MJF COLLEGE OF VETERINARY AND ANIMAL SCIENCE, CHOMU, JAIPUR

DEPARTMENT OF VETERINARY PATHOLOGY

Clinically important Gram positive bacilli

Spore forming

1. Bacillus
2. Clostridium

Non spore forming

1. Corynebacterium
2. Listeria
3. Rhodococcus
4. Erysipelothrix
5. Arcanobacterium

Bacilli branching filaments

1. Actinomyces
2. Nocardia
3. Dermatophillus

BACILLUS

Introduction:

- **Robert Koch –** First time isolated in pure culture.
- **Louis pasture-** Prepare Attenuated vaccine

- This genus contains number of species, which are found in air, soil, dust, water etc.

Most important species:

B.anthracis, - *Anthrax*
B.cereus, - *Food poision in human*
B.piliformis-Tyzzer's Disease
B.licheniformis- Sporodic abortion
B.mycoides
B.subtilis,
B.megaterium,

Anthrax

Synonym

- Malignant Pustule,
- Malignant Edema,
- Woolsorters' Disease,
- Ragpickers' Disease,
- Hide porters Disease
- Splenic Fever

Etiology agent: *B.anthracis*

- Anthrax is a severe zoonotic disease that affect all mammalian species
- The disease occur world wise
- Ruminant are highly susceptible
- Pig and Horses are moderately susceptible
- Carnivores are comparatively resistant
- Birds are totally resistant

Morphology

- *G*ram-positive bacilli (size 1 µm in width x 5-10µm in length)

- Arrangement-Bamboo stick like appearance (in culture)
  Singly or in short chains. (In tissue/Blood)

- End of bacilli- Truncated/Square cut
-
- Aerobic or facultative anaerobic.

- Capsule – Polypeptide capsule (polymer of d-glutamic acid)

- Motility-Non motile

- Spore- Endospore (Spherical and Central)

- Catalase- Positive (*Clostridium*- Negative)

- Sporulation occur outside body in the presence of oxygen
- Spore formed in culture medium or soil
- Spore never formed inside animal body
- Spore are present in soil for 60 yr (P.M Stricly prohibited)
- Increase survival spore in Alkaline, calcium and Nitrogen rich, moisture soil

