



# HLS SYSTEM

**Sub:** *Micro Biology*

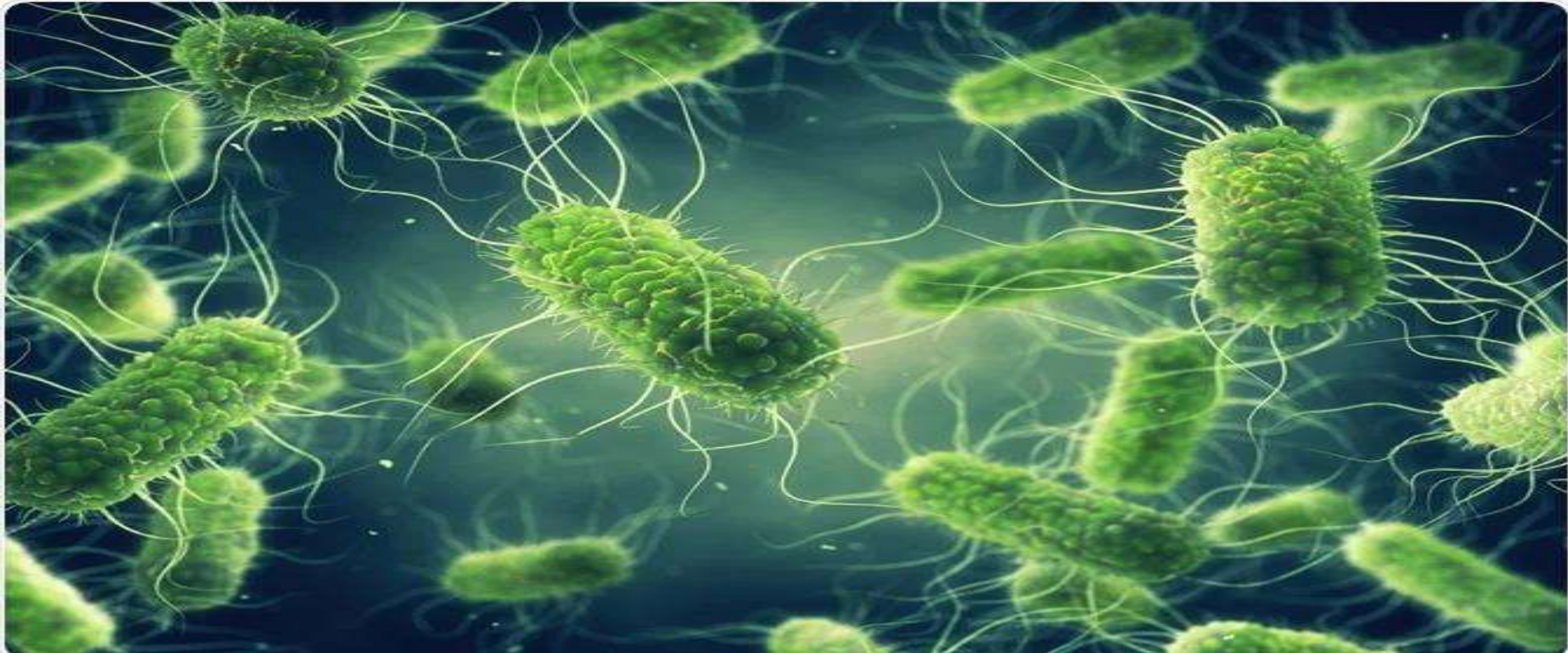
**Done by:** *Yousef Bassam AL Sabateen*

**Lec no:** *4*

**Title:** *Salmonella and Brucella*

# SALMONELLA

By Prof. Hala Tabl



# Classification

There are more than 2400 serotypes. The main species of medical importance are :

## 1- Salmonella causing enteric fever (Typhoidal salmonella): → Major Cause of Bacteremia

- *Salmonella typhi*. Causes enteric fever also called typhoid fever and its the most virulent
  - *S. paratyphi A.*
  - *S. paratyphi B.*
  - *S. paratyphi C.*
- Causes mild form of enteric fever  
typhoid fever وفي بعض الاوقات يطلق عليها اسم

## 2. Salmonella causing food poisoning (Non-typhoidal salmonella): → not imp in this system

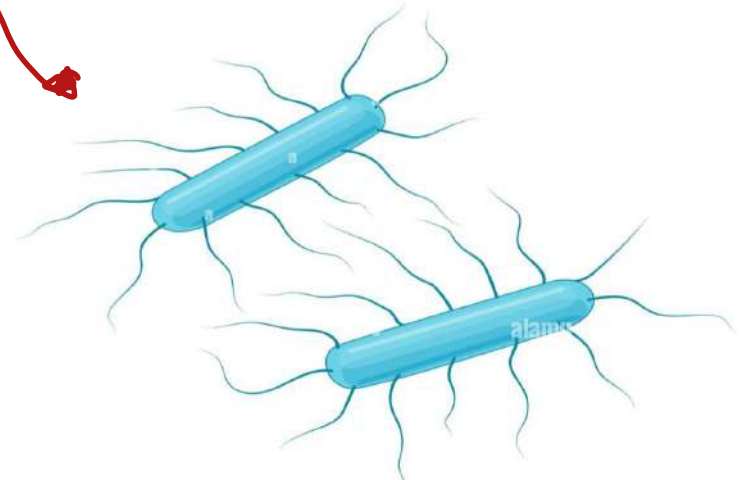
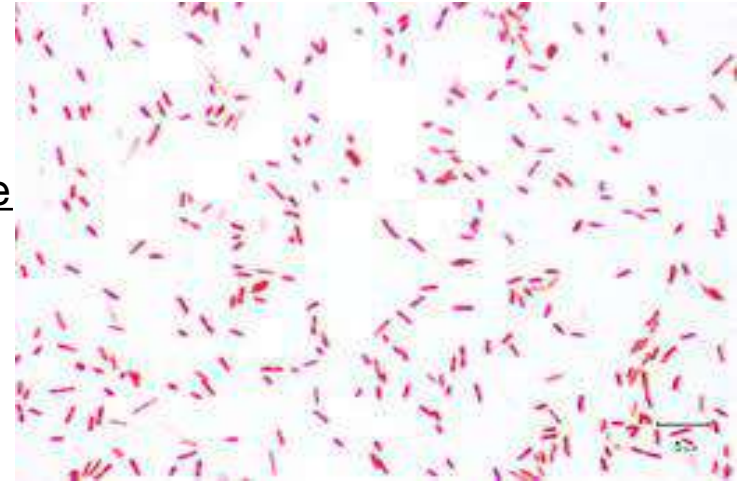
- *S. typhimurium.*
- *S. enteritidis.*

# Morphology

- Gram **negative** bacilli. Covers the whole surface
- Motile with **peritrichate flagella**.
- Non spore forming.
- **Virulent strains are capsulated.**

→ typhi

↳ only one



# Cultural characters

➤ Aerobic and facultative anaerobes. Can be cultured on any medium

➤ Optimum temperature 37°C.

➤ **MacConkey and DCA:** They produce **pale yellow colonies (non lactose fermenters).**

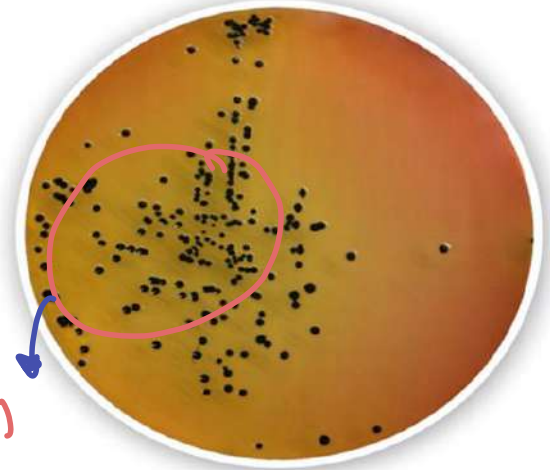
This agar is selective for gram (-) and used as a differential agar between lactose and non lactose fermenting bacteria

➤ **Salmonella-Shigella agar (SS)** is superior to

MacConKey's and DCA medium in **detecting H<sub>2</sub>S**

**producing salmonella.** Ss agar is selective and deferential  
Salmonella appears as black colonies

\* Salmonella is lactose fermenting (-) / Some strains are H<sub>2</sub>S (+)



# Biochemical Reactions

- All salmonellae ferment glucose, maltose and mannite while they are non-lactose, non-sucrose fermenters and indole negative.

Specific in every Salmonella

- *S.typhi* produce acid only and H<sub>2</sub>S positive.

- *S.paratyphi* produce acid and gas:

S.typhi and S. Paratyphi B and C are h<sub>2</sub>s +

- SPA is H<sub>2</sub>S, citrate negative.

