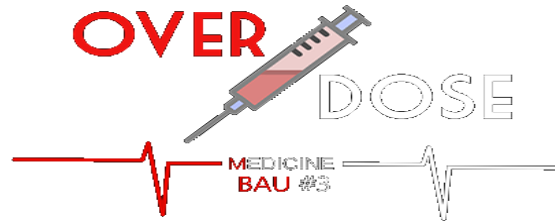




Al- Balqa Applied University
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Microbiology of HLS

Sheet #1.

Lecture Title: Salmonella and Brucella.

Lecture Date: 11-3-2019.

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Academic Team - Medicine BAU - Overdose

If you come by any mistake (whether it be spelling, grammatical or scientific) while browsing this sheet, kindly report it to the academic team

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Sheet notes are either in boxes or within text highlighted yellow and started with #

Link for prozone phenomenon:

<https://drive.google.com/open?id=1cAqwe9ZqIn3G87gkc85LCkgm3Zle3yHt>

Lecture one

Salmonella and Brucella



The Salmonella

Sheet Notes:

- ❖ *Salmonella* revision:
- ✓ Gram negative rods, member of Enterobacteriaceae family, oxidase negative, catalase positive (facultative anaerobes, but prefer aerobic conditions), reduce nitrate to nitrite (by reductase enzyme).
- ✓ Possess 3 major groups of antigens: H antigen, O antigen, and capsular antigen (K). O and H antigens are more important and are used in serologic classification.
- ✓ Some strains are encapsulated, others are not. (unlike *Klebsiella* that are all encapsulated).
- ✓ Normal flora in animals, but never in humans.

Salmonellae are often pathogenic for humans or animals when acquired by the oral route. They are transmitted from animals and animal products to humans, where they cause

1. Enteritis
2. Systemic infection
3. Enteric fever. (Typhoid fever)

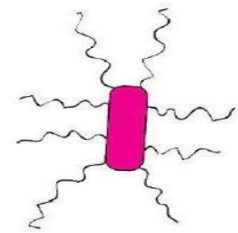
Sheet Notes:

- ❖ Each one of them is caused by different strain.
- ❖ Enteritis and enteric fever are the most common in Jordan, with enteritis more often.
- ❖ Enteritis occurs through ingestion of contaminated food.

Morphology and Identification

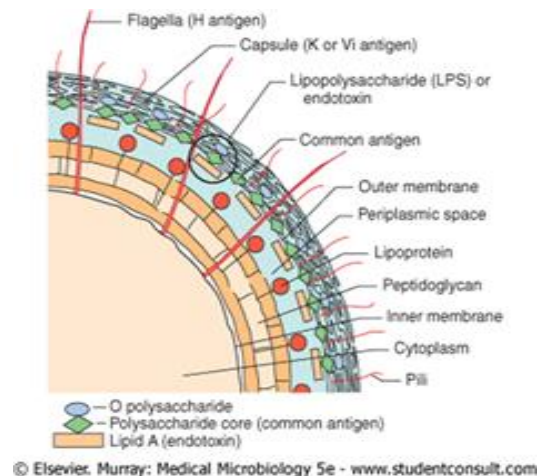
- ✚ Most isolates are motile with peritrichous flagella.
- ✚ Never ferment lactose or sucrose.
- ✚ Form acid and sometimes gas from glucose and mannose.
- ✚ **Usually produce H₂S.**
- ✚ Survive freezing in water for long periods.
- ✚ Resistant to certain chemicals
- ✚ Gram-negative, facultative anaerobic rods
- ✚ Oxidase negative
- ✚ Lipopolysaccharide consists of outer somatic O polysaccharide, core polysaccharide (common antigen), and lipid A (endotoxin)
- ✚ More than 2500- O serotypes (commonly referred to as individual *Salmonella* species)

Sheet Note: Peritrichous flagella:



Sheet Notes:

- ❖ A distinguishing feature between *Salmonella* and *Shigella* (as they are both lactose non-fermenter and grow on same culture {s-s agar}) is *Salmonella*'s motility.
- ❖ Another example of peritrichous flagella bacteria is *Proteus Spp* (swarming motility).
- ❖ In the picture beside, is the antigens of *salmonella*'s cell wall. Somatic O polysaccharide is responsible for stimulation of immune response.