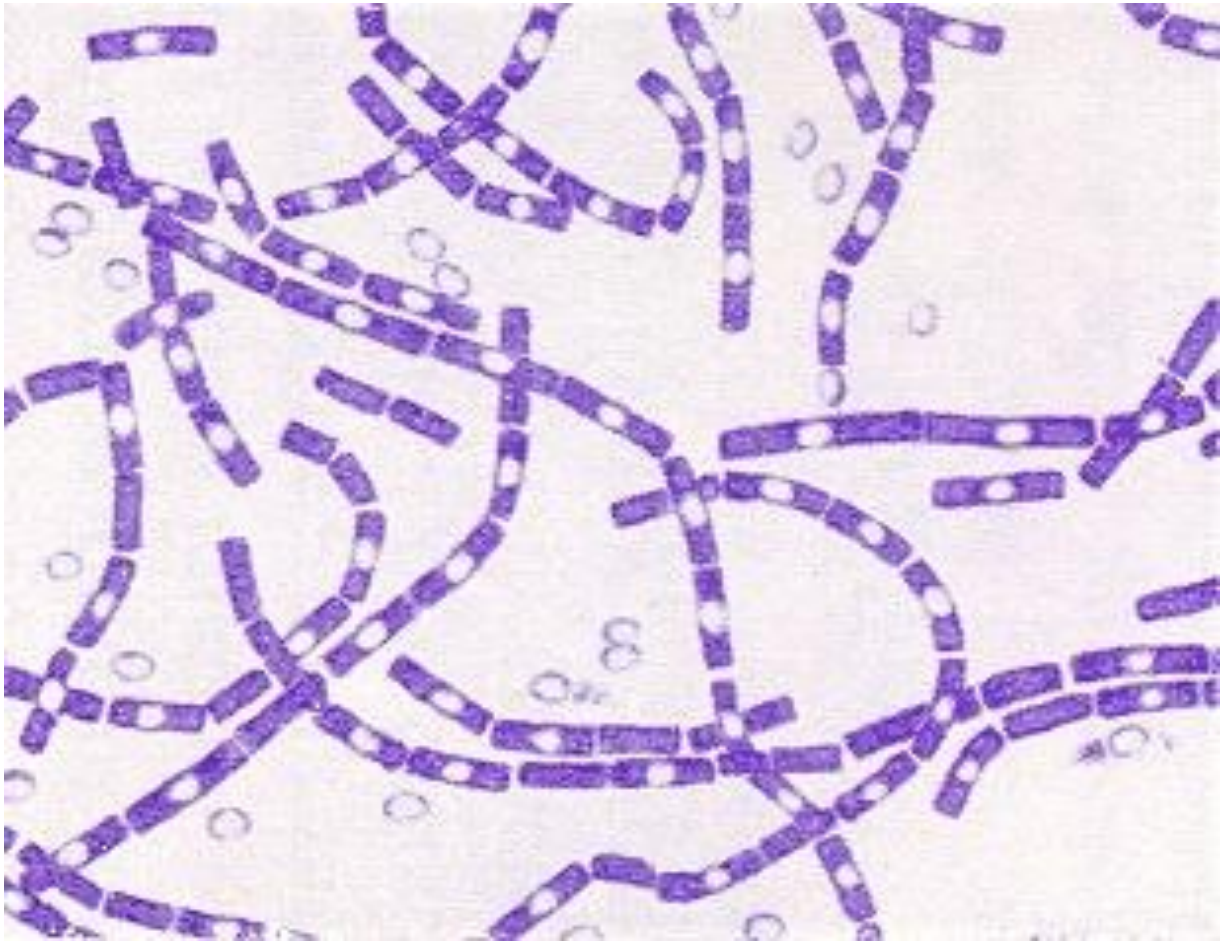
(Calcium combine with dipicolonic acid to forms a lattice that stabilizes the DNA and Enzymes in the spore)

- The spores of the organisms are considerably resistant than the vegetative cells to the physical and chemical agents.

- Boiling for 10 minutes and by dry heat at 140^{o}C for 3 hours destroy the spores.

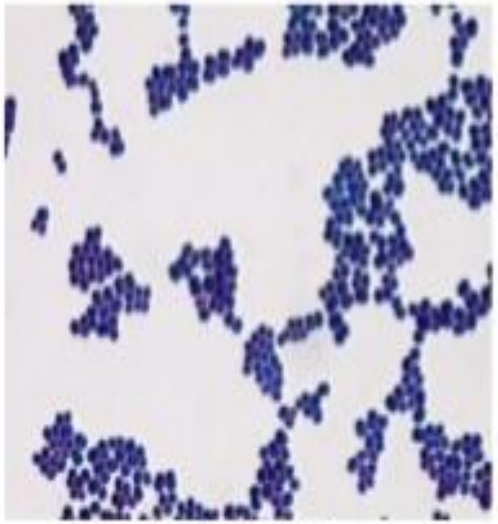
- 5% phenol for 2 days, 10-20% formalin in 10 minutes and autoclaving destroys the spores completely

