

Salmonella

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Classification

- **Family**
 - **Lactose non fermenters, Enterobacteria?**
- **Genus**
 - ***Salmonella*, named after United States Department of Agriculture vet. Daniel E Salmon.**
- **Species**
 - *Salmonella enterica* , 5 subspecies, 2500 serovars
 - *Salmonella bongori*

- Most of the human pathogenic *Salmonella* serovars belong to the *S. enterica* subspecies. These subspecies include
 - *Salmonella* Typhi, (enteric fever)
 - *Salmonella* Paratyphi A, B, C (enteric fever)
 - *Salmonella* Enteritidis, , (food poisoning),
 - *Salmonella* Typhimurium, (food poisoning)
 - *Salmonella* Choleraesuis (septicemia)

Enterobacteriaceae

Shigella

Salmonella

Escherichia

Vibrionaceae

S. cholerae suis

S. enteritidis

S. typhimurium

S. paratyphi A. B. C.

S. typhi

Paratyphoid fever

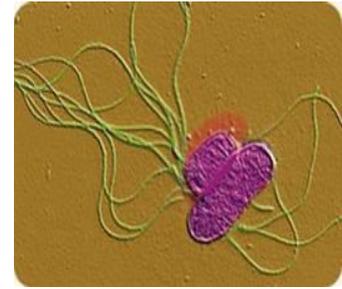
Typhoid fever

Septicaemia

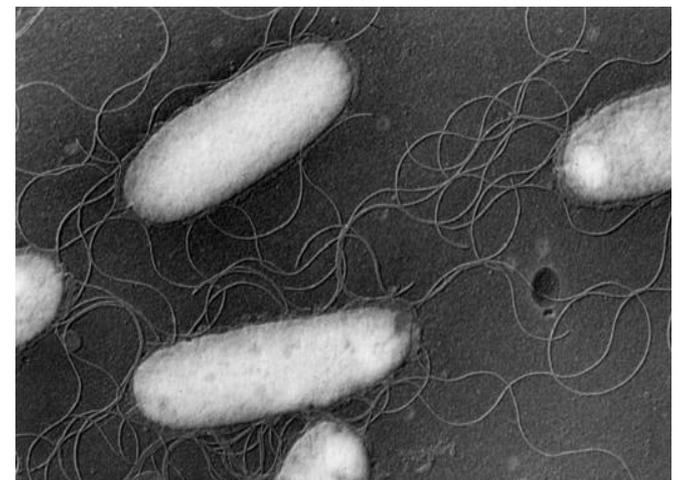
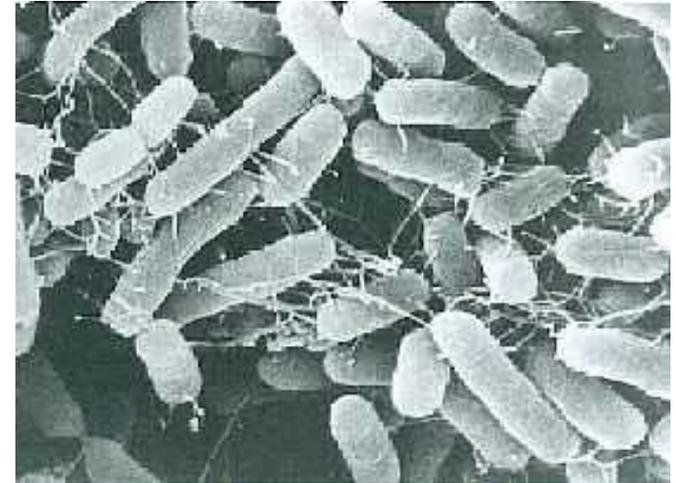
Gastroenteritis

