:** small gram-negative coccobacilli
3. **Culture:**
 - Grow on commonly used media, including chocolate and blood agar
 - Brucella agar medium is highly enriched selective media that grow *Brucella* species bacteria very well
 - All cultures should be incubated in 8–10% CO₂ at 35–37°C and should be observed for 3 weeks before being discarded as negative
 - Colonies: small, convex, smooth colonies appear on enriched media in 2–5 days

4. Biochemical tests:

Catalase positive

Oxidase positive

Urease positive

5. Serology:

- Plate agglutination test (Brucella ring test)
 1. Drop of serum mixed with drop of Brucella antigen
 2. Clumping indicates infection
 3. If the mixture remains clear, the result is negative
 - Antibodies that agglutinate suspensions of heat-killed organisms typically reach titers of 1: 640 or more in acute disease
6. **ELISA:** detects specific IgG and IgM antibodies

Treatment and Prevention

- Treated with combination of tetracycline and doxycycline
- Prevention:
 1. Serology & confirmatory bacterial culture to identify infected animals
 2. Positive animals are destroyed
 3. Vaccination is available but is not a 100% effective and is costly to cattle ranchers
 4. Milk, milk products and meat need to be boiled or cooked properly



Thank you...