The S.paratyphi A is h<sub>2</sub>s -

- SPB is H<sub>2</sub>S, citrate positive.

B and C are the same.

# Antigenic structures

**1- Somatic (O) antigen:-** Immune response is based on it

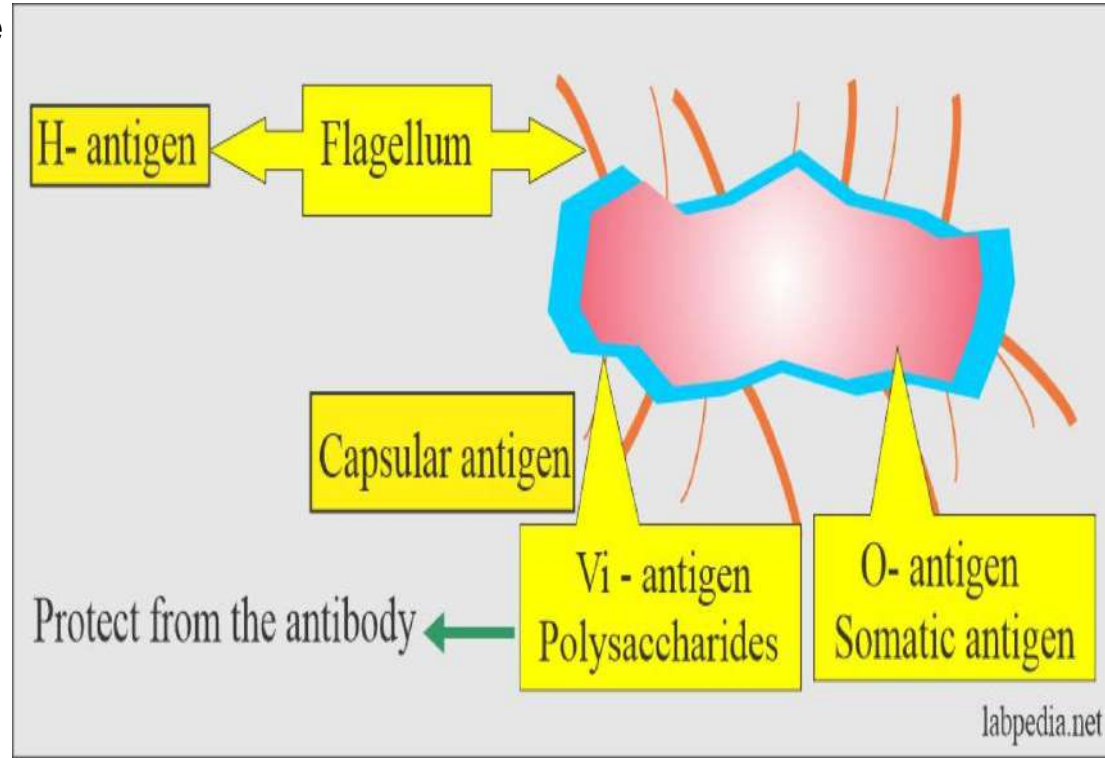
- Heat stable.
- It is a common antigen.
- Its antibodies are mainly IgM.

**2- Flagellar (H) antigen:-**

- Heat labile.
- Specific for each serotype of salmonella.
- Its antibodies are mainly IgG.

**3- Virulence (Vi) antigen:-**

- Heat labile.
- Produced only by some virulent members e.g. *S.typhi*. → only on Capsule
- It is capsular polysaccharide and protect bacteria from phagocytosis



## 1- Somatic (O) antigen:

Antigen of the body of salmonella, its the same in every type that's why its called the common antigen the antibody against is an IGM antibody which means it's an indication of an active infection

## 2- Flagellar (H) antigen:-

It's a specific antigen which means it changes from one type to the other and it's antibody is IGG which is imp in secondary infections

## 3- Virulence (Vi) antigen:-

This antigen is only present in S.typhi (the virulent type) and it's the antigen on the capsule



A magnifying glass with a black handle and silver frame is positioned over the text 'TYPHOID FEVER'. The text is written in a bold, dark blue, sans-serif font. The background is split vertically: the left side is a solid light blue, and the right side is white. On the light blue side, there are three white, oval-shaped pills. On the white side, there are two light blue, oval-shaped pills. The magnifying glass's lens is centered over the text, and its handle extends towards the right edge of the frame.

**TYPHOID  
FEVER**

**Typhoid fever** is caused by *S.typhi* & is strictly a human disease.

**Paratyphoid fever** is caused by *S.paratyphi* A or B or C.

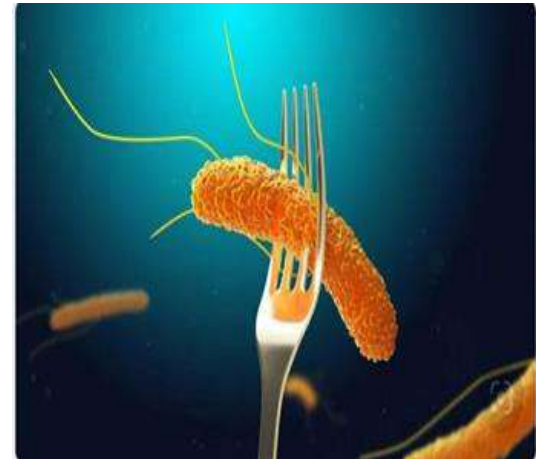
The source of infection is the stool or urine of **cases or carriers**.

A **carrier** state is common; thus a **food handler** is very important source.

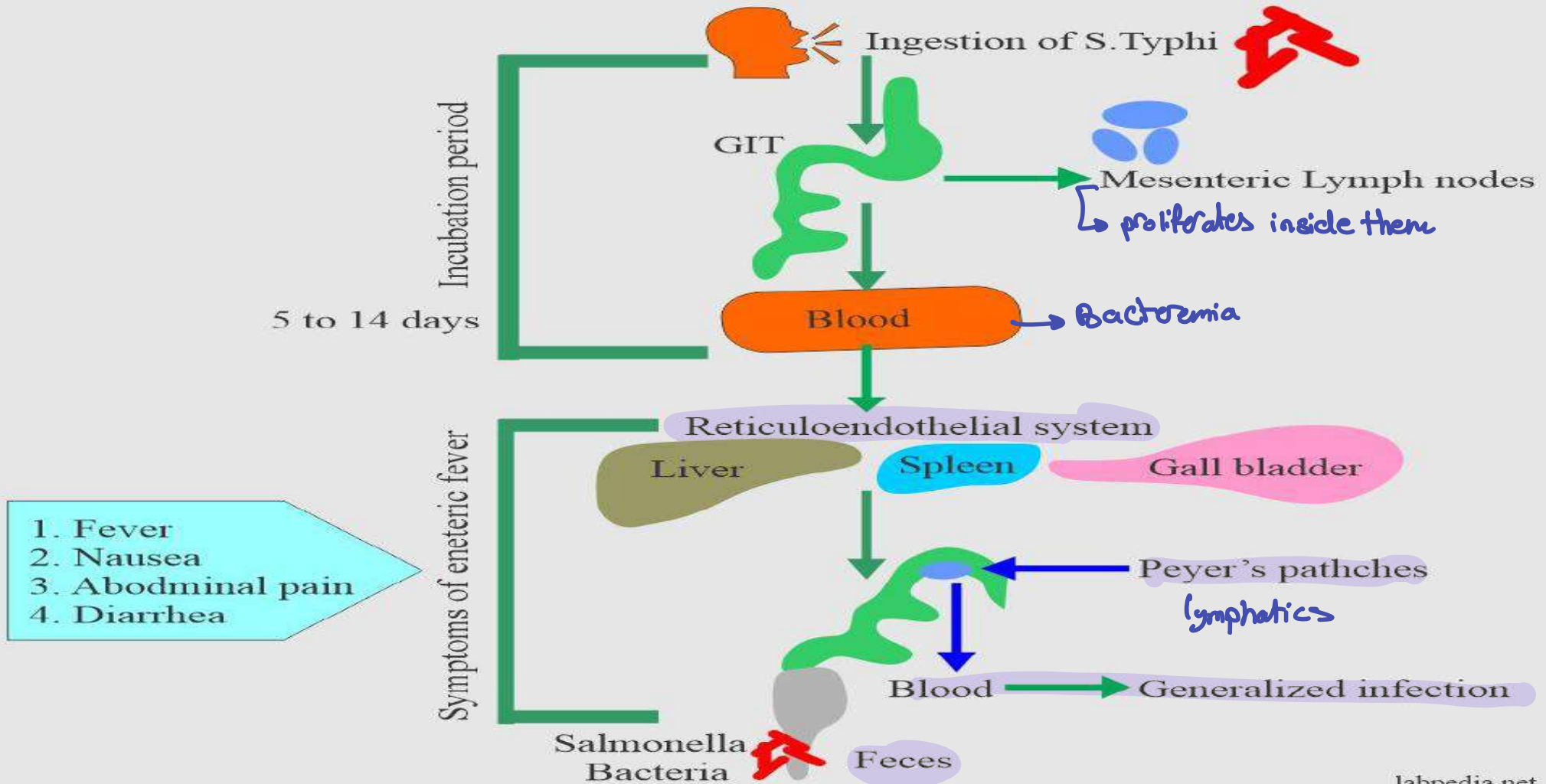
→ Feco oral route

Transmitted by ingestion of **contaminated water or food**.