Enteric fever

Overview/ Bacteriology

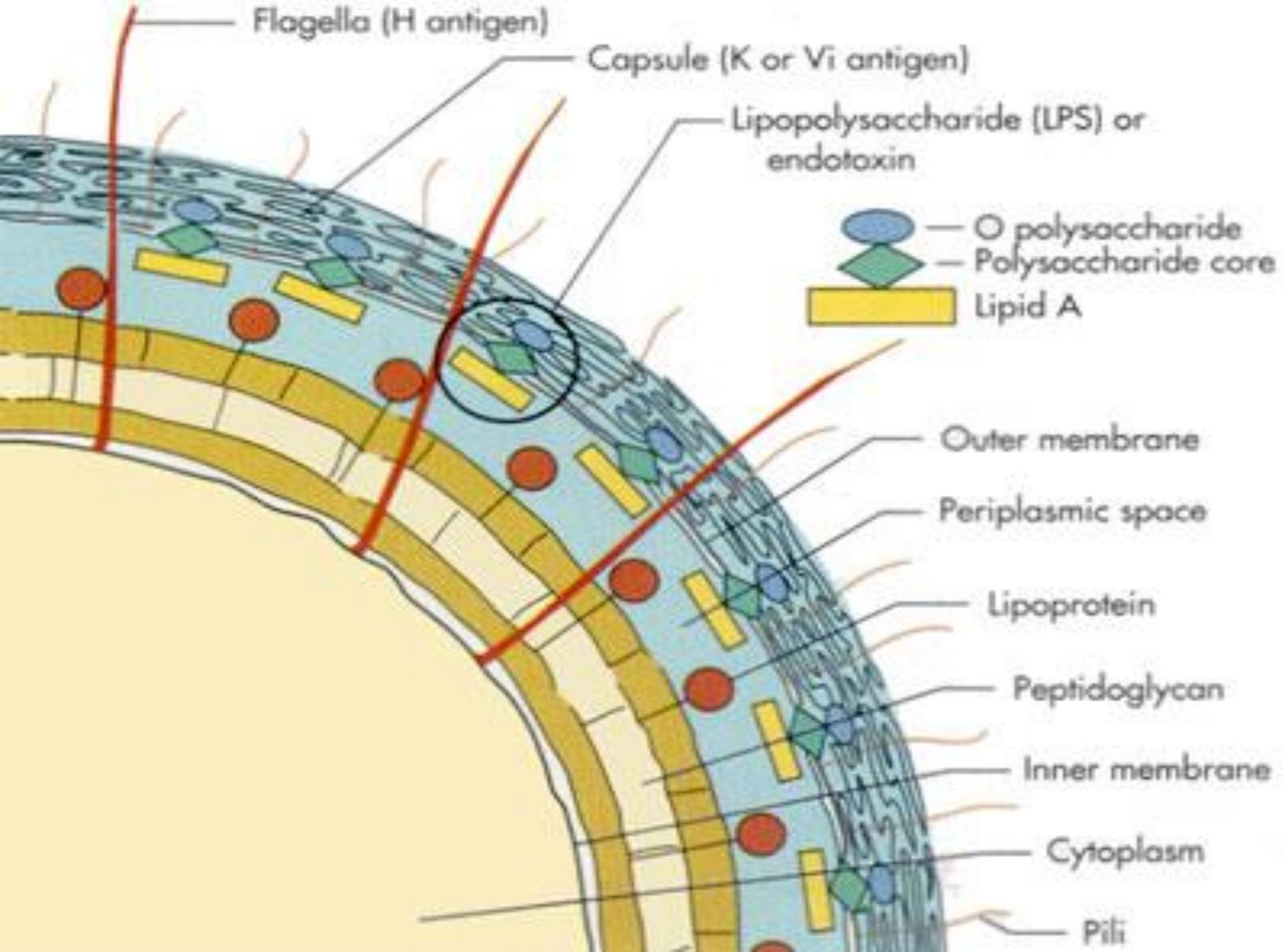


- Salmonella is a rod-shaped, non-spore-forming Gram-negative bacterium, Aerobic and facultative anaerobe
- Predominantly Motile by peritrichous flagella (H antigen).
- Non capsulated except for S. Typhi
- biochemical characteristics;
 - Glucose-fermenting
 - Non lactose fermenters
 - Non sucrose fermenters



Antigenic Structure

- Kauffmann-White antigenic scheme
- For classification of salmonella
- Done by agglutination reactions with specific antisera against *Salmonella* antigens
- Scientists determine the serotype based on the distinct combination of O and H antigens.
 - O antigens
 - O polysaccharide unit in LPS. It is the variable part where the antibody binds to. In *s. typhi* only
 - H antigens
 - flagellar antigens (protein) and may occur in one of two phase variations. For *s. typhi* and *para typhi*
- Vi or K antigen
 - a capsular polysaccharide produced by some virulent members of *S. Typhi* , protect from phagocytosis



History of Salmonella

- Alexander the Great died mysteriously in 323 B.C.
- During the Victorian era, an estimated 50,000 cases per year occurred in England.
- Typhoid Epidemic in the Spanish-American War (1898)



Epidemiology

Enteric fever (*S. Typhi* and *paratyphi*)

- Only in humans
- person-to-person spread
 - contamination with human faeces
 - usual vehicle is contaminated water, milk and food
 - occasionally, contaminated food (usually handled by an individual who harbours *S. typhi*)
 - and sometimes also by flying insects feeding on faeces.
- asymptomatic carrier (Typhoid mary)

Infectious dose

- typically about 1,000,000 bacteria
- much lower if the stomach pH is raised
- much lower if the vehicle for infection is chocolate
 - protects the bacteria in their passage through the stomach
 - an infectious dose of about 100 bacteria

Epidemiology

carrier states

- carrier state may last from many weeks to years with faecal shedding
- chronic carrier, Asymptomatic carrier
 - ~3% of persons infected with *S. typhi*
- Typhoid Mary” Mallone. She was the first person in the United States identified as an asymptomatic carrier of the pathogen associated with typhoid fever. She was presumed to have infected some 53 people over the course of her career as a cook

“Typhoid Mary” Mallone



“TYPHOID MARY” MOST HARMLESS

The Extraordinary Predicament of Mary Mallon, a Prisoner on New York's Quarantine Hospital Isle

