Salmonella

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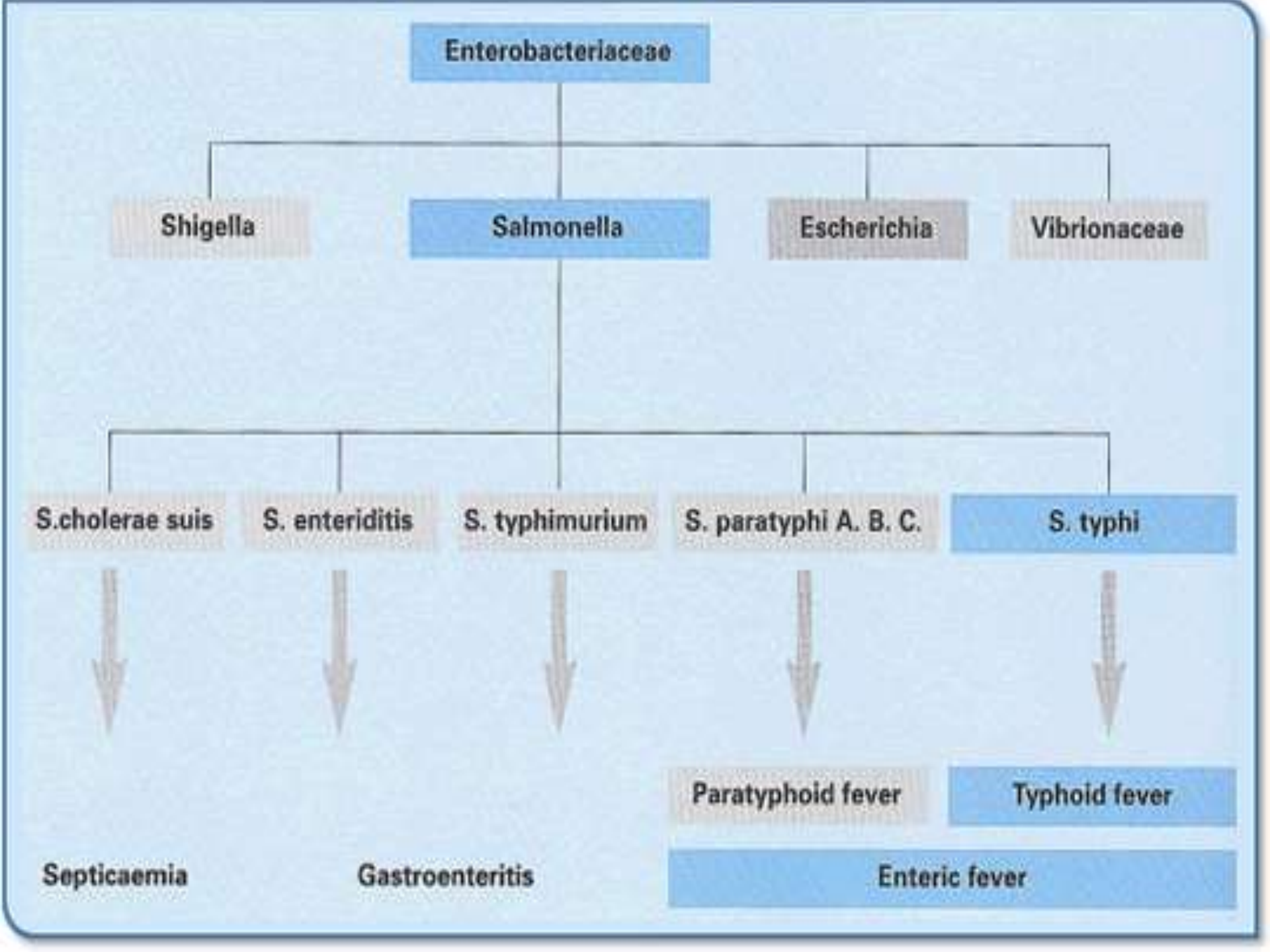
Classification

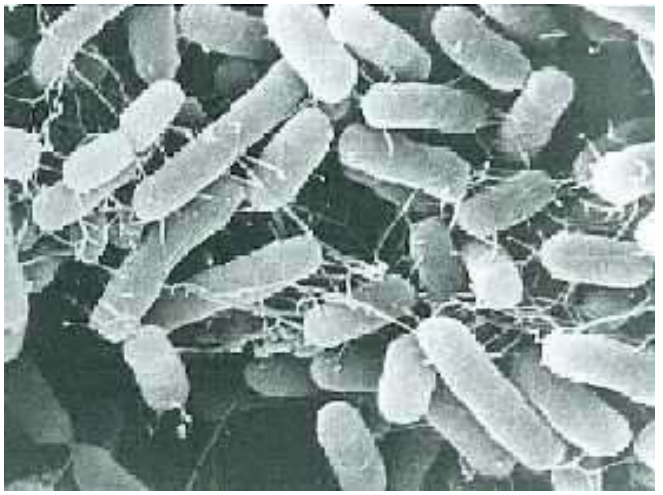
- **Family**
  - **Lactose non fermenters, Enterobacteria?**
- **Genus**
  - ***Salmonella*, named after United States Department of Agriculture vet. Daniel E Salmon.**
- **Species**
  - *Salmonella enterica* , 5 subspecies, 2500 serovars
  - *Salmonella bongori*

- 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)