In the case of a chronic carrier the bacteria stays inside the gall bladder and urinary tracts with No symptoms but are still excreted in stool and urine so he is still a source of infection (mostly virulent strains cause this chronic case)



# Enteric Fever Pathogenesis



# Clinical picture

- FAHMM *M* Fever, anorexia headache  
malaise myalgia → *prolonged fever*
- Diarrhea or constipation. *more diarrhea patient but also could be patients with both (alternating )*
- Enlargement of the liver & spleen.
- **Rose spot may appear on abdomen.**
- In about 2-5% of convalescents, the salmonella organism persists in the body often for many years; such persons are called **chronic carriers** and carry the bacilli most commonly in the **gall bladder** or in the **urinary tract** with excretion of the bacteria in the feces and urine for long periods.

# Symptoms of Typhoid Fever



**High Fever**



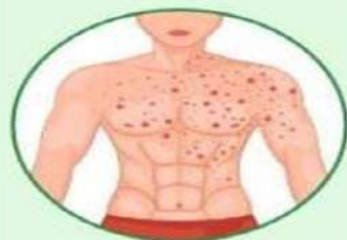
**Chills**



**Loss of  
Appetite**



**Stomach  
(Abdominal)  
Pain**



**"Rose Spots"  
Rash  
(usually on  
Chest/Stomach)**



**Cough**



**Muscle  
Aches**



**Headache**



**Nausea,  
Vomiting**



**Diarrhea or  
Constipation**

# Methods of diagnosis

Looking for the  
bacteria

**Direct**

**Isolation of the  
causative organisms  
from the blood, faeces  
or urine**

*↳ samples*

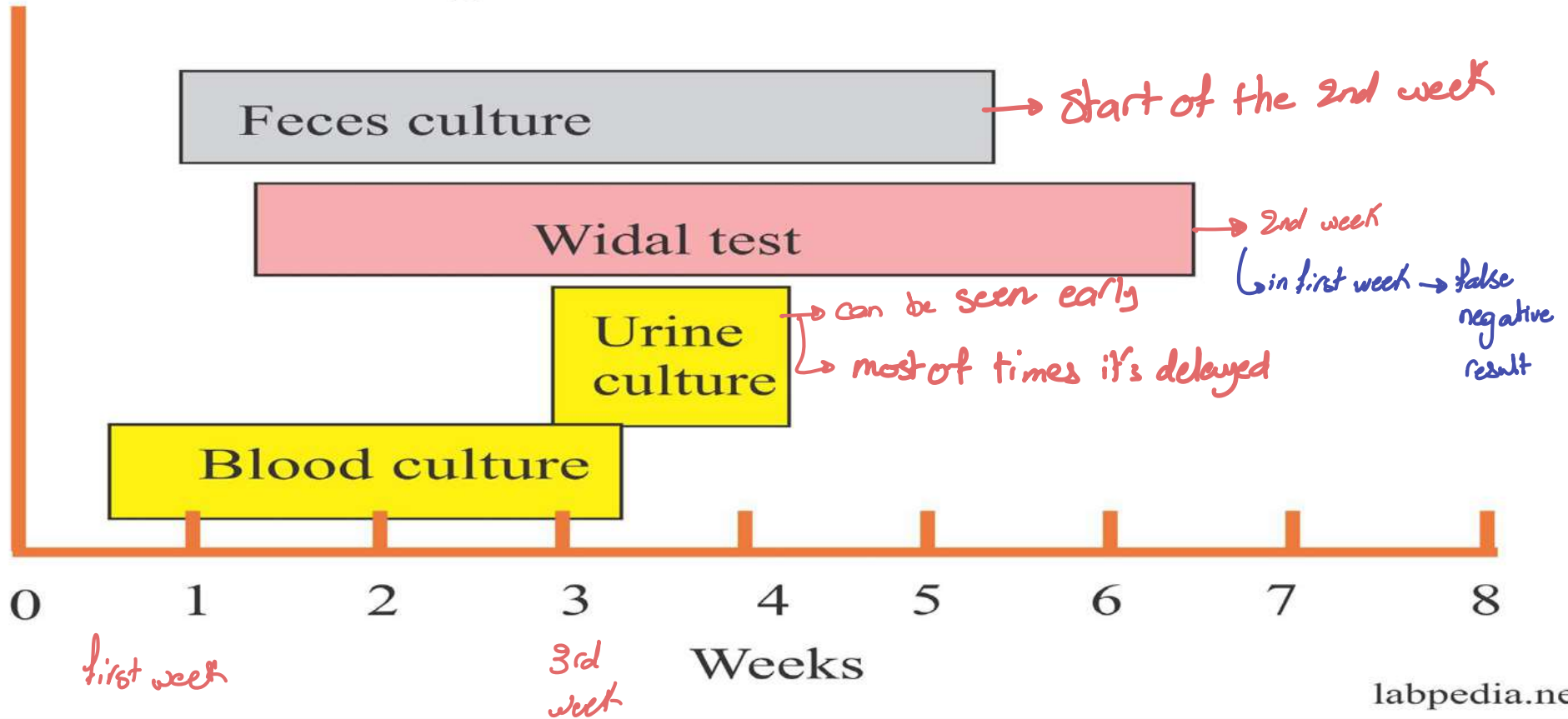
Looking for the Anti body

**Indirect**

**Serology  
(Widal test)**

# 1- Specimen Collection

## Diagnosis of Enteric fever



# A -Isolation from blood :

➤ In the **first** week of illness. The levels of bacteria in blood decreases with time

➤ **Blood culture** is the most reliable diagnostic method. *not specific*  
*↳ patient's blood with broth → culture for growing any type of bacteria*

➤ Subculture is made on MacConkey's or DCA or SS agar.

➤ Complete identification:

Any pale yellow colonies are picked and further identified by:

*↳ colony* ① *glucose/lactose ....* ② *AB against the Ag* ③

Morphology, biochemical characters and serologic typing by slide agglutination.

➤ If no growth appears, subcultures are repeated daily up to **7 days** before discharging as negative. It one of the tests is positive then the patient has salmonella but it after 7 days all tests came back negative then The patient doesn't have it





## B - Isolation from stool :

Stool is not sterile → has normal flora in it

- In the 2<sup>nd</sup> and 3<sup>rd</sup> week of illness.
- Enrichment media: **selenite broth**, **tetrathionate broth** (which inhibit multiplication of the normal intestinal flora and permit multiplication of salmonella).
- Subculture on macConkey or DCA or SS agar.
- Complete identification.

→ Test is made once unlike Blood tests

## C- Isolation from urine : *sterile → No need for any Bath*

- In the 2<sup>nd</sup> and 3<sup>rd</sup> week.
- Centrifugation and inoculation of the deposit on macConkey or DCA or SS agar.
- Complete identification.