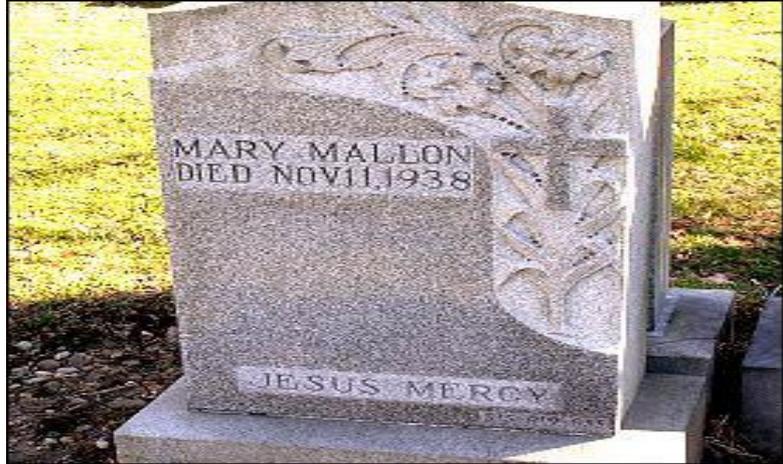
It is evident that Mary Mallon is a prisoner for the... and yet she has committed no crime, she never been... guilty of any offense or crime, and she never... been a prisoner in any way, for she has been... imprisoned by the State.

By Dr. Wm. H. Parks, New York Board of Health.

The Extraordinary and Disease

The Official Report of Dr. Wm. H. Parks, New York Board of Health.

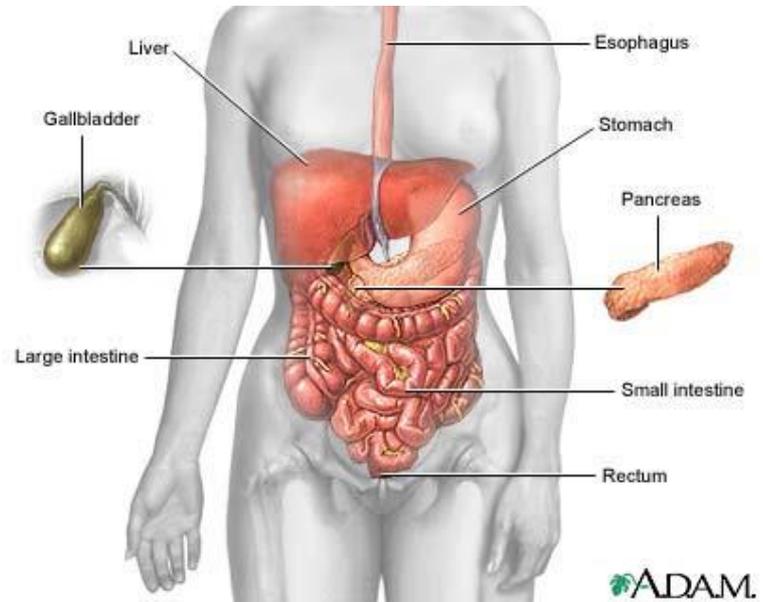
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AUTHOR

Infection Enteric Fever

- **INCUBATION PHASE** It ranges from 10-14 days. When reaches the bacilli in the abdomen most of them will be destroyed, but some of them are revived and attaches to the epithelial cells of villi. These cells then enters to the mesentric lymph nodes and multiplication occurs. After multiplication the bacteria enters to the blood stream and causes primary infection.