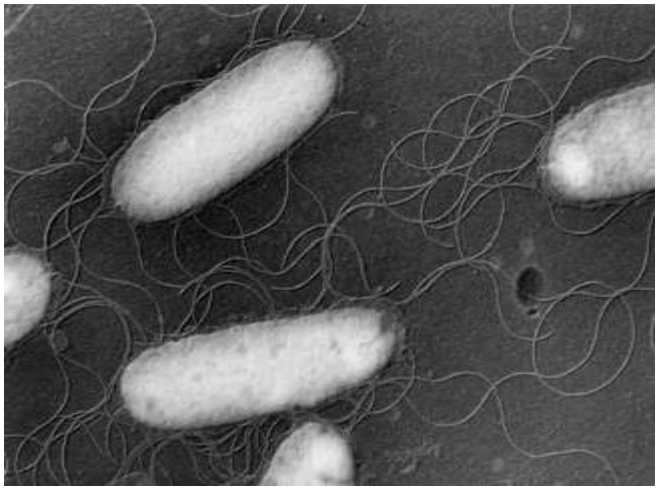

Enterobacteriaceae
Shigella
Salmonella
Escherichia
Vibrionaceae
S.cholerae suis
S. enteriditis
S. typhimurium
S. paratyphi A. B. C.
S. typhi
Paratyphoid fever
Typhoid fever
Septicaemia
Gastroenteritis
Enteric fever

Overview/ Bacteriology

- Salmonella is a rod-shaped, non-spore-forming Gram-negative bacterium, Aerobic and facultative anaerobe



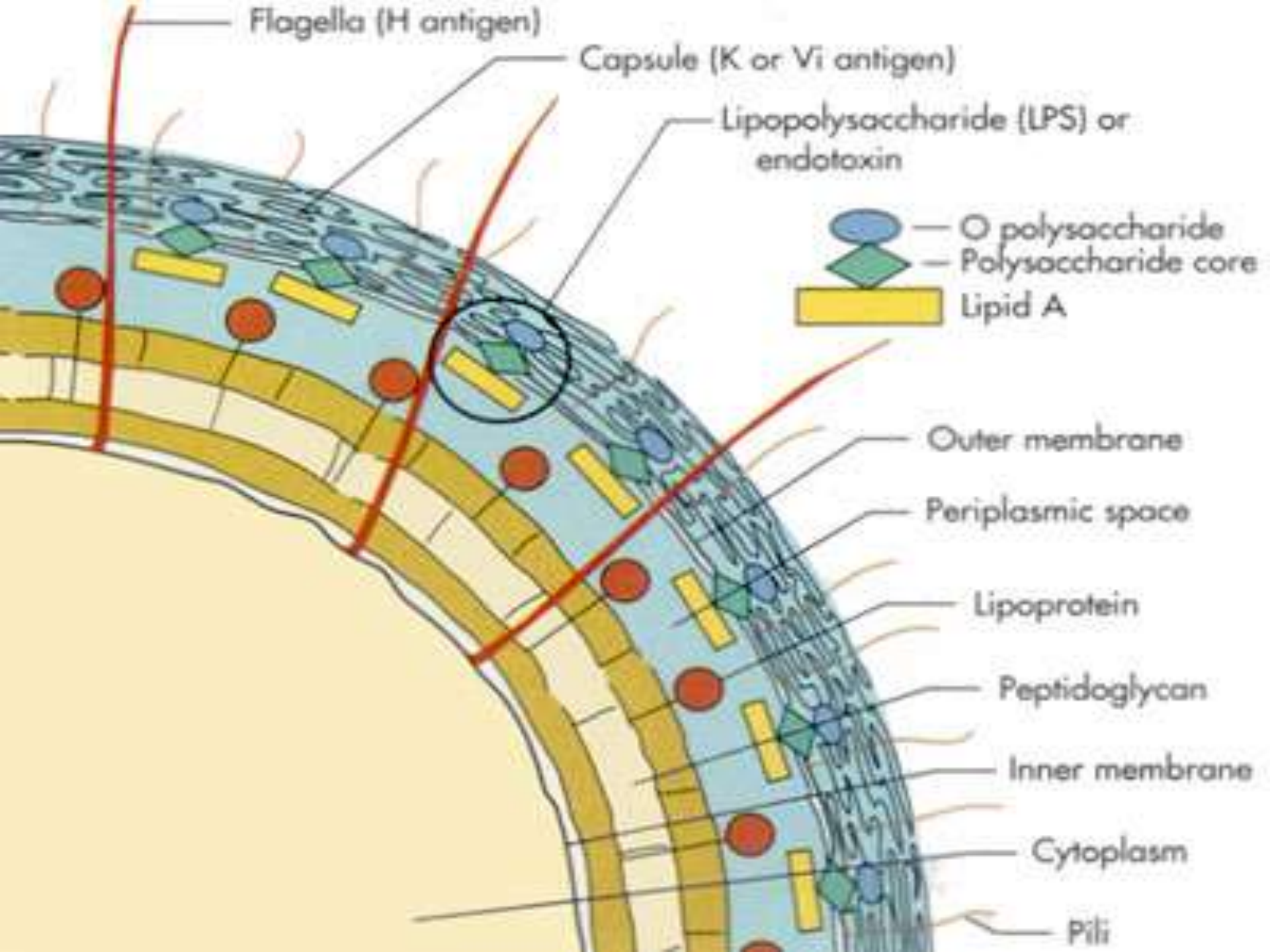
- Predominantly Motile by peritrichous flagella (H antigen).