Bacillus anthracis. Gram stain showing squared ends



Gram Positive cocci

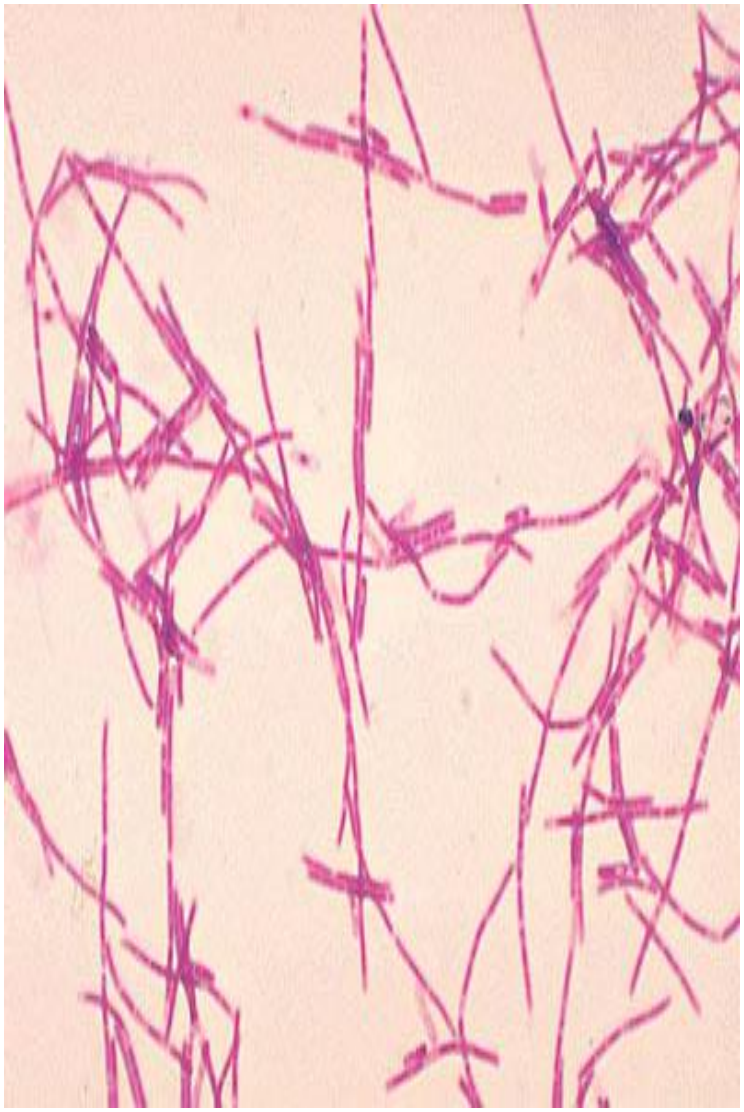
- 1- staphylococci
- 2- streptococci
- 3- pneumococci