Pathogenesis

Enteric Fever

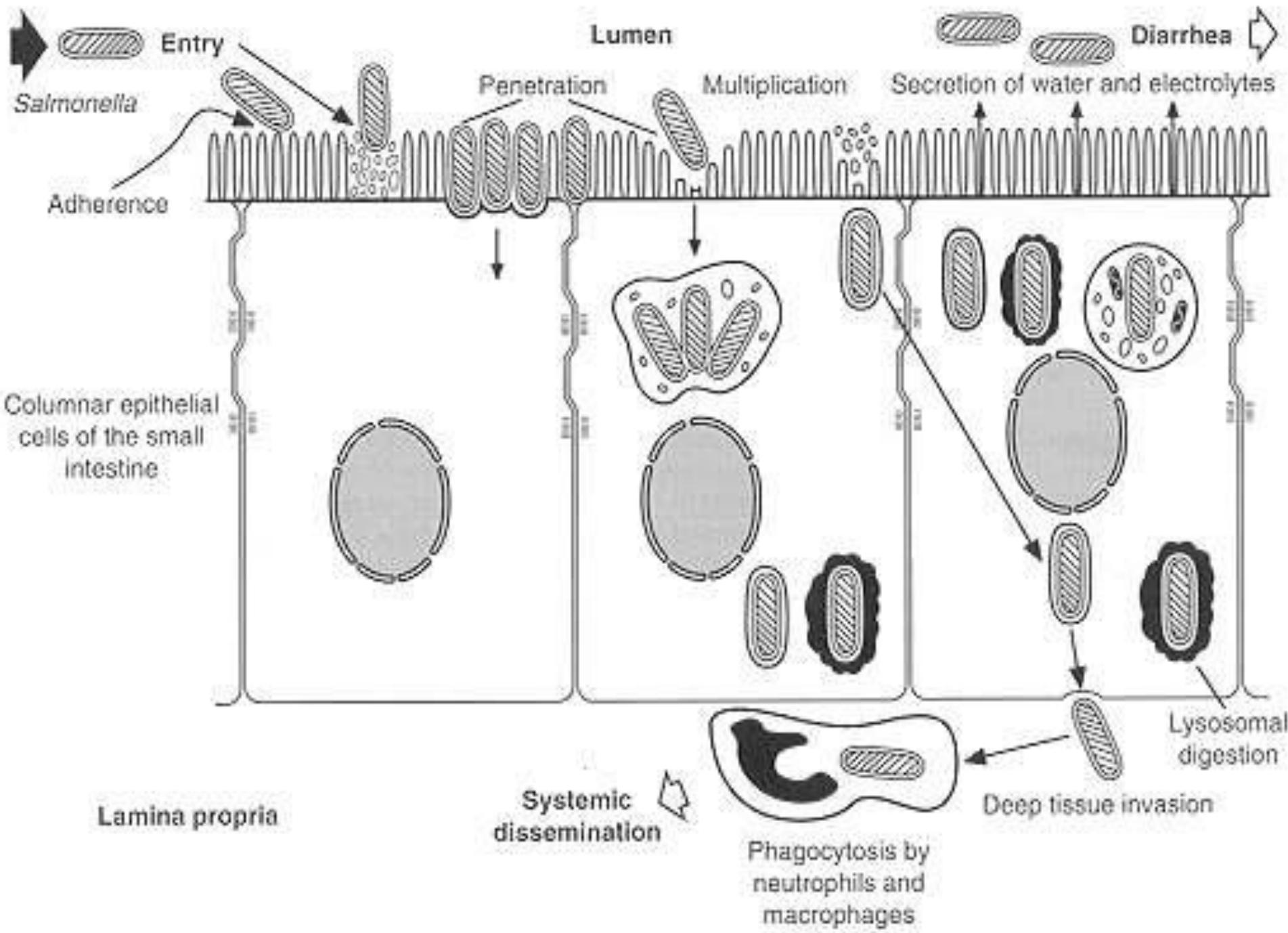
- Septicaemic phase;
 - Bacteria invade mucosa or Peyer's patches of small intestine,
 - where they survive and multiply within macrophages (facultative intracellular),
 - they pass into mesenteric lymph nodes
 - then enter the blood stream via the thoracic duct

During this stage the bacilli are entered to the gall bladder, spleen, liver bone marrow, lungs and kidney and the further multiplication occurs

Pathogenesis

Enteric fever

- **LOCALIZATION PHASE** Some of the fully grown bacilli will be localized in the organs like liver, gall bladder, spleen etc and producing the toxins. So that the tissue becomes inflamed, undergoes necrosis and results in typhoid ulcer. Later this ulcer leads to hemorrhage and perforation.



Enteric fever (typhoid fever) Illness phase

- **EARLY ILLNESS** Once signs and symptoms do appear, you're likely to experience: Fever that starts low and increases daily, possibly reaching as high as 104.9 F (40.5 C) Headache Weakness and fatigue. Muscle aches Sweating Dry cough Loss of appetite and weight loss Abdominal pain Diarrhea or constipation Rash Extremely swollen abdomen
- **LATER ILLNESS** If you don't receive treatment, you may: Become delirious Lie motionless and exhausted with your eyes half-closed in what's known as the typhoid state
- 3 weeks disease

Typhoid fever

- A number of complications can occur:
 - Intestinal hemorrhage
 - Intestinal perforation in the distal ileum this is a very serious complication and is frequently fatal.
 - Encephalitis
 - Neuropsychiatric symptoms (described as "delirium"),
 - Metastatic abscesses ,cholecystitis , endocarditis and osteitis:
- . Dehydration causes the patient to be delirious (typhoid state).
- If untreated the mortality rate because of dehydration and other complications may reach 30% in the 3rd week

Clinical Features

Enteric Fever (paratyphoid A)

- incubation period 10 to 14 days Paratyphoid fever resembles Typhoid Fever but presents with milder symptoms and a shorter course
- Paratyphi A illness
 - myalgia and headache
 - fever
 - splenomegaly
 - leukopenia
 - abdominal pain
 - Rose spots (macular rash on abdomen) in 30%
 - constipation
- 1% fatal in paratyphi A

Maculopapular rash



S. Paratyphi

S. Paratyphi B.

- There is diarrhea and vomiting and the entire intestinal tract can be inflamed especially in type B infections.

S. Paratyphi C causes:

- Mainly cause septicemia
- Complications include abscesses, arthritis & inflammation of the gall bladder.