- 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 (more antigenic)
      - 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 and pratyphi , protect from phagocytosis


Flagella (H antigen)
Capsule (K or Vi antigen)
Lipopolysaccharide (LPS) or endotoxin
O polysaccharide
Polysaccharide core
Lipid A
Outer membrane
Periplasmic space
Lipoprotein
Peptidoglycan
Inner membrane
Cytoplasm
Pili

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)

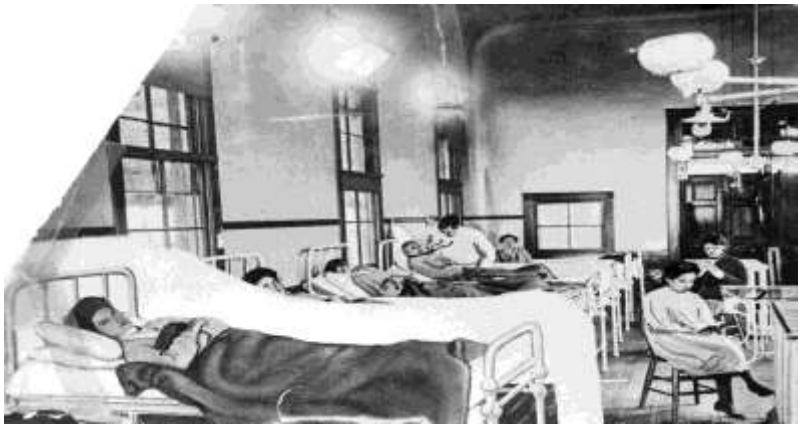
Infectious dose

- 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

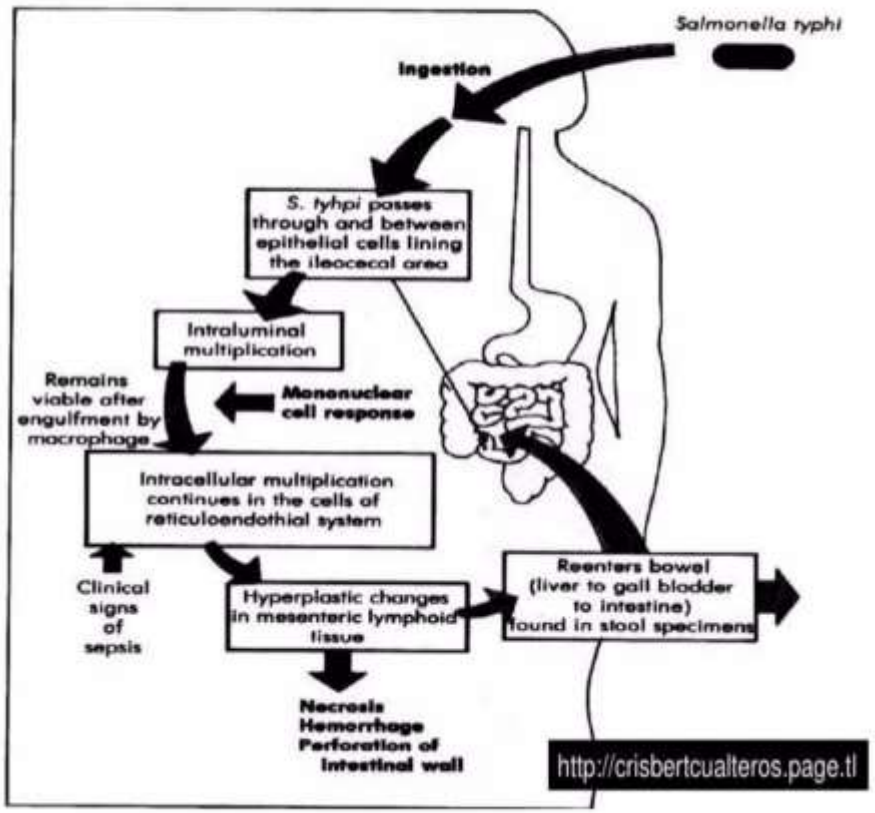
“Typhoid Mary” Mallone

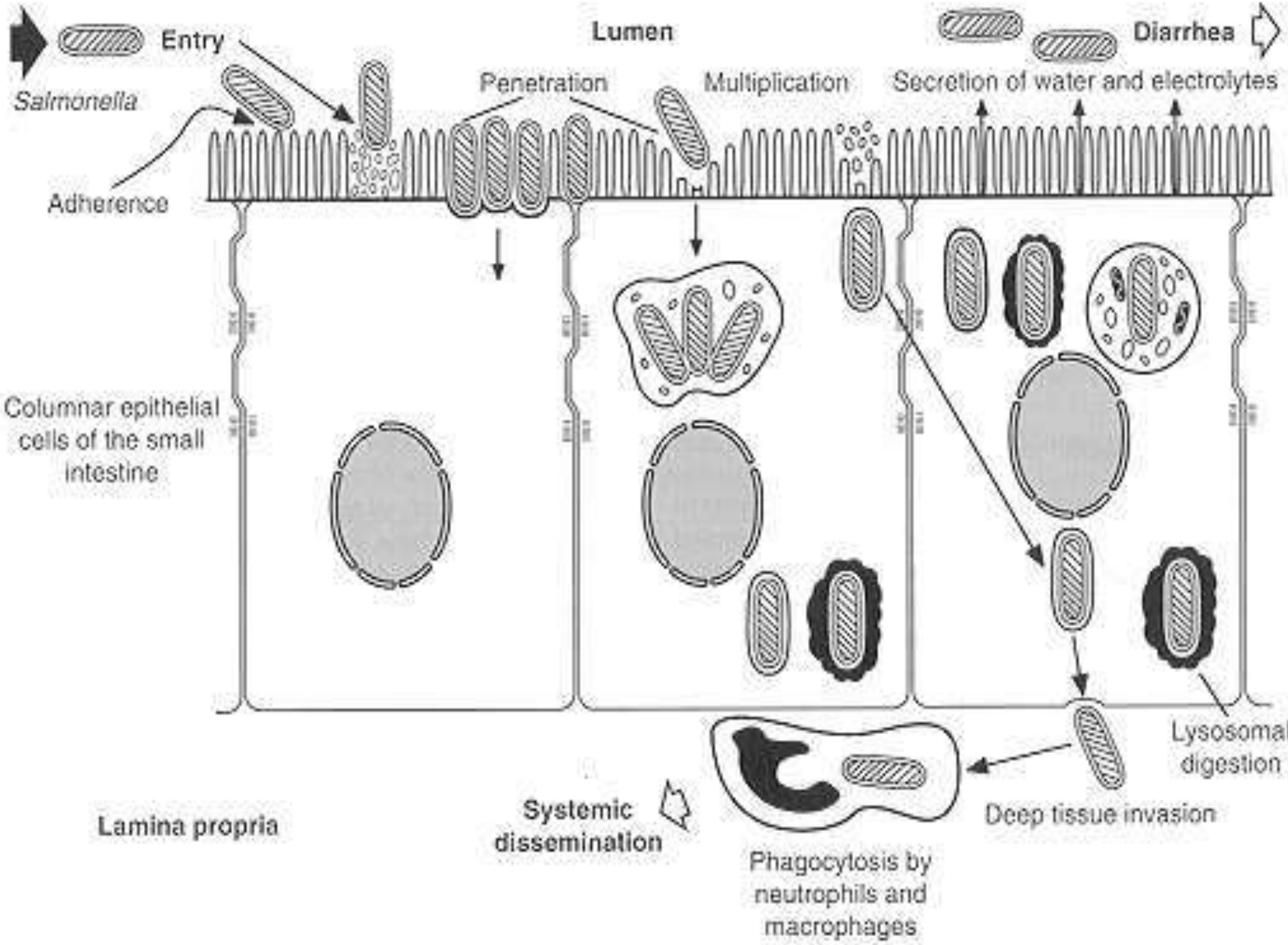


ENTERIC FEVER

- Caused by salmonella typhi and salmonella para typhi A, B and C
- Source of infection is ingestion of contaminated food or water
- Incubation period 7-14 days
- In the gut, the organisms get attached to the epithelial cells of the intestinal villi and penetrate to the lamina propria and submucosa
- Phagocytosed by neutrophils and macrophages, but resist the intracellular killing and multiply within the cells