Classification

- Currently, the genus *Salmonella* is divided into Two Species each with multiple subspecies and serotypes.
- **The two species are *Salmonella enterica* and *Salmonella bongori***
(*bongori* is not infectious of humans)
- ***S enterica* contains five subspecies, which are**
 - subspecies *enterica* (subspecies I)
 - subspecies *salamae* (subspecies II)
 - subspecies *arizonae* (subspecies IIIa)
 - subspecies *diarizonae* (subspecies IIIb)
 - subspecies *houtenae* (subspecies IV)
 - subspecies *indica* (subspecies VI)
- Most human illness is caused by the subspecies I strains, written as:
S enterica* subspecies *enterica. The subsp consists of many serotypes, so the accepted nomenclature for classification will be as follows:
S. enterica subspecies *enterica* serotype Typhimurium, which can be shortened to : ***S. typhimurium***

Four serotypes of salmonellae that cause Enteric fever can be identified in the clinical laboratory by biochemical and serologic tests. These serotypes should be routinely identified because of their clinical significance.

They are as follows:

- | | |
|---|---|
| <ol style="list-style-type: none"> 1. <i>Salmonella paratyphi A</i> (serogroup A) 2. <i>Salmonella paratyphi B</i> (serogroup B) 3. <i>Salmonella choleraesuis</i> (serogroup C1) 4. <i>Salmonella typhi</i> (serogroup D). | <ul style="list-style-type: none"> ❖ Serogroup C1 is not as important as the others and is not common in Jordan. ❖ Each of these serogroups has different O and H antigens. |
|---|---|

Variation

Organisms may lose H antigens and become nonmotile. Loss of O antigen is associated with a change from smooth to rough colony form. Vi antigen may be lost partially or completely. Antigens may be acquired (or lost) in the process of transduction. **#Through bacteriophage or lysogenic cycle.**

In humans, Salmonella are the cause of two diseases called salmonellosis:

- 1. Enteric fever (typhoid), resulting from bacterial invasion of the bloodstream,***
- 2. Acute gastroenteritis, resulting from a foodborne infection/intoxication.***

Sheet Notes:

- ❖ Infection: microorganisms are the cause.
- ❖ Intoxication: by enterotoxins.

Pathogenesis and Clinical Findings



- Animal reservoir for human infection is the **poultry, pigs, rodents, cattle, pets (from turtles to parrots), and many others.**
- The organisms almost always enter via the **oral route**, usually with contaminated food or drink. The mean infective dose to produce clinical or subclinical infection in humans is **10^5 – 10^8 salmonellae (but perhaps as few as 10^3 S. Typhi organisms).**

The “Enteric Fevers” (Typhoid Fever)

- This disease is produced by only a few of the salmonellae, of which **S Typhi** is the most important.
- The ingested salmonellae reach the SI **#but don't cause infection at this time (the reason is unknown)**, from which they enter the lymphatics and then the BS. They are carried by the blood to many organs, including the intestine.
- After an incubation period of 10–14 days, **fever, malaise, headache, constipation, bradycardia, and myalgia** occur. The fever reach a high plateau **#may reach 40 celsius**, and the **spleen and liver become enlarged**. **Rose spots** usually on the skin of the abdomen or chest, are seen briefly in rare cases.
- Hyperplasia and necrosis of lymphoid tissue (eg, Peyer's patches); focal necrosis of the liver; and inflammation of the gallbladder **#they resist bile and stay in gallbladder some years if the patient stops antibiotic the patient now is asymptomatic but infection will reactivate if our patient become immunocompromised**, periosteum, lungs, and other organs.