Laboratory Diagnosis

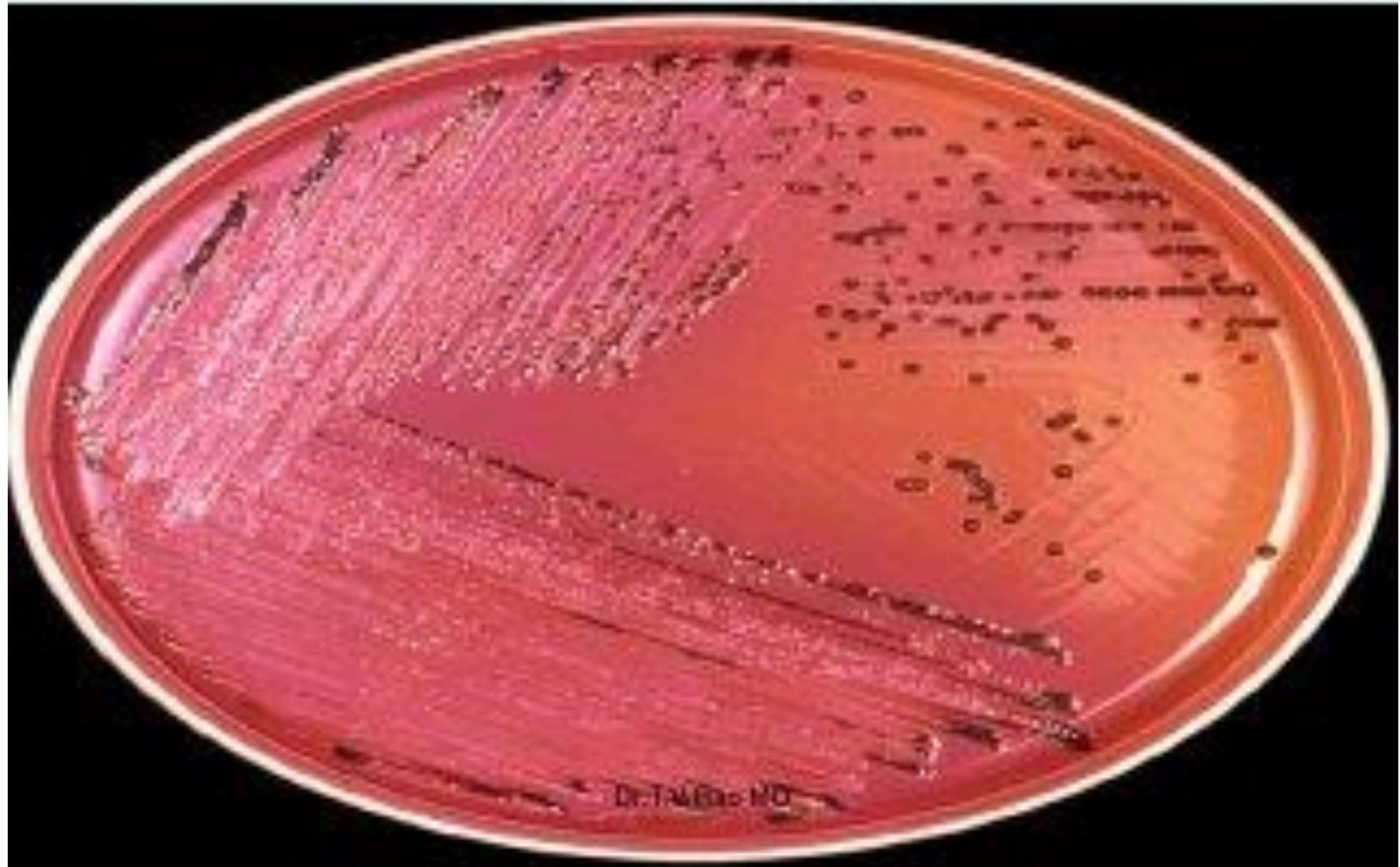
- Blood culture The blood should be collected before starting the treatment. Blood culture is positive in 80-90% of patients in first week. Culture result will get negative after 3 hours of administration of chloramphenicol.
- Appears as a non-lactose fermenter
 - Put the sample in nutrient broth for multiplication
 - Then Subculture in MacConkey agar produce circular convex colonies
 - similar selective agar are
 1. WILSON&BLAIR BISMUTH SULPHITE MEDIUM It is a selective medium for salmonella. The colonies will be black in color with metallic surface appearance.
 2. XLD AGAR MEDIUM Red color colonies will be formed with black centers.
 3. DEOXYCHOLATE CITRATE AGAR MEDIUM It is also a selective medium for salmonella. In this colorless colonies will be produced after 24hrs of incubation
 - 90% positive in 1st week to 25% in 4th week

Salmonella on Mac Conkey's agar



Dr. T.V. Rao MD

Salmonella on XLD agar



Laboratory Diagnosis

- Biochemical tests and serological tests must be done in parallel
 - Some other bacteria, e.g. Citrobacter, may have similar serological profiles

Widal Test

- This tests for O & H antibodies in the patients serum and comes in handy when culturing facilities are not available in developing countries (unreliable).
 - Positive only by the beginning of the second week onwards.
 - Should have 2 positive
 - May have false positive in immunization
 - Titer O>1/80, H>1/160

Phage typing done for epidemiological purposes

- E.g. to find source of outbreak

LAB diagnosis

- Hematological investigations The leucocytes, lymphocyte and monocyte counts will be elevated.
- Clot culture In this test 5ml of blood is allowed to clot. The clot is added to a bottle of bile broth containing streptokinase. Lysis of the clot and release of the clot will occur.
- Stool culture will be positive during second week of illness. The stool sample is cultured in tetrathionate broth and incubated for 24 hrs, the growth of bacilli can be observed.
- Urine culture Will be positive in second and third week of infection
- Duodenal juice or bile culture Performing to identify whether the bacilli are present over intestine or liver.