→ *Test is made once unlike Blood tests*



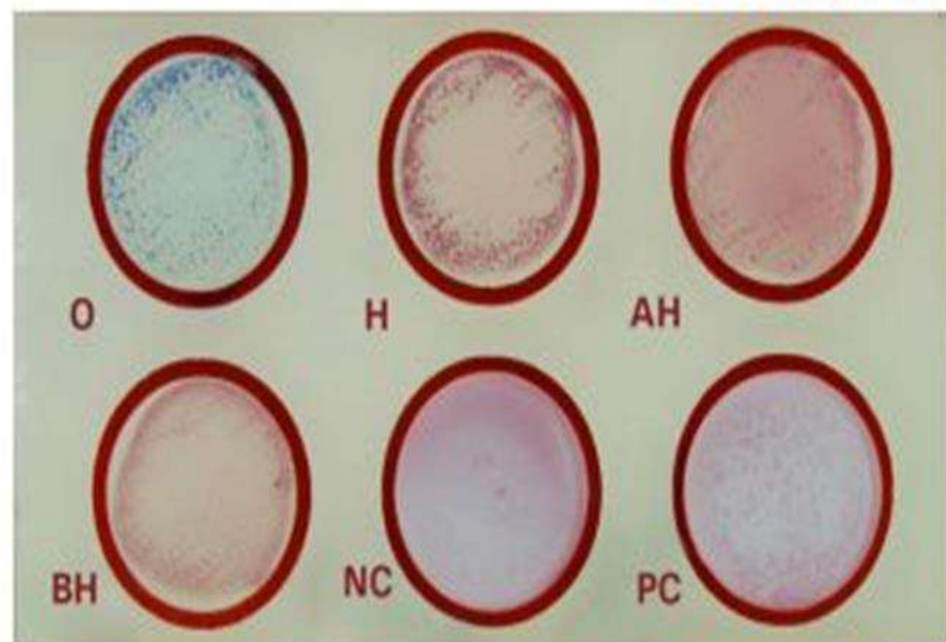
Serological

## • Serodiagnosis (Widal test)

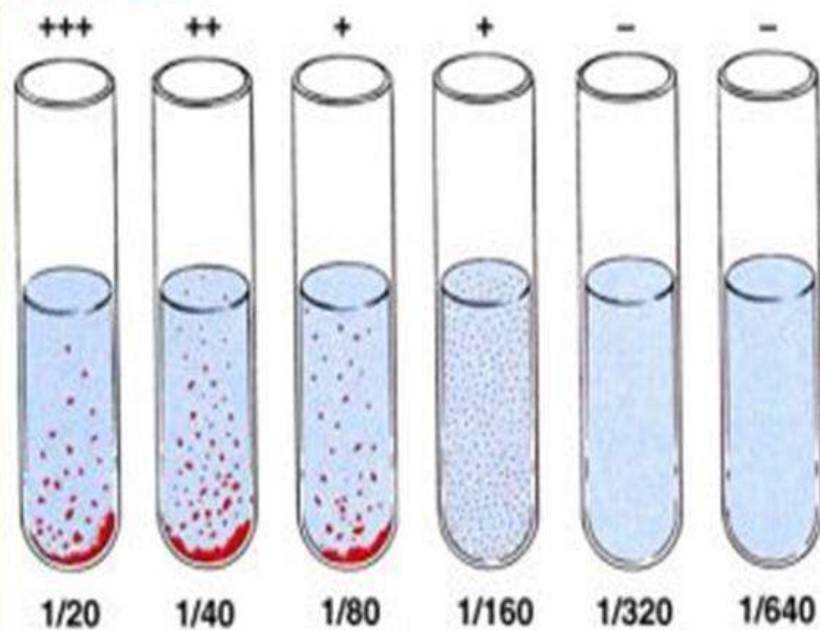
- Widal test is an **agglutination** test used in the **indirect** diagnosis of enteric fever.  
↳ AB - Ag complex
- Agglutinating antibodies begin to **appear in the serum of the patient during the second week of fever and reach maximum about the end of the third week and persist for several months.**
- The test is positive only by the beginning of the **second week** onwards. So if **the test is done during first week it gives false negative results** →→(1)

False negative :The patient has salmonella but he tested negative for the serological test because antibodies were not formed

# Widal Test



Rapid Slide Test



Quantitative Tube Test

Amount of Anti Body  
in the Serum

## Interpretation of Widal test

- The **diagnostic titre (DT)** depends on the endemicity of the disease. In non-endemic area, 1/160 is diagnostic, however, in endemic area, the DT is  $>1/160$ .
- In suspected cases with a titre below 1/160, a second serum sample is taken after 7-10 days later, if there is a rising titre, this indicate infection.
- If patient received antibiotic therapy early in the disease, the drug will reduce the antigenic mass and subsequently the patient's antibody response will be suppressed giving **false negative result** → → (2)
- The O-antibodies disappear **faster** than H- antibodies, so its presence is more indicative of **current or recent infection**.

O → IgM

H → IgG

بشكل عام في مناطق بتكون فيها salmonella منتشرة بشكل كبير زي مصر ام الدنيا فطبيعي يكون الشخص جسمه كون antibodies تجاه هاي البكتيريا بسبب تاثره الكبير فيها عشان هيك ال DT عندهم بكون اعلى بس ما بكونو مصابين او بتكون عندهم ب كميات قليلة جدا (subclinical)

واذا كان شخص في شك كبير انه تعرض ل salmonella ولسا ما ظهر على النتائج لازم نعمل كمان فحص بعد ٧-١٠ ايام عشان تكون ال antibodies تكونت عنده ونتأكد انه مصاب او لا

اي شخص باخذ antibiotics ك anaphylaxis جسمه ما يلحق يعمل antibodies فهيكون عنده ال DT اقل من المطلوب ويعطي false negative

## Diagnosis of carrier <sup>more imp</sup> <sub>harder</sub>

- In order to label a person as a typhoid carrier, the organism should be **isolated from urine or faeces**.
- **Vi antibodies** present in a titre of more than 1:10 is also suggestive of chronic typhoid carrier. Virulent strain is the one that causes this chronic infection
- The excretion of the organisms in the urine or faeces may be **intermittent**, so **repeated examination** is necessary to give more accurate results.

Not every sample of urine will have the bacteria in it so we need to do multiple tests on multiple samples

# Prevention

1) Proper sanitary measures to prevent contamination of food and water by organism:

a. Proper sewage disposal and chlorinated water supply.

b. Hand washing before food handling

b. Periodic examination of food handlers and treatment of carrier cases.

2) Immunization:  Attenuated

a) Oral typhoid vaccine :containing avirulent mutant of the *S. typhi*.

b) Intramuscular vaccine: contain the Vi capsular polysaccharide of *S. typhi*.



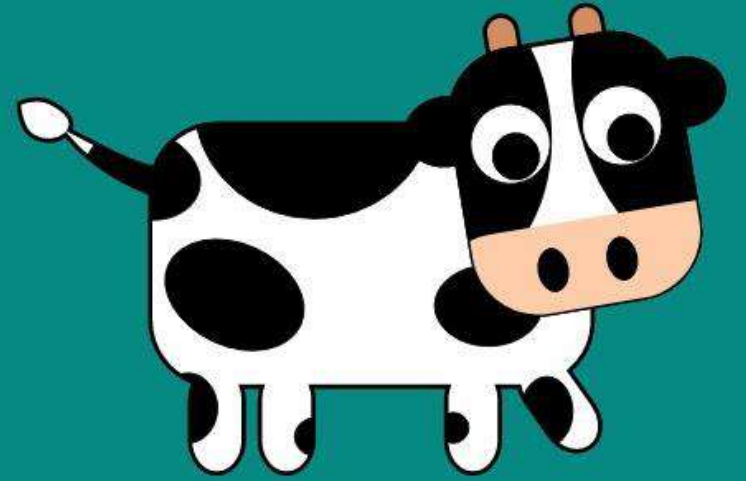
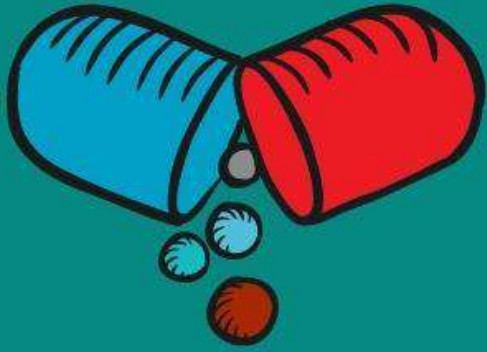
# Treatment

- Fluoroquinolones e.g. ciprofloxacin, may be a first choice
- Ampicillin and ciprofloxacin should be used for chronic carriers

*↳ combination for people  
with chronic carriers*

للاسف ما رح احط محاضرة sketchy هون لانه الدكتور ما شرحت امراض  
السالمونيلا فما رح تفيدنا كثير الصورة





# Brucellosis



HD

# Introduction:

- Brucella, **obligate intracellular**, Gram-negative coccobacilli, that infect animals and humans.
- Brucellosis is a **zoonotic** disease, primarily affecting goats, sheep, cattle, and other animals and **transmitted to humans**.
- It has various names: **Malta fever, undulant fever**.
- Named **undulant fever** because of wave-like or undulant nature of febrile response. Named **Malta fever** because it first discovered in Malta during the Crimean war in the 1850s.

## Four closely related species are described in genus brucella:

- 1- *Brucella melitensis*; infect mainly goats and sheep.
- 2- *Brucella abortus*; causes abortion in cattle.
- 3- *Brucella suis*; causes abortion in pigs.
- 4- *Brucella canis* : infect mainly dogs.

**Morphology:** Cocccbacilli: very short bacillus

- Gram-negative coccobacilli, non-motile and non-spore forming.

## Cultural character :

- Aerobes; optimum temperature 37°C; *B.abortus* requires 10%

CO<sub>2</sub> for its growth. Capnophils

Cant grow on normal agar needs

- Growth is improved by the addition of serum or liver extract.
- The media employed currently are serum dextrose agar, liver - extract agar trypticase-soy agar.
- The growth is relatively slow. The colonies appear in 2-5 days.