Sheet notes:

- 1- in first week we cant use stool culture (it is -ve culture) and widal test but we can use blood culture (+ve culture) , after 10 days we can use stool culture(now it is +ve).
 - 2- if the patient has developed enteric fever with o antigen , they will not be reinfected by o antigen (unless reactivation occur) but they may be infected by h antigen .
 - 3- people don't developed salmonella typhi & salmonella paratyphi, salmonella paratyphi A& salmonella typhi B, salmonella typhi/paratyphi O & salmonella typhi/paratyphi H at the same time.
- #why people don't develop salmonella h and o antigens at same time ??
- Salmonella surfaces have many epitopes but our body can recognize only 1 epitope , salmonella h is easier to develop because h epitope protrudes externally

Enteric fever: M.O  mouth  SI  Lymphatic and blood stream

Infect liver, spleen, bone marrow  multiplies and passes into blood

bacterimia  onset of clinical illness  Colonization of gallbladder 

invasion of the intestine. 

Mechanism of pathogenicity

Bacterial products involved in virulence:

- Salmonellae owe their pathogenicity largely to their ability to **invade tissue** and to **survive within macrophages**.
 - The VI antigen is a **capsule** that affords salmonellae some protection from phagocytosis.
 - Once phagocytosed, S.typhi **inhibits generation of oxidative free radicals** and intraphagosomal killing.
 - Additionally, salmonellae have **endotoxic lipopolysaccharide**, which is responsible for septic shock in patients with bacteriemia.
- #salmonella are facultative intracellular , the can live extracellularly or intracellularly (or inter phagocytic cells)

Bacteremia with Focal Lesions

- This is associated commonly with ***S. choleraesuis***
- After oral infection, there is early invasion of the bloodstream (with possible focal lesions in lungs, bones, meninges, and so on), but intestinal manifestations are often absent.

Diagnostic Laboratory Tests

Specimens: Blood , Urine, Faeces , Aspirated duodenal fluid, etc..

Bacteriologic Methods for Isolation of Salmonellae

1. Differential medium cultures

- **Enrichment broth:** Tetrathionat, selenit broth
- **Selective media:** MacConkey, S-S agar, Hektoen Enteric Agar, Bismuth sulfide agar



2. Final identification: Biochemical tests



API identification kit

Serologic Methods: Agglutination test (Widal test)

Sheet Notes: (of diagnostic laboratory tests)

- Blood or stool culture are easier than aspirated duodenal fluid, therefore preferred
- Isolation of salmonella is a little more complicated because the stool contains many other gram negative normal floras. Enrichment broth is used at first, supporting growth of many enterobacteria, including salmonella. Then a selective media for salmonella (ex. SS.agar).
- Bismuth agar can differentiate salmonella typhi from other salmonella, it appears as black colonies because of H₂S production.
- API identification kit (analytical profile index) is a manual method for fast identification of some enterobacteriaceae species, and it is based on using many test tubes for different enzymatic activities and comparing the positive and negative results with the profile of the expected species... details in GI system.
- There is automatic test for fast identification but it is expensive. One should be familiar with manual, semiautomatic and automatic methods, to handle any situation.
- Widal test is dependent on antigen antibody reactions (O and H antigens).

Immunity

- **Active immunity**
- **Passive immunity-** the temporary immunity that an infant acquires from its mother

Sheet notes:

- Active immunity could be artificial (vaccines).
- Passive immunity is acquired from your mother through placenta, breast milk after birth and Ig. That is given for immunocompromised people mainly when they are travelling to where endemic typhoid fever is (this serum is prepared in horse, and it is given only once because the next time there will be antibodies against some horse plasma ... allergy).