Biochemical Reactions

- They grow On MacConkey's or Deoxycholate-citrate agar (DCA) medium, they produce pale yellow colonies being non lactose fermenters.
- Salmonella growing on XLD agar; Xylose Lysine Deoxycholate agar is a selective growth medium used in the isolation of Salmonella (black dots) and Shigella species from clinical samples and from food
- S. Typhi ferment glucose, mannitol and sorbitol to produce acid or acid and gas
- Some S. paratyphi ferments these with production of acid and gas
- To differentiate between S paratyphi A and B;
 - A is H₂S -& citrate –
 - B is H₂S + & Citrate +
- Indole – (in tryptophane broth)
- Methyl red (MR)+ (change to red)
- Urea – (no ureas production)
- S paratyphi C, S. typhomurium and enteritides are similar to S. paratyphi B. To differentiate use serological testing (slide agglutination test)
- VP test -

Treatment of enteric fever

- Third generation cephalosporins or quinolones is the current treatment
- Severe typhoid fever (altered consciousness, septic shock): dexamethasone treatment
- Chronic carriers: 6 weeks of treatment with either oral amoxicillin, ciprofloxacin, norfloxacin
- Surgical intervention to remove damaged cells
- When untreated, typhoid fever persists for three weeks to a month. Death occurs in 10% to 30% of untreated cases

Prevention

- Remove source
 - Salmonella free life-stock
 - Vaccinate chicks
- Interrupt transmission
 - Good food hygiene
 - Cook food properly
 - Keep raw and cooked foods apart
 - Public Health: clean water
- Strengthen host

- **WASH YOUR HANDS WITH SOAP AND WATER!!!**

Salmonella vaccines

- Vaccination of travellers and who at risk
- Three licensed vaccines
 - Traditional heat-killed; TAB vaccine, a combined vaccine used to produce immunity against the diseases typhoid, paratyphoid A, and paratyphoid B.
 - Vi subunit vaccine; is made from the purified Vi capsular polysaccharide from the Salmonella Typhi strain; it is a subunit, it lasts for 2-3 years (single dose)
 - live oral vaccine; Typhoral oral vaccine, live-attenuated Ty2 strain of S. Typhi in which multiple genes, including the genes responsible for the production of Vi, have been mutated chemically so as to render it harmless but nevertheless immunogenic.

Salmonella septicemias

- S.cholera suis
- Deep abscess, Endocarditis
- Isolation from Blood and Pus.
- Chloramphenicol highly effective





Brucellosis

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Hemo. Module, 3rd year medical
students



○ Other names

- Undulant fever, Malta fever, Mediterranean fever (humans)
- Contagious abortion, Bang's disease, epizootic abortion (animals)

Brucellosis

- Causative organisms and their hosts
 - *Brucella abortus* (abortion in cattle)
 - *B. melitensis* (goats)*
 - *B. suis* (swine) *
 - *B. canis* (dogs)
 - *B. ovis* (sheep)
- * More virulent for humans

Brucellosis: History

- David Bruce – English doctor
 - Discovered in 1887
 - British soldiers sick in Malta
 - Identified in goats' milk
- Pasteurization
 - Eliminates organism
 - Reduced human cases





Brucellosis: Microbiology

- Small, gram-negative cocco-bacilli
Non-motile
- Facultative intracellular organism(
in side macrophages)
- non-encapsulated



Antigenic structure

- The 3 species (melitensis, abortus and suis) share 2 antigens A and M
- Typical melitensis contain an excess of M
- Typical abortus has an excess of A
- Suis has nearly equal distribution of both
- The antigens can be detected by specific antibodies

Characteristics

- Slow growing
- Need enriched medium as liver –extract agar and glucose-serum agar
- Killed at temperature of 60 c in 10 mins,
- Vary in their ability to live in media containing dyes which is used to differentiate between them; suis inhibited by(basic fuchsin). Abortus (thionin) and melitensis (not inhibited by either.
- Biochemical reaction; H₂S is produced by abortus and some suis. Melitensis is H₂S -