## Biochemical reaction:

- Catalase positive.
- Oxidase positive
- Urease positive.
- *B.abortus* and *B.suis* are H<sub>2</sub>S positive. The others are not

## Virulence factors:

- LPS is the principle virulence factor. No exotoxins are produced.
- Intracellular location of the bacteria makes them resistant to killing by antibodies and also by phagocytes and explains the relative resistance of the bacterium to chemotherapy.

 LPS is an endotoxin

## Resistance & Sensitivity:

➤ Brucellae are destroyed by (Sensitive to):

- Heat at 60 °C in 10 minutes.
- Pasteurization of milk.
- Sunlight.



It is sensitive to heat which makes it sensitive pasteurization this is good because the main source of infection is milk and it's derivatives

➤ The organism survives for 10 days in refrigerated milk, one month in ice cream.



# Brucecelosis

Fiebre de malta





➤ Brucellosis is a **zoonotic** disease in which sheep, goats, cattle and pigs are the sources of infection.

➤ Modes of transmission are:

❖ Direct contact with infected animal discharges

(particularly uterine discharge after abortion).

❖ Consumption of infected milk. Cheese made from unpasteurized goats' milk & ice cream are particularly common vehicles.

❖ Inhalation of dust from wool or infected aerosols of uterine discharge.

➤ Human such as farmers, slaughterhouse workers, laboratory workers and veterinarians are important risk groups.



*very imp*

*vets*

*any abrasions*

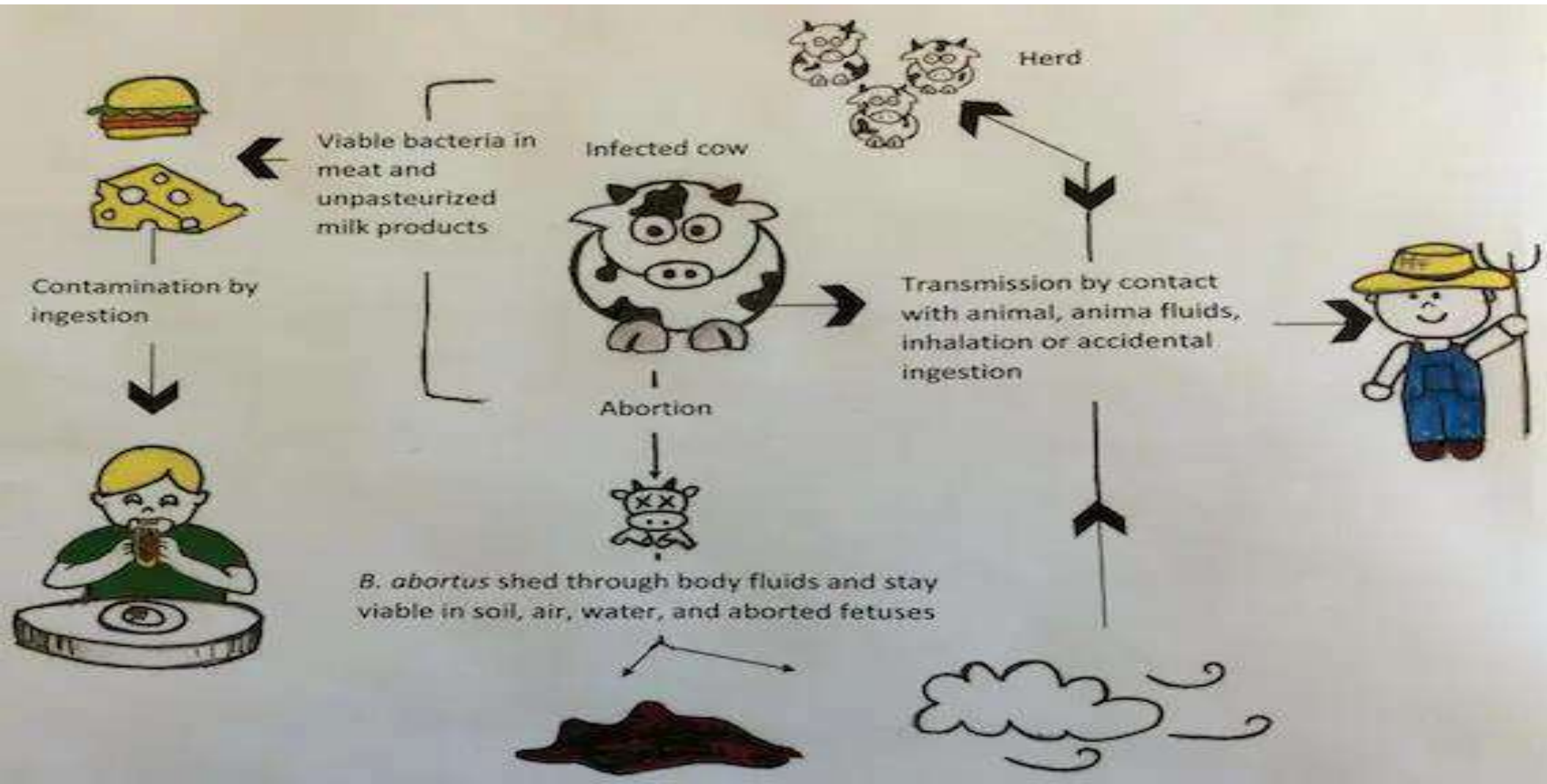
البيطري والمزارعين والرعاة اكثر الناس عرضة للاصابة وهذا بسبب طريقة  
العدوى

١. اجهاض عند الحيوانات والسوائل التي تخرج من الرحم قد تدخل بأي  
فتحة بالجلد وتحدث infection


٢. اي رذاذ متطاير

٣. حليب غير مبستر

# Brucella Transmission



## Pathogenesis:

- The Brucellae enter the body through abraded skin, mucous membranes of the alimentary and respiratory tracts and sometimes through the conjunctiva.
- Then ingested by polymorphonuclear leucocytes (PMNLs). Some Brucella killed but 10% resist intracellular killing by PMNLs.  Neutrophils
- Brucella that are not killed, spread from the site of infection to the regional lymph nodes to the bloodstream which distributes them to the organs of reticuloendothelial system, such as liver, spleen, bone marrow, lymph nodes.
- In these organs, the lesions are in the form of granulomatous nodules that may develop into abscesses. Granuloma around the bacteria which could cause central necrosis inside it

Salmonella can cause osteomyelitis but not as the main complication

- Osteomyelitis is the most common complication.
- The brucellae that infect humans have apparent differences in pathogenicity:
  - *B abortus* usually causes mild disease without suppurative complications.
  - *B canis* also causes mild disease. ↳ Abscess
  - *B suis* infection tends to be chronic with suppurative lesions.
  - *B melitensis* infection is more acute and severe.

**N.B.** Placentas of cattle, pigs, sheep, and goats contain erythritol, a growth factor for brucellae which is not found in human placentas, and this explain why abortion is not part of brucella infection of humans.

# Pathogenesis

a. **Virulent brucellae**

*Brucella* organisms

↓  
Entry via lesions or cuts, ingestion or inhalation

↓  
Phagocytosed by macrophages

↓  
Survive and replicates with phagocytes and monocytes (much of the pathogenesis of brucellosis is associated with intracellular survival)

↓  
Infected macrophages localize in reticuloendothelial system namely lymph nodes, liver, spleen and bone marrow

↓  
Results to formation of granuloma with lymphocytes and epitheloid gaints cells, which can progress to form focal abscesses and caseation

## Clinical findings:

*long period*

Incubation period: 1 to 3 weeks.

### Acute brucellosis:

Fever stays like this everyday of the week and then there's a week break and this cycle keeps on happening

1) FAHMM

2) Fever: of wavy or undulating (rising-and-falling) pattern. The fever rises in the afternoon and fall during the night accompanied by drenching sweat.

3) Hepatosplenomegaly and lymphadenopathy are frequently found.