- They enter the mesenteric lymph nodes, multiply there and reach the blood stream
- Internal organs like liver, gall bladder, spleen, bone marrow, lungs, lymph nodes and kidney are affected
- A massive bacteremia occurs with the onset of clinical disease

Pathogenesis




Entry
Salmonella
Lumen
Diarrhea
Penetration
Multiplication
Secretion of water and electrolytes
Adherence
Columnar epithelial cells of the small intestine
Lysosomal digestion
Lamina propria
Systemic dissemination
Deep tissue invasion
Phagocytosis by neutrophils and macrophages

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

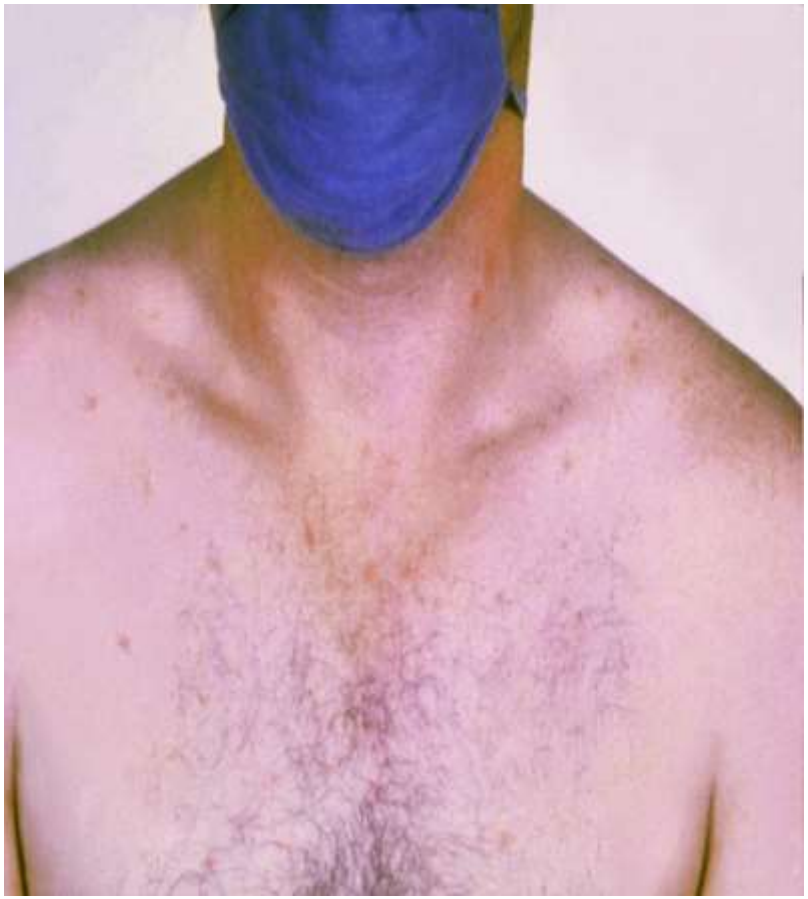
- A number of complications can occur:
  - Intestinal hemorrhage
  - Intestinal perforation in the distal ileum this is a very serious complication and is frequently fatal.
  - Encephalitis
  - Neuropsychiatric symptoms (described as "delirium"),
  - Metastatic abscesses ,cholecystitis , endocarditis 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