Prevention and control

- Sanitary measures.
- Carriers must not be allowed to work as food handlers.
- Strict hygienic precautions for food handling.
- Vaccines against *S. Typhi*:
 1. **Purified Vi antigen**
 2. **Oral, live attenuated vaccine.**

The Brucellae



Sheet notes:

- Appears bipolar with stain.
- Unlike salmonella, which facultative intracellular, brucella is obligate intracellular (depends on the metabolic machinery of the host cell)
- It causes splenomegaly, hepatomegaly ...
- Mostly fecal oral route of transmission.
- Brucellosis occurs more in summer, by brucella melitensis

- ❖ *Small (0.5×0.6 to $1.5 \mu\text{m}$), nonencapsulated*
- ❖ *Strictly aerobic*
- ❖ *Does not ferment carbohydrates*
- ❖ *Varies from cocci to rods, with short coccobacillary forms predominating.*
- ❖ *Grows slowly*
- ❖ *Intracellular parasite*
- ❖ *The organisms are phagocytosed by macrophages and monocytes . Phagocytosed bacteria are carried to the spleen, liver, bone marrow, lymph nodes*
- ❖ *The bacteria secrete proteins that induce granuloma formation*
- ❖ *Obligate parasites of animals and humans, inactive metabolically.*
- ❖ *Species of Brucella include the following:*
 1. ***Brucella melitensis* typically infects goats**
 2. ***Brucella suis*, swine**
 3. ***Brucella abortus*, cattle**
 4. ***Brucella canis*, dogs.**

The disease in humans, brucellosis (undulant fever, Malta fever), is characterized by an acute bacteremic phase followed by a chronic stage that may extend over many years and may involve many tissues.

Growth Characteristics

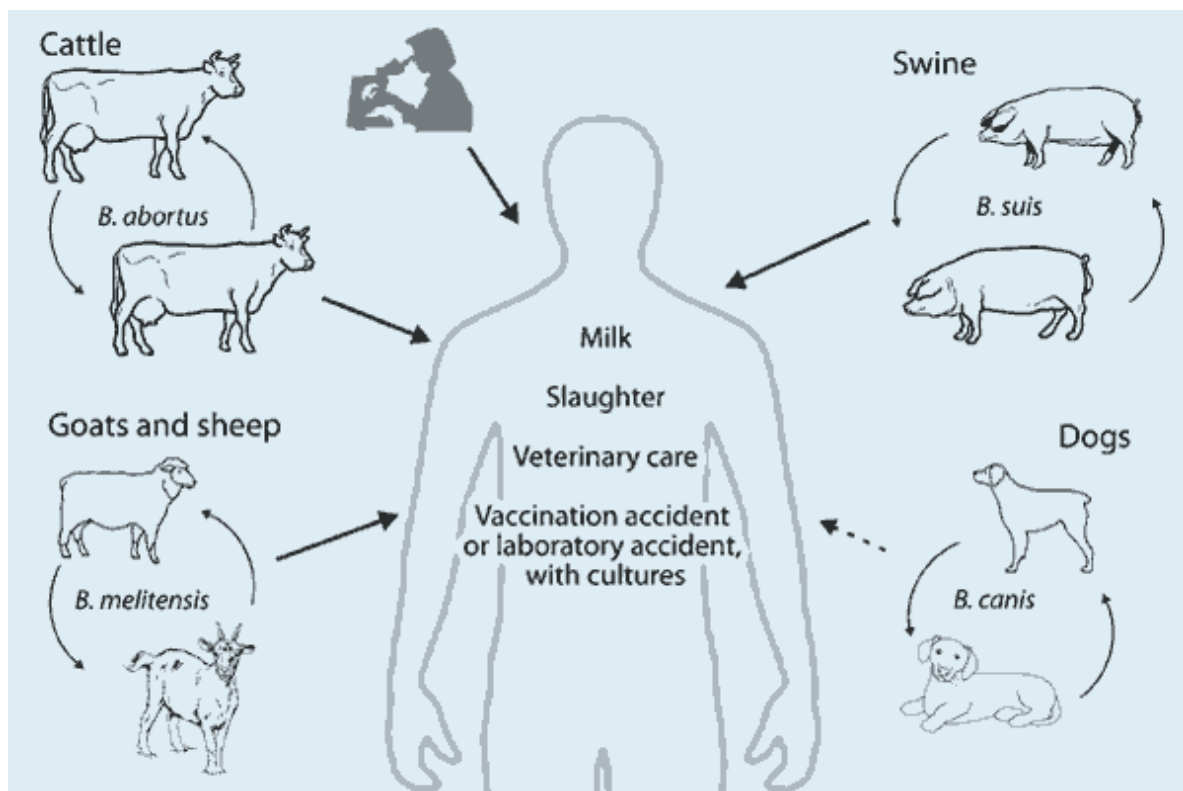
- *Fresh specimens inoculated on trypticase-soy agar or blood culture media.*
- *Brucellae use carbohydrates but produce neither acid nor gas.*
- *Catalase and oxidase are produced by the four species.*
- *Hydrogen sulfide is produced by many strains, and nitrates are reduced to nitrites.*
- ***killed in milk by pasteurization.***