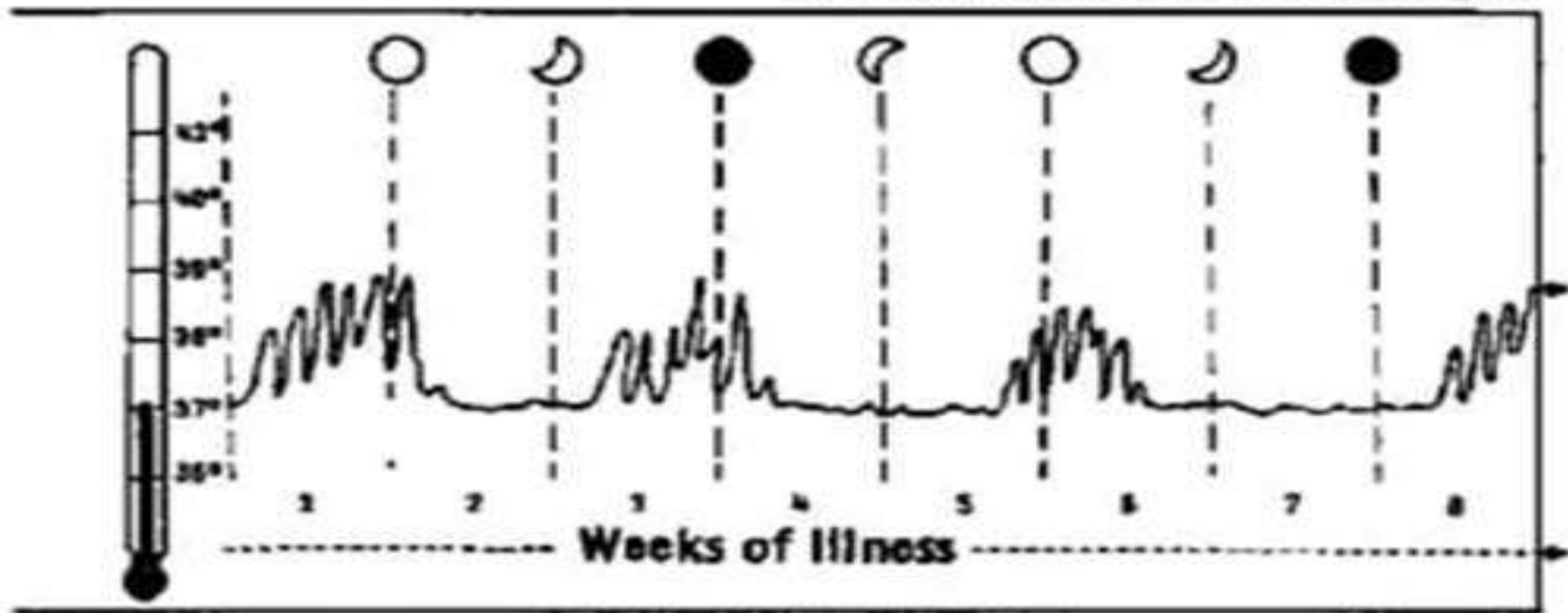
4) Pancytopenia occurs.

5) Gastrointestinal symptoms (disturbances of motions).

6) Deep pain particularly in vertebral bodies, suggest osteomyelitis.

# Fever pattern of Brucellosis

Fever comes in waves. Rises in the afternoon and falls at night.





## Chronic brucellosis:

Mainly suis

Following the initial infection, a chronic stage may develop, characterized by:

- 1) A low-grade & prolonged fever that may lasts for years with periodic exacerbations; bouts of fever lasting 3-4 weeks alternating with afebrile periods of a similar duration .
- 2) Weakness, aches with psychoneurotic symptoms. *with time*

# Diagnosis:

\* Specimens: Blood and urine for culture, serum for serologic tests.

**A) Blood Culture** Bacteremia happens in acute phases so it's hard to test positive in chronic with a blood sample

- Blood culture gives positive results in acute cases.
- Better done during the febrile attacks while the fever is rising <sup>no best time</sup> → during afternoon
- The blood culture incubated in the presence of **10% CO<sub>2</sub>** which is essential for the growth of *Br abortus*.
- Subcultures on liver extract agar are made every few days.
- The blood culture should be retained for **3-4 weeks** before being discarded as negative.

↳ slow growing organism

## من هون لعند ال *brucellin test* الدكتور اجلته للاب

### **B) Culture of urine:**

- The organisms may be excreted in the urine particularly in *Br. melitensis* infection.
- Centrifuged urinary sediment is inoculated on plates of liver extract agar.

### **N.B.**

- ❖ Negative cultures for brucella do not exclude the disease because brucellae can be cultivated from patients only during the acute phase of the illness or during recurrence of activity.
- ❖ Because brucellae are hazardous in the laboratory, tests should be performed using biologic safety cabinets.



## **C) Identification:**

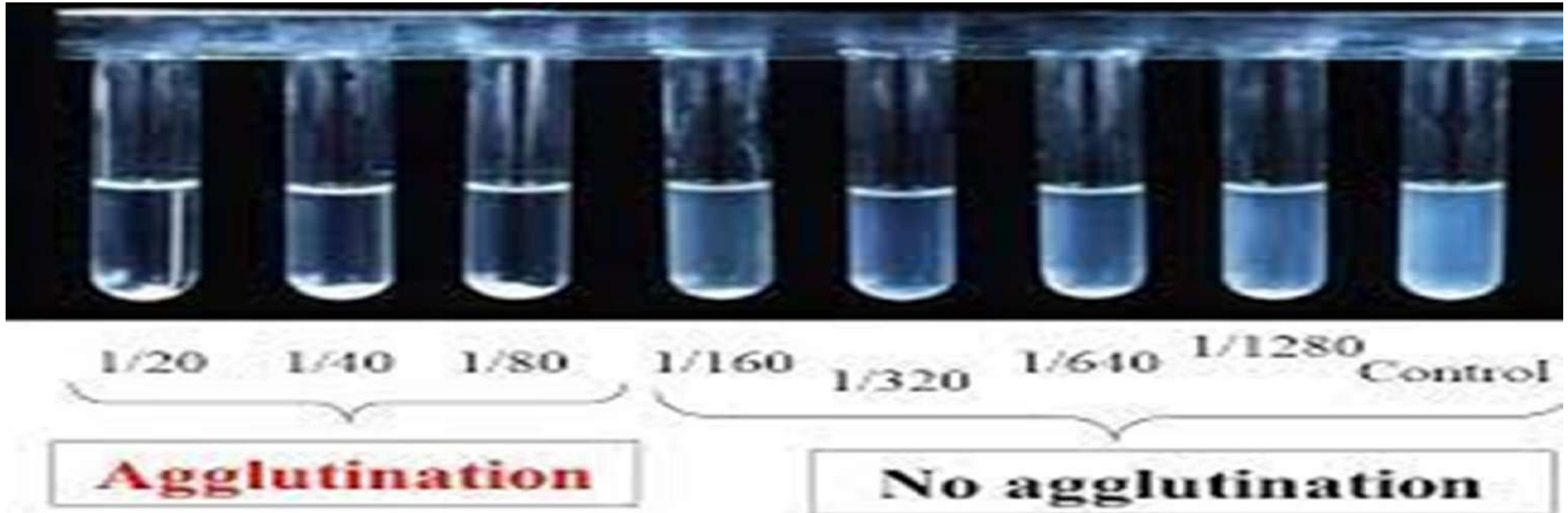
Any growth is identified by:

- Colonial morphology.
- Microscopic examination.
- Biochemical reactions: catalase +ve, oxidase +ve urease +ve reduce nitrates.
- Agglutinates with brucella monoclonal antibodies.
- Species can then be identified according to CO<sub>2</sub> requirements, H<sub>2</sub>S production and dye sensitivities.

## D) Serology:

### (1) Standard tube agglutination test (STAT):

- For total antibody (IgG & IgM)
- Is the commonest test used for diagnosis of brucellosis.
- A single titre of **>160** or a **four fold** rise or greater is considered significant.



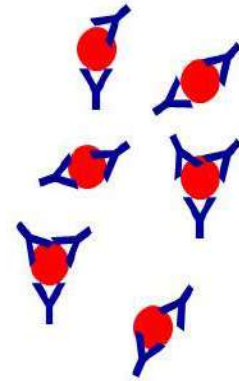
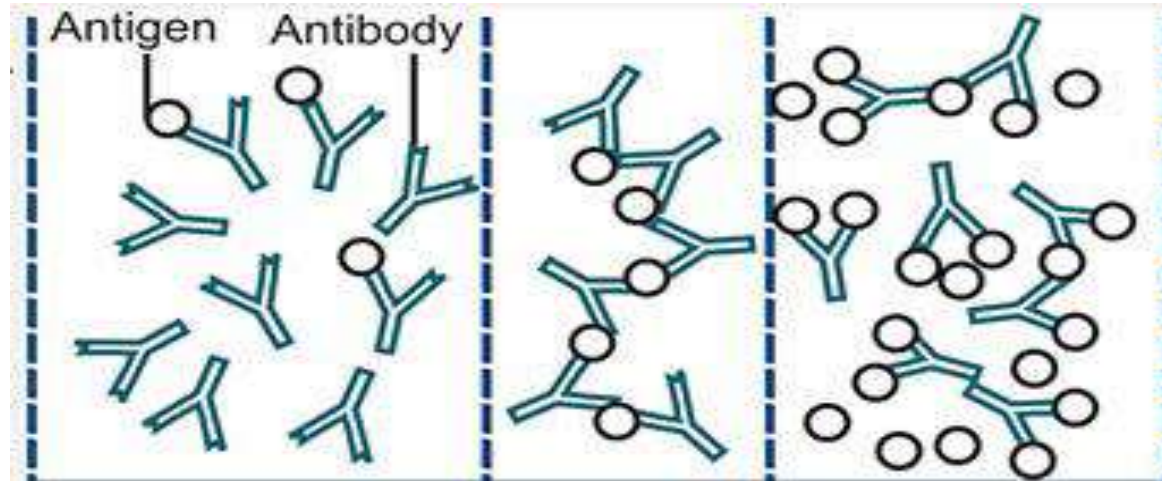
## Prozone phenomenon

- Absence of agglutination in low dilutions of serum.
- Common in brucellosis.
- It may be due to:

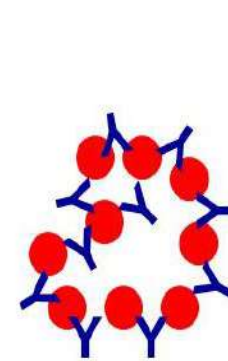
1)Antibodies excess.