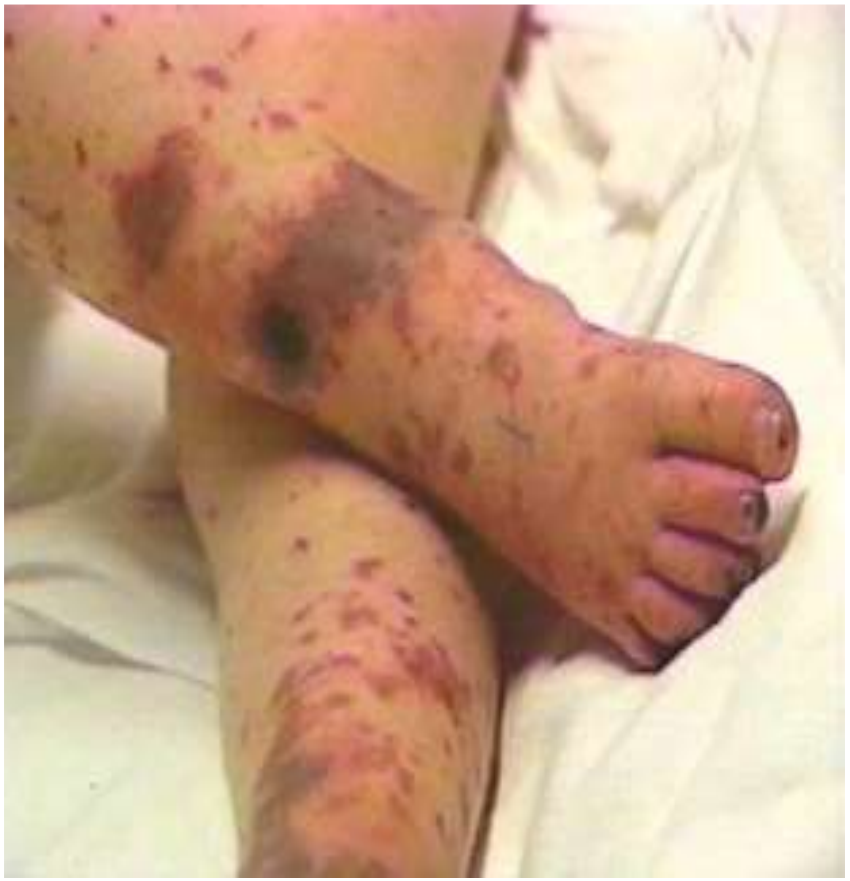
Clinical Features

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 paretyphi 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.