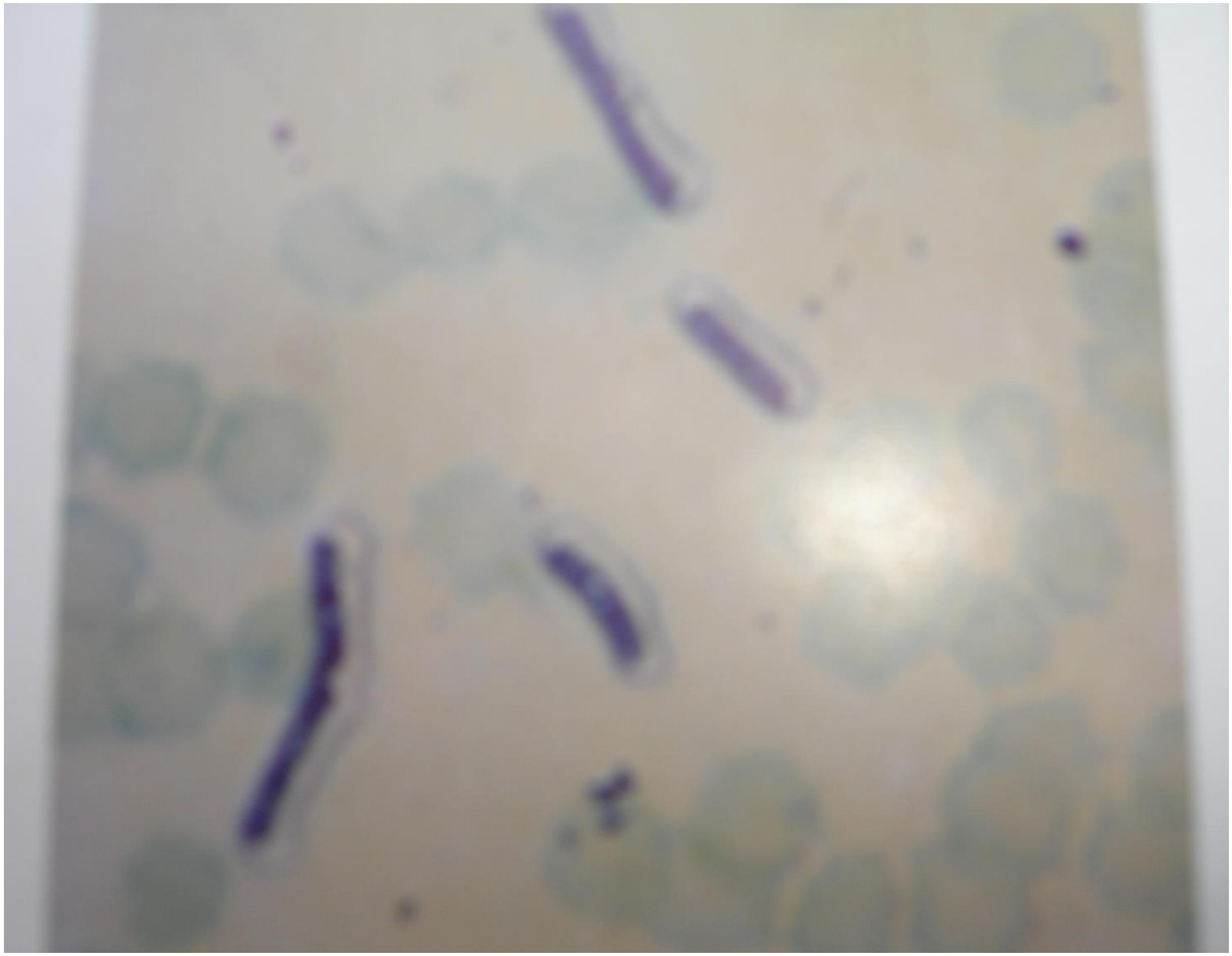


Staphylococcus

streptococci

Streptococcus in chains and Bacillus





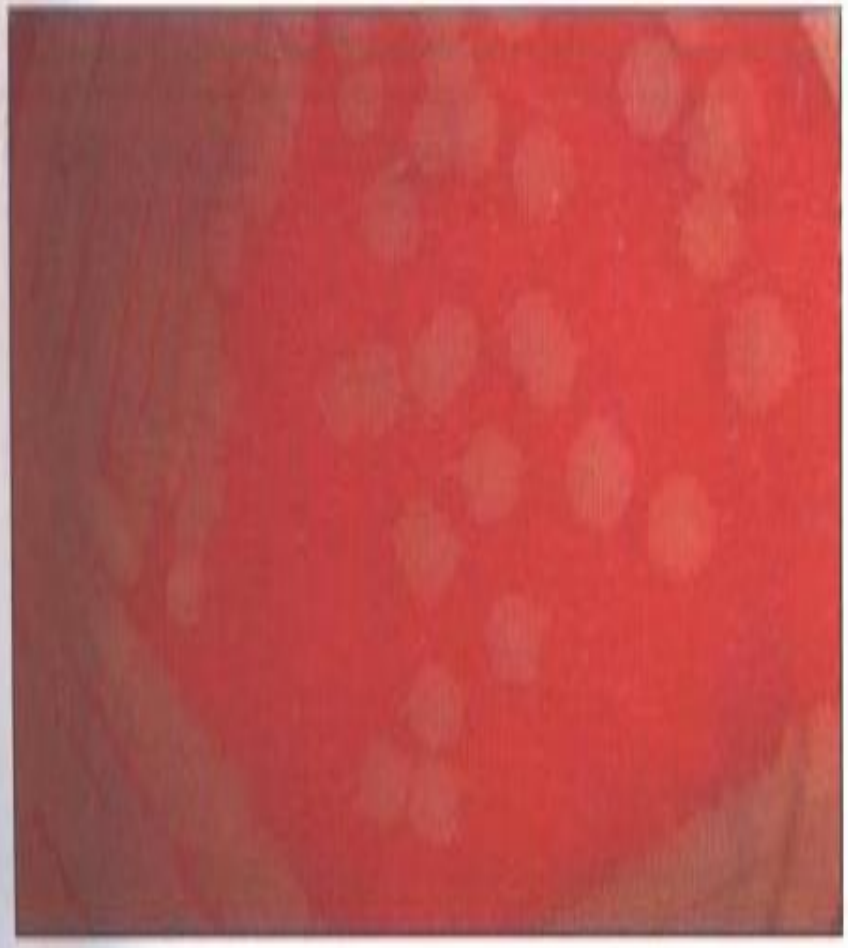
Habitat

- Majority non pathogenic Bacillus distributed in the environment because they produce highly resistant endospore

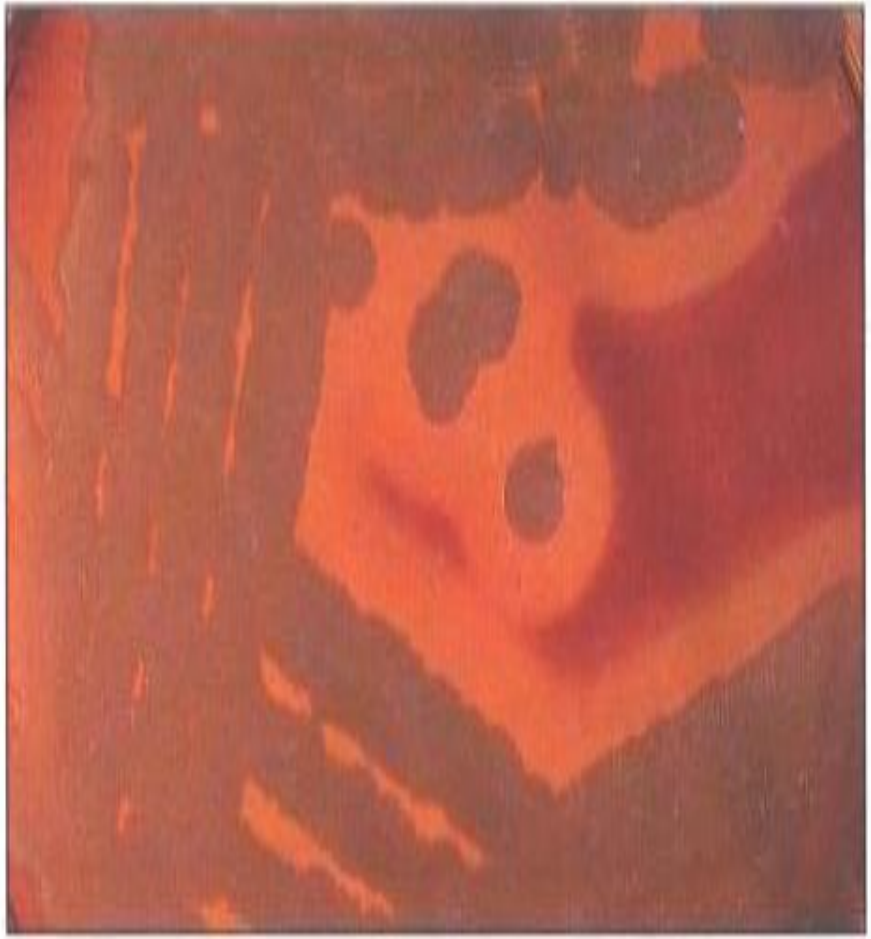
Cultural characteristics:

- BHI- Flat Dry, Opaque just like
  (Ground glass like appearance)
- Under low power microscope- Edges of the colonies appear as Medusa heads / judges wig / women's curly hair type
- B.A- *B. anthracis*-Slight or non haemolytic rough colony
  B. cereus/anthracoid- complete haemolytic, smooth
- Gelatine stab- **Invert fir tree type growth**
- **Mac-conkey-No growth**

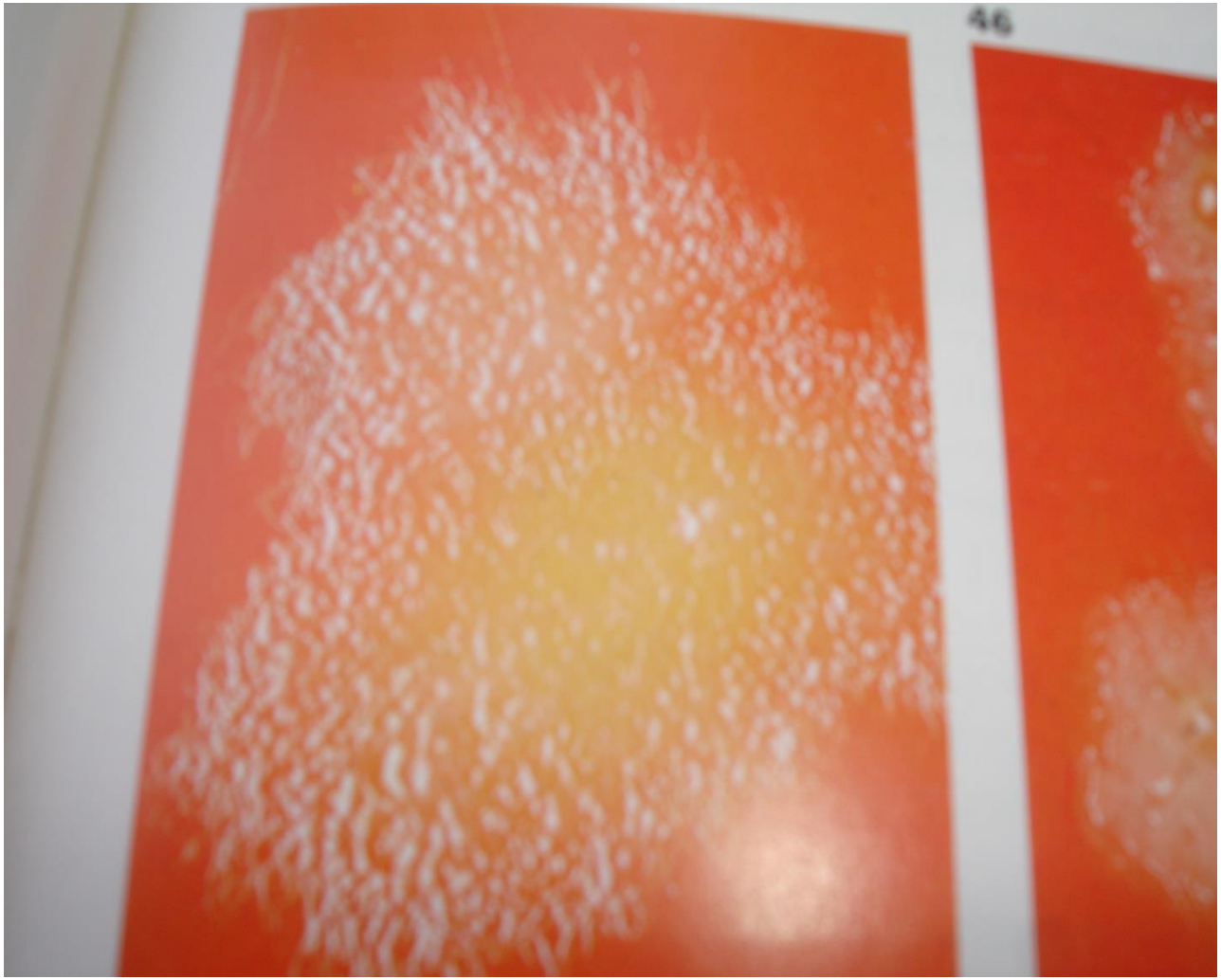
(Rough strain- Virulent)



216 *B. anthracis* on sheep blood agar illustrating non-haemolytic, flat, 'ground-glass', dry colonies with irregular edges.



217 *B. cereus* on sheep blood agar. The morphology resembles that of *B. anthracis* but the colonies are usually strongly haemolytic.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