2)Antigen excess.

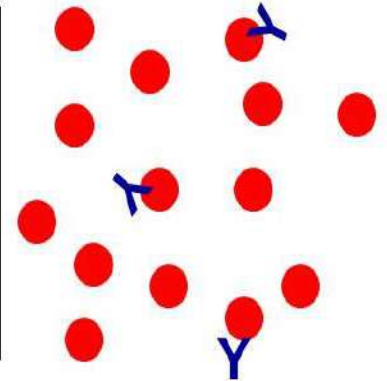
3)Blocking Ab (IgA).



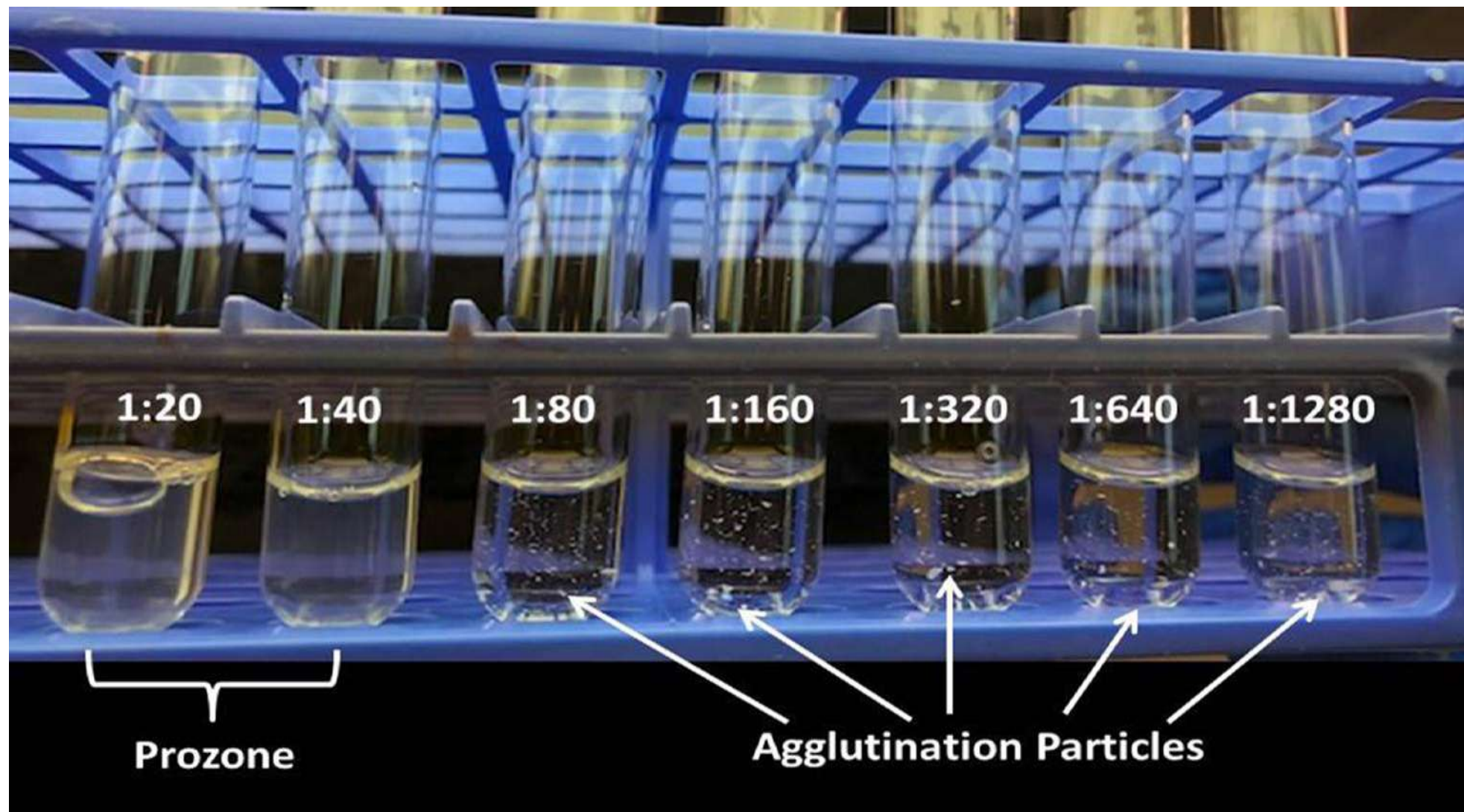
Antibody excess  
(Prozone)



Zone of  
equivalence



Antigen excess  
(Postzone)



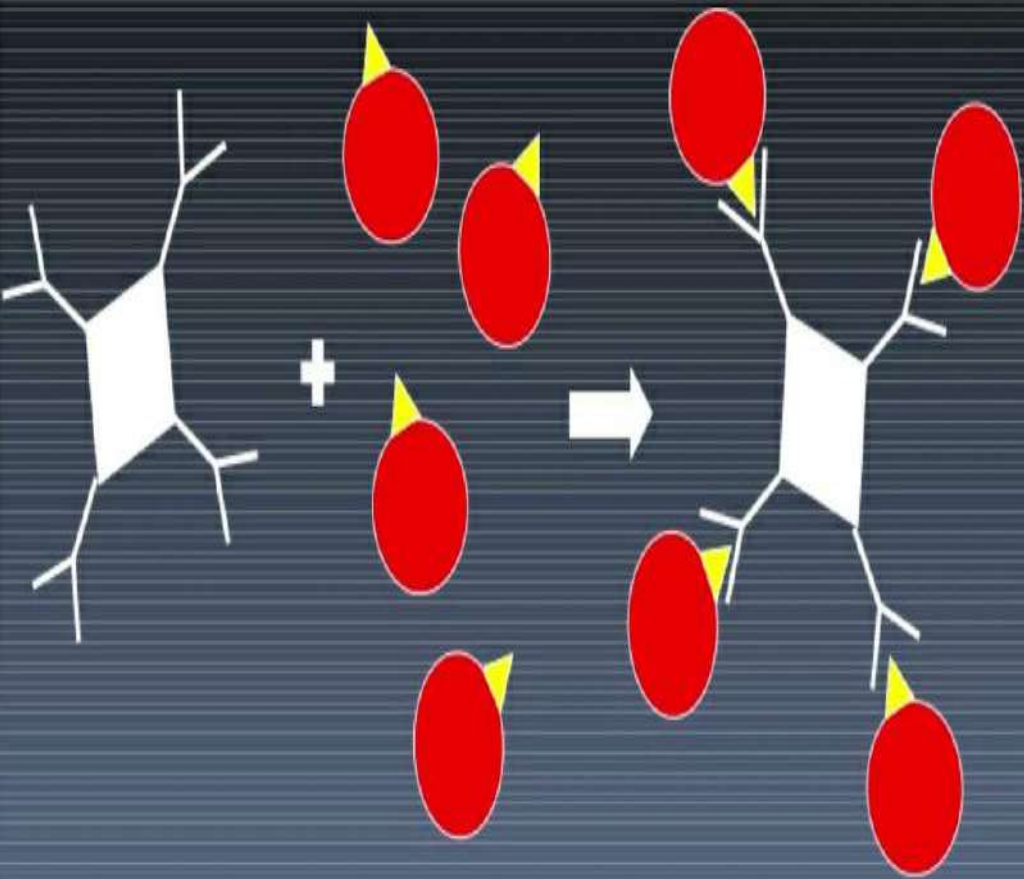




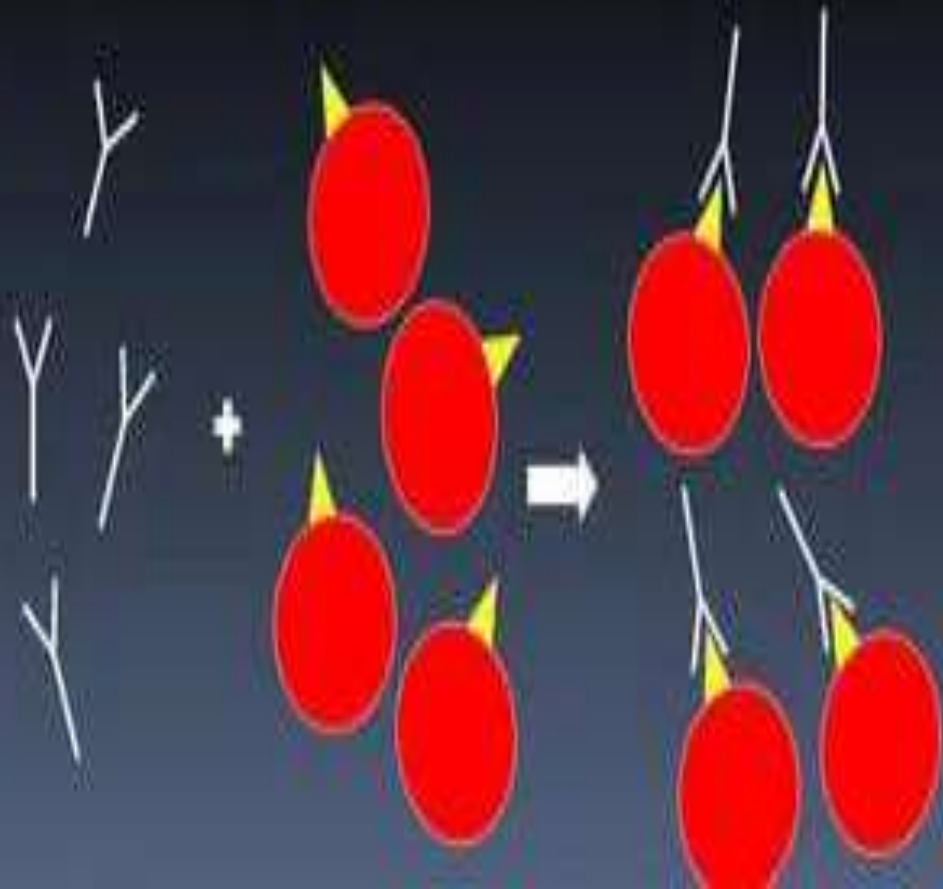
## **Blocking Antibodies (Incomplete Antibodies):**

- These are IgA antibodies that interfere with agglutination by IgG and IgM and cause a serologic test to be negative in low serum dilutions (prozone) although positive in higher dilutions.
- These antibodies appear during the subacute or chronic stage of infection, tend to persist for many years.
- If the serum agglutination test is negative in patients with strong clinical evidence of brucella infection, tests must be made for the presence of "blocking" antibodies.
- They can be detected by the **Coomb's test**.

# Complete Antibodies

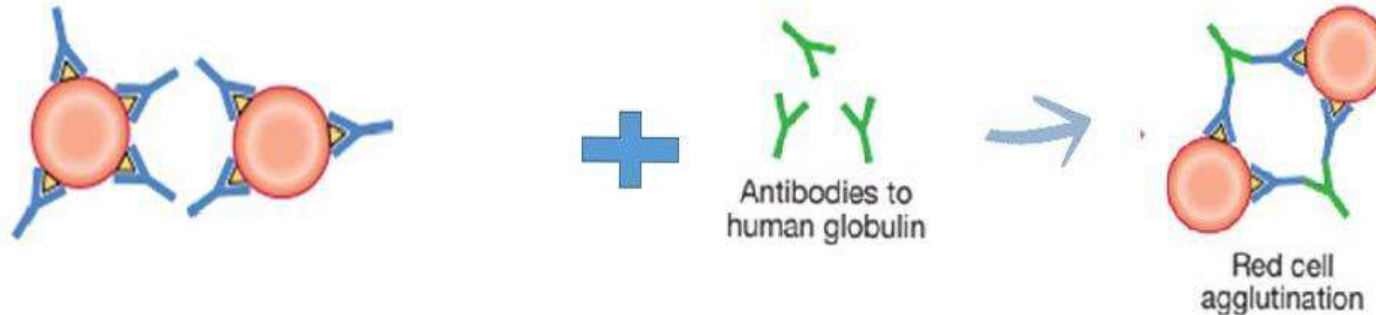


# Incomplete Antibodies



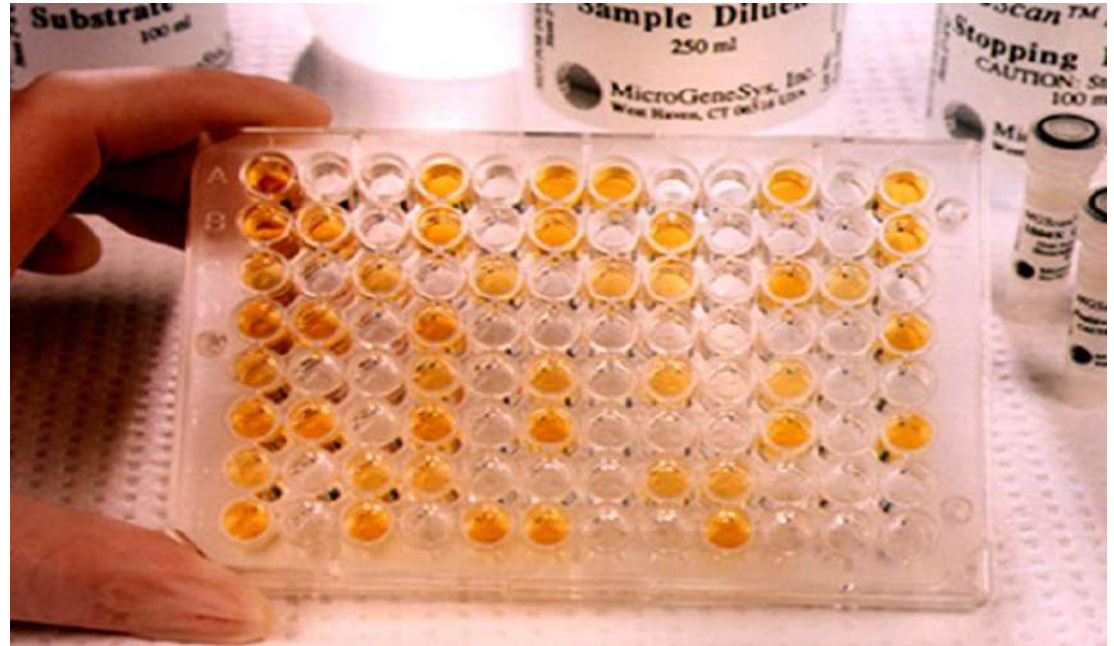
## (2) Indirect agglutination test (Coomb's test):

- Incomplete antibodies which combine with the organisms but are unable to agglutinate them can be detected by **Coomb's test**.
- Tubes from a negative STAT are centrifuged to deposit the bacteria; antihuman globulin is added and if incomplete antibody is present, which has coated the bacteria, agglutination will now take place.



### (3) ELISA Assays:

- IgG, IgA, and IgM antibodies may be detected using ELISA assays.
- These assays tend to be more sensitive and specific than the agglutination test.




### ***Acute brucellosis:***

At the time of presentation, IgM and IgG levels are almost always high and direct agglutination test is positive.

### ***Chronic brucellosis:***

levels of IgG and IgA are elevated; the Coomb's test and complement fixation test may be positive and the direct agglutination test is negative. Similar findings are encountered in healthy individuals whose immunity is being repeatedly stimulated by contact with brucella organisms and positive serological tests are common in those at occupational risk.

## Brucellin test: *→ same as leichman test*

- The intradermal allergic test done by injection of a killed brucella suspension.
- Positive result may mean past or present infection.
- Often positive in healthy people in agricultural areas.
- **Not** reliable for diagnosis. 

Can't tell if someone was or is infected

# Wright test, False negative results

① • First week of disease → Still not formed

② • Disease due to Br. Canis

③ • Blocking Ab. (Chronic brucellosis)

④ • Prozone phenomenon



## Prophylaxis:

- **Pasteurization** of milk has been found to be an effective measure in the prevention.
- Care in handling animals and their discharges specially uterine exudates in aborting animals.
- Live attenuated vaccine for cattle.
- There is **NO** human vaccine.



## Treatment:

Because of **intracellular** location, the organisms are not readily eradicated completely from the host.

For best results, treatment must be **prolonged & combined**.

Combined treatment with a **tetracycline and streptomycin** for **2–3 weeks** or **tetracycline and rifampicin** for 6 weeks is recommended.

الصفحة الي بعدها مش داخلة ومش الكل رح يقتنع بالفكرة او يحبها بس في  
شخص رائع اسمه sketchy بشرح ويعمل mnemonic وعمل عن البروسيا تحط  
الصورة وشرحها تحت



Thank  
you