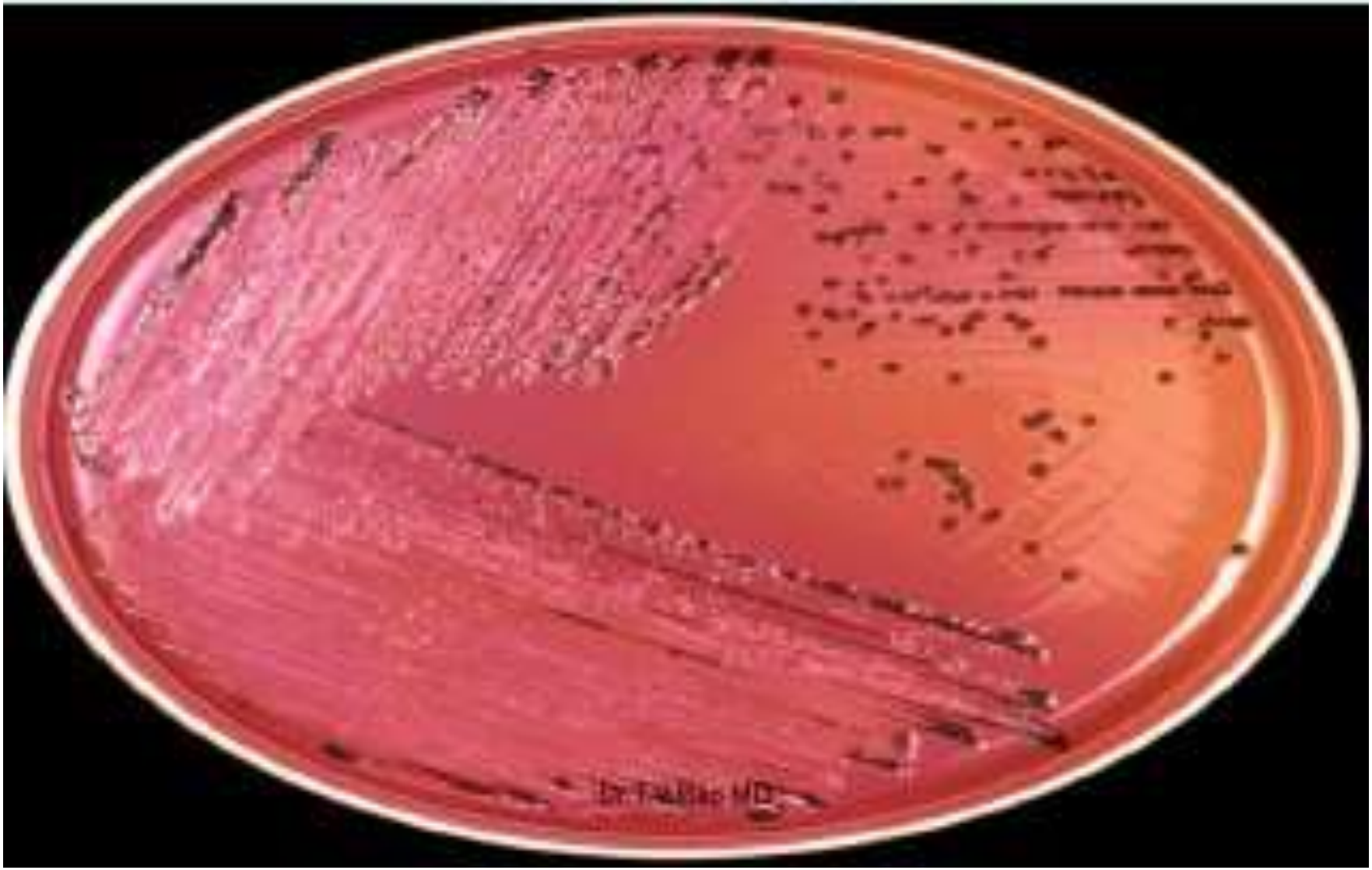
Laboratory Diagnosis

- Blood culture (common in early infection) 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
  - They grow On MacConkey's or Deoxycholate-citrate agar (DCA) medium, they produce pale yellow to colourless 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
  - similar selective agar is

  1. WILSON&BLAIR BISMUTH SULPHITE MEDIUM It is a selective medium for salmonella. The colonies will be black in color with metallic surface appearance.

Salmonella on Mac Conkey's agar

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 weak 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

Biochemical Reactions

- **S. Typhi ferment glucose, mannitol and sorbotol to produce acid**
- **Some S. paratyphi ferments these with production of acid and gas**
- **To differentiate;**
  - **A is H2S -& citrate –**
  - **B is H2S + & Citrate +**
  - **Typhi 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 –**
- **Catalase + oxidaze -**

Laboratory diagnosis of Enteric Fever

❖ **Biochemical Identification of Isolates-**

Tests	S. typhi	S. Paratyphi
Catalase	+ve	+ve
Oxidase	-ve	-ve
Nitrate Reduction	+ve	+ve
Glucose	+ve (Acid only)	+ve(Acid and Gas)
Mannitol	+ve (Acid only)	+ve(Acid and Gas)
Lactose	-ve	-ve

Laboratory diagnosis of Enteric Fever

- The test of choice depends on the duration of disease-

Duration of disease	Specimen	Positivity (%)
1st week	Blood culture	90
2nd week	Blood Culture Feaces culture Widal test	75 50 Low titre
3rd week	Widal test Blood culture Feaces culture	80-100 60 80

LAB diagnosis

- Hematological investigations The leucocytes, lymphocyte and monocyte counts will be elevated.
- Stool culture will be positive during second week of illness and in carriers. 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.

Treatment of enteric fever

- Third generation cephalosporins or quinolones is the current treatment
- Sever 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

ANTIBIOTIC THERAPY FOR ENTERIC FEVER IN ADULTS

INDICATION	AGENT	DOSAGE (ROUTE)	DURATION, DAYS
Empirical Treatment			
	Ceftriaxone[a]	1–2 g/d (IV)	7–14
	Azithromycin	1 g/d (PO)	5
Fully Susceptible			
	Ciprofloxacin[b] (first line)	500 mg bid (PO) or 400 mg q12h (IV)	5–7
	Amoxicillin (second line)	1 g tid (PO) or 2 g q6h (IV)	14
	Chloramphenicol	25 mg/kg tid (PO or IV)	14–21
	Trimethoprim-sulfamethoxazole	160/800 mg bid (PO)	14
Multidrug-Resistant			
	Ciprofloxacin	500 mg bid (PO) or 400 mg q12h (IV)	5–7
	Ceftriaxone	2–3 g/d (IV)	7–14
	Azithromycin	1 g/d (PO)[c]	5
Nalidixic Acid–Resistant			
	Ceftriaxone	1–2 g/d (IV)	7–14
	Azithromycin	1 g/d (PO)	5
	High-dose ciprofloxacin	750 mg bid (PO) or 400 mg q8h (IV)	10–14

[a]Or another third-generation cephalosporin [e.g., cefotaxime, 2 g q8h (IV), or cefixime, 400 mg bid (PO)].
[b]Or ofloxacin, 400 mg bid (PO) for 2–5 days.
[c]Or 1 g on day 1 followed by 500 mg/d PO for 6 days.