Pathogenesis and Pathology

- *The common routes of infection in humans are*
 1. ***The intestinal tract (ingestion of infected milk)***
 2. ***Mucous membranes (droplets)***
 3. ***Skin (contact with infected tissues of animals).***
- *Cheese made from unpasteurized goats' milk is a particularly common vehicle.*

- The organisms progress from the portal of entry **via lymphatic channels and regional lymph nodes** to the **bloodstream**, which distributes them **to the organs**.
- Incubation period 1-6 weeks
- A chronic state may occur

Placentas and fetal membranes of cattle, swine, sheep, and goats contain erythritol, a growth factor for brucellae. The proliferation of organisms in pregnant animals leads to placentitis and abortion in these species.



Clinical Findings

- malaise, fever, weakness, and sweats.
- The fever usually rises in the afternoon; its fall during the night is accompanied by drenching sweat.
- Granulomatous nodules that may develop into abscesses form in lymphatic tissue, liver, spleen, bone marrow.
- Lymph nodes enlarge, SM, Hepatitis, Osteomyelitis, may be gastrointestinal and nervous symptoms.

Diagnostic Laboratory Tests

A. Specimens

Blood should be taken for culture, biopsy material for culture (lymph nodes, bone, and so on), and serum for serologic tests .

B. Culture

- Brucella agar was specifically designed to culture Brucella species bacteria.

- *Enriched blood agars*
- *Microscopic and colonial morphology*
- *Positive oxidase and urease reactions*
- *B. abortus and B. melitensis, B. abortus, and B. suis will react with antisera prepared against B. abortus or B. melitensis*

Immunity

- *IgM, IgG, IgA (Rose Bengal test)*
- *Titer 1/80 indicate active infection*
- *If agglutination negative “blocking antibodies”*

Sheet notes:

- *Rose Bengal stain*
- *There is cross reactivity between the four species in serology tests.*
- *Differentiate acute and chronic infections by rose Bengal test (IgM for acute while A and G for chronic).*
- *“Blocking antibodies” is when the concentration of antibodies in the serum is very high while the result is negative because the positive result is well appeared when there is equivalence between the antibody and antigen... HOW DO WE SOLVE THIS???*
- **EXTRA NOTE:** The doctor explained her question in the lab, this is called prozone phenomenon and is caused by the excess of either antibodies or antigens (because they must be equivalent). To solve the problem you must dilute the serum before using it (usually 1:5) and multiply the titer at the end by 5 (dilution of the serum) to calculate the concentration of antibodies. other steps remain the same . **I added the link of the record that explains this at the beginning...Ban**

Epidemiology, Prevention, and Control

*The common sources of infection for humans are **unpasteurized milk, milk products, and cheese as well as occupational contact** (eg, farmers, veterinarians, and slaughterhouse workers) with infected animals.*