Brucellosis: Transmission to Humans

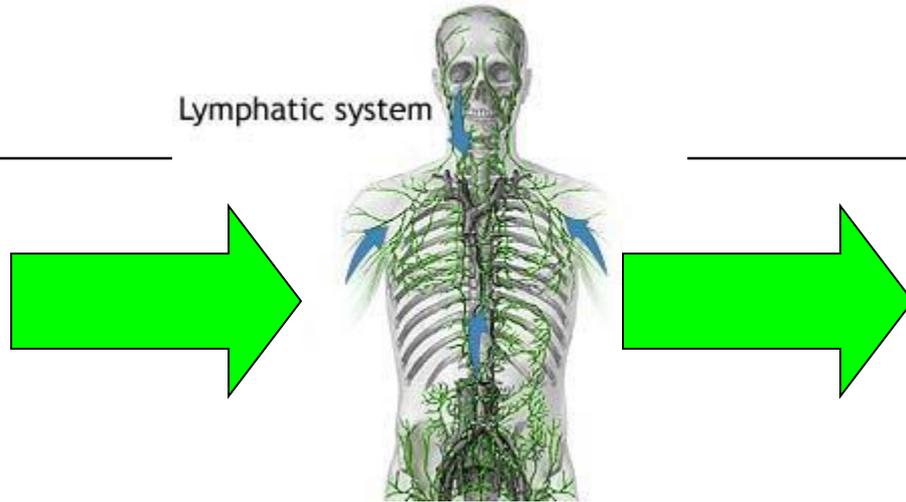
- Direct Contact
 - conjunctiva or broken skin in contact with infected tissues as animal Blood, urine, vaginal discharges, aborted fetuses, placentas
- Ingestion
 - Raw milk & unpasteurized dairy products
 - Rarely undercooked meat
- Inhalation of infectious aerosols
 - Pens, stables, slaughter houses
 - Laboratory transmission
- Inoculation with animal vaccines
 - *B. abortus* strain 19
 - *B. melitensis* Rev-1
- No evidence of person-to-person transmission



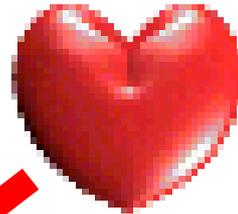
Brucellosis: Pathogenesis

- Organism enters lymphatics and replicates within regional lymph nodes reach to thoracic duct then to blood (septicemic phase)
- Survives and multiplies within phagocytic and monocyte cells
- Hematogenous dissemination results in localization, often liver, spleen, bone marrow with granulomatous lesions.
- The main way of recovery is by cell mediated immunity
- And some immunity is mediated by IGM.
- Chronic infection or relapse mediated by IGG

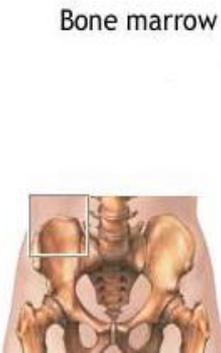
GOT BRUCELLOSIS?



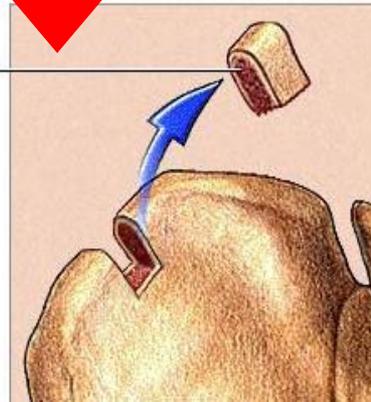
Phagocytic Cells



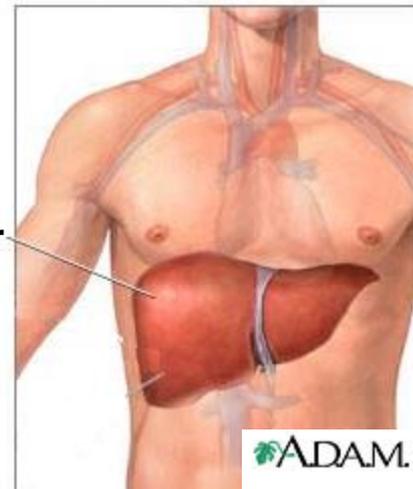
Spleen



Bone marrow



Liver



Brucellosis

Clinical Manifestations

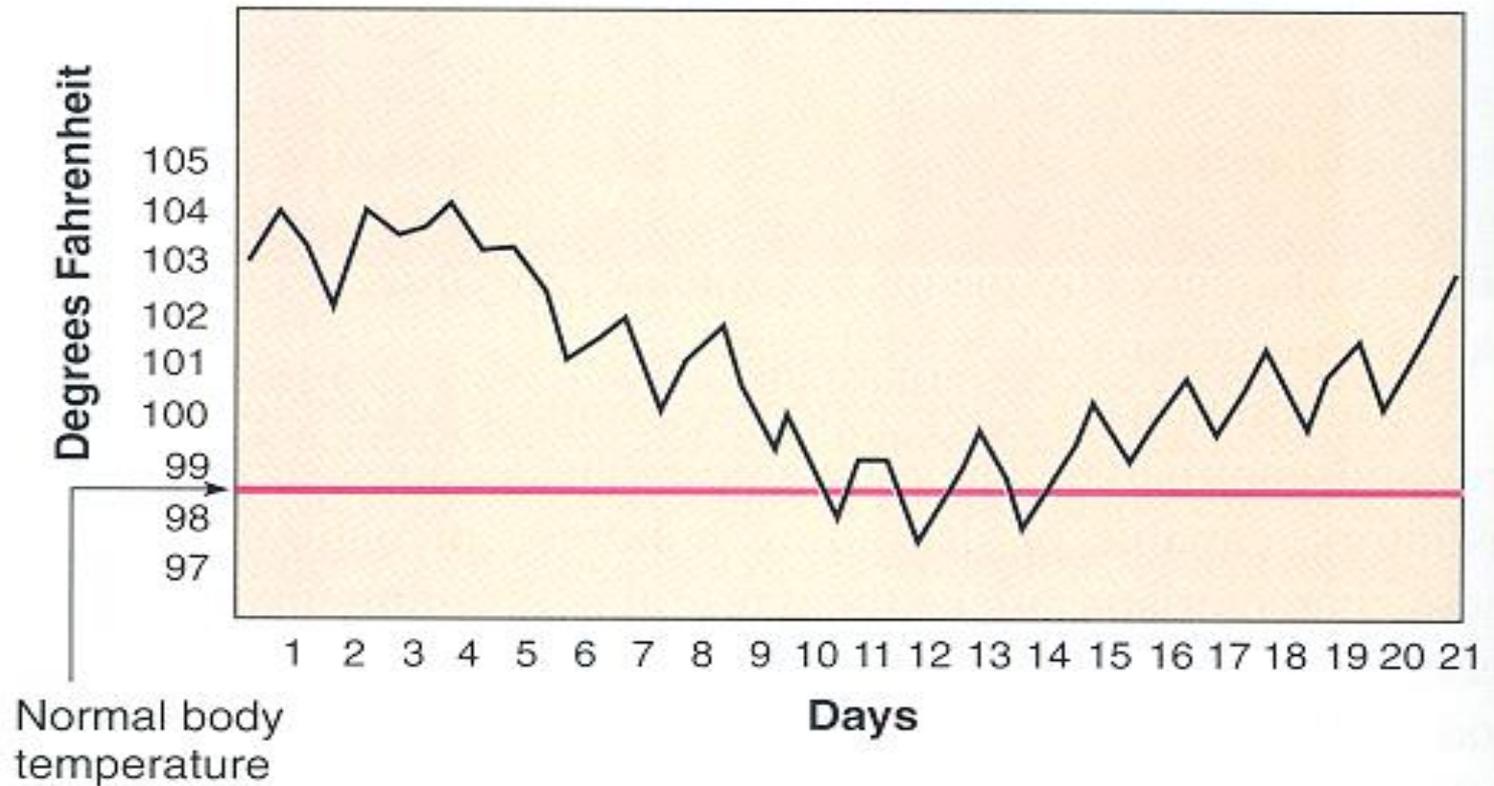
- **Incubation period of 1-3 weeks to several months**
- **Undulant fever characteristic**
 - **Intermittent or irregular fever with variable duration. The organism appear in the blood with the start of fever and even secreted in urine.**
- **Nonspecific and variable symptoms**
 - **Headache, weakness, arthralgia, depression, weight loss, fatigue, liver dysfunction**
- **Focal (or localized) disease**
 - **Osteoarticular, hepatobiliary disease**
 - **Orchitis**
 - **Endocarditis (most common cause of death), meningoencephalitis**

Chronic infection

- Chronic infection
 - Relapse common within 3-6 mon.-
 - The disease's sequelae are highly variable and may include granulomatous hepatitis, arthritis, spondylitis,
 - anaemia, leukopenia, thrombocytopenia
 - uveitis, optic neuritis,
 - various neurological disorders collectively known as neuro-brucellosis

Brucellosis

Classic Temperature Cycle



KP Talaro, A Talaro; *Foundations in Microbiology*, 4th Ed. (2001)

Brucellosis: Diagnostics

- Isolation of organism
 - Blood, urine and serum
- Blood culture (during febrile attack) on tryptose broth. Prolonged incubation (up to 6 weeks) may be required
- Serum
 - Direct agglutination test. Antibodies appear within 7 to 14 days after infection and identification of *B. abortus*, *B. melitensis*, and *B. suis* is achieved through this test
 - Fourfold or greater rise in titer
 - Samples 2 weeks apart
 - Indirect agglutination test (coomb's test) for the negative tubes
 - Complement fixation test
 - ELISA

Diagnosis

- Histological evidence of granulomatous hepatitis (hepatic biopsy)
- Radiological alterations in infected vertebrae: the Pedro Pons sign (preferential erosion of antero-superior corner of lumbar vertebrae) and marked osteophytosis are suspicious of brucellic spondylitis

Pedro pons sign



Brucellosis: Treatment

- Combination therapy has the best efficacy
 - Doxycycline for six weeks in combination with streptomycin for 2-3 weeks or rifampin for 6 weeks
- CNS and endocarditis
 - Doxycycline in combination with 2 or more other drugs
 - Treat for many (6-9?) months
 - Endocarditis may also require surgical replacement of valves