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 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 most pathogenic, abortus is the least 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(Methyl violet or basic fuchsin). Abortus inhibited by (thionin) and melitensis (not inhibited by either.
- Biochemical reaction; H2S is produced by abortus and some suis.
- Melitensis is H2S -

Brucellosis: Transmission to Humans

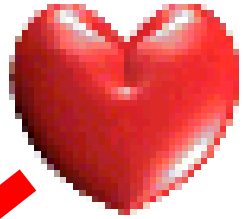
- Direct Contact
  - conjunctiva or broken skin in contact with infected tissues as animal Blood, urine, vaginal discharges, aborted fetuses, placentas
- Ingestion
  - Raw milk & unpasteurized dairy products
  - Rarely undercooked meat
- Inhalation of infectious aerosols
  - Pens, stables, slaughter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

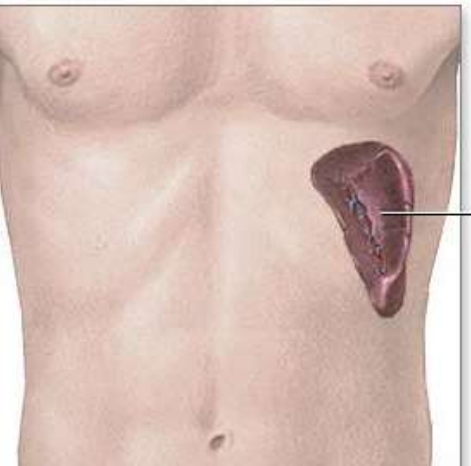
Brucellosis: Pathogenesis

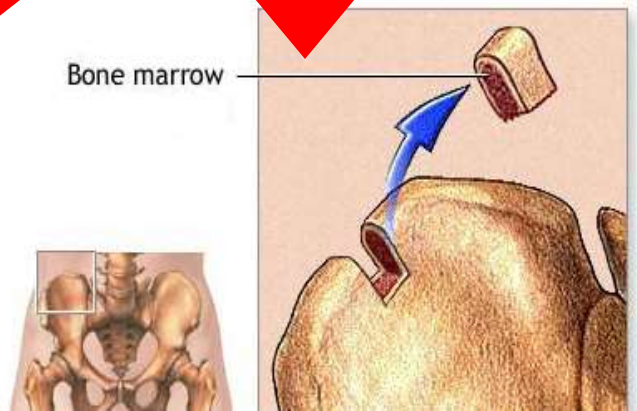
- Organism enters lymphatics and replicates within regional lymph nodes reach to thorasic duct then to blood (septicemic phase)
- Survives and multiplies within phagocytic and monocyte cells
- Hematogenous dissemination results in localization, often liver, spleen, bone marrow with granulomatous lesions.

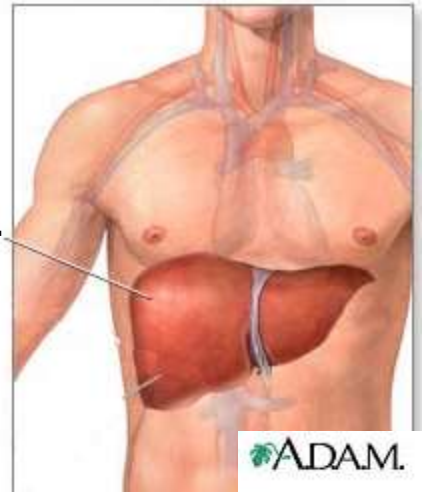
GOT BRUCELLOSIS?

Phagocytic Cells









Brucellosis

Clinical Manifestations

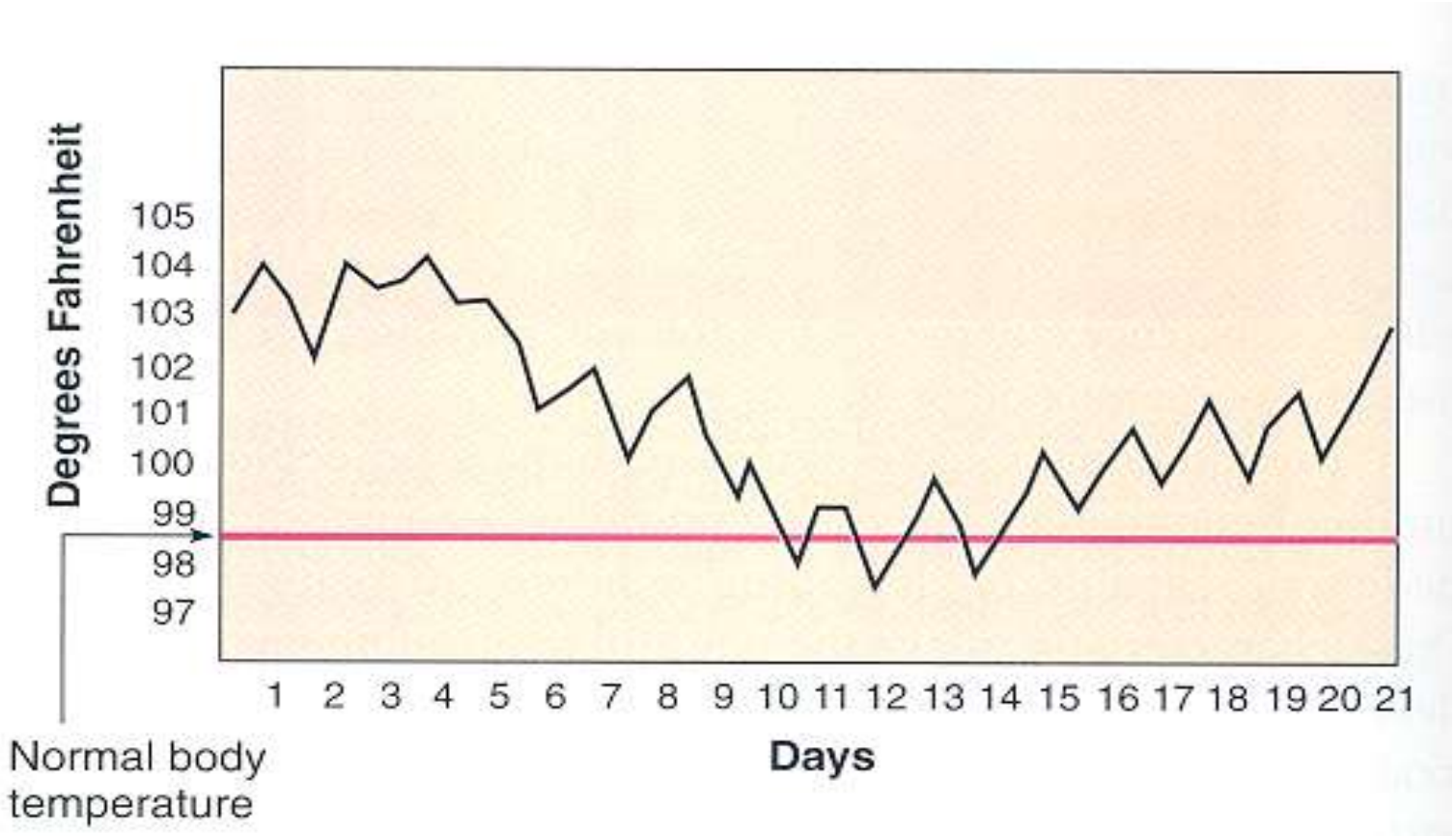
- **Incubation period of 1-3 weeks to several months**
- **Undulant fever characteristic**
  - **Intermittent or irregular fever with variable duration. The organism appear in the blood with the start of fever and even secreted in urine.**
- **Nonspecific and variable symptoms**
  - **Headache, weakness,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

Chronic infection

- Chronic infection
  - Relapse common within 3-6 mon.-
  - The disease's squeal 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)

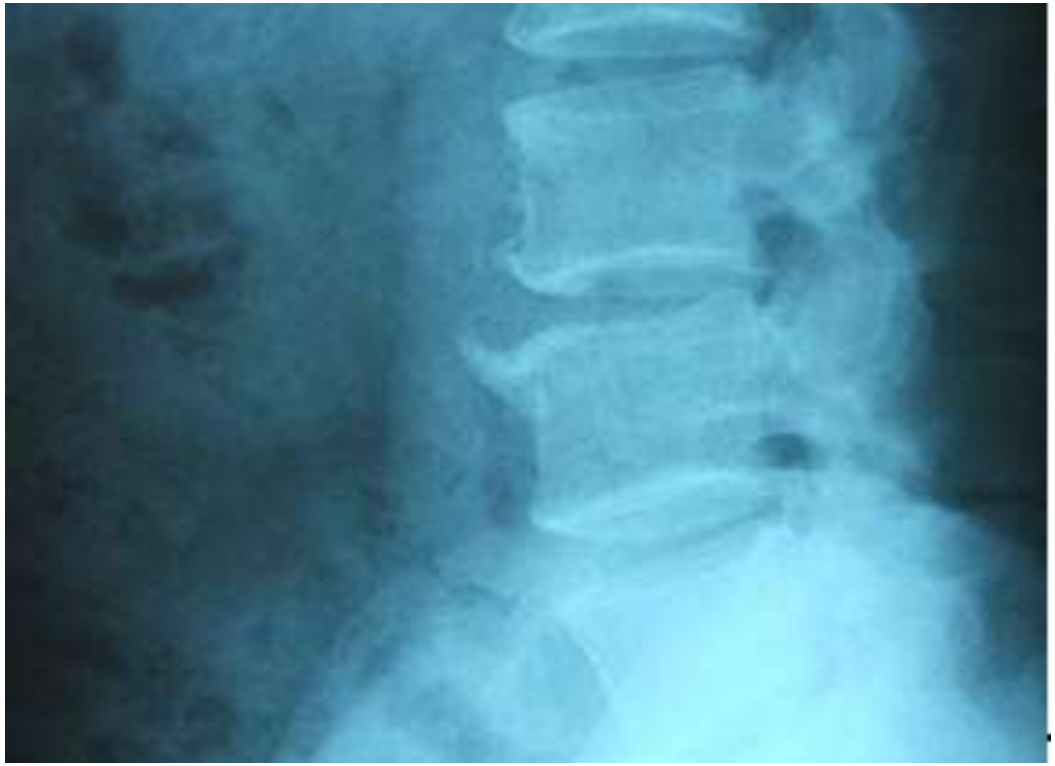
Brucellosis: Diagnostics

- Isolation of organism
  - Blood, urine and serum
- Blood culture (Common) (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
    - More than 1/160 titer is significant
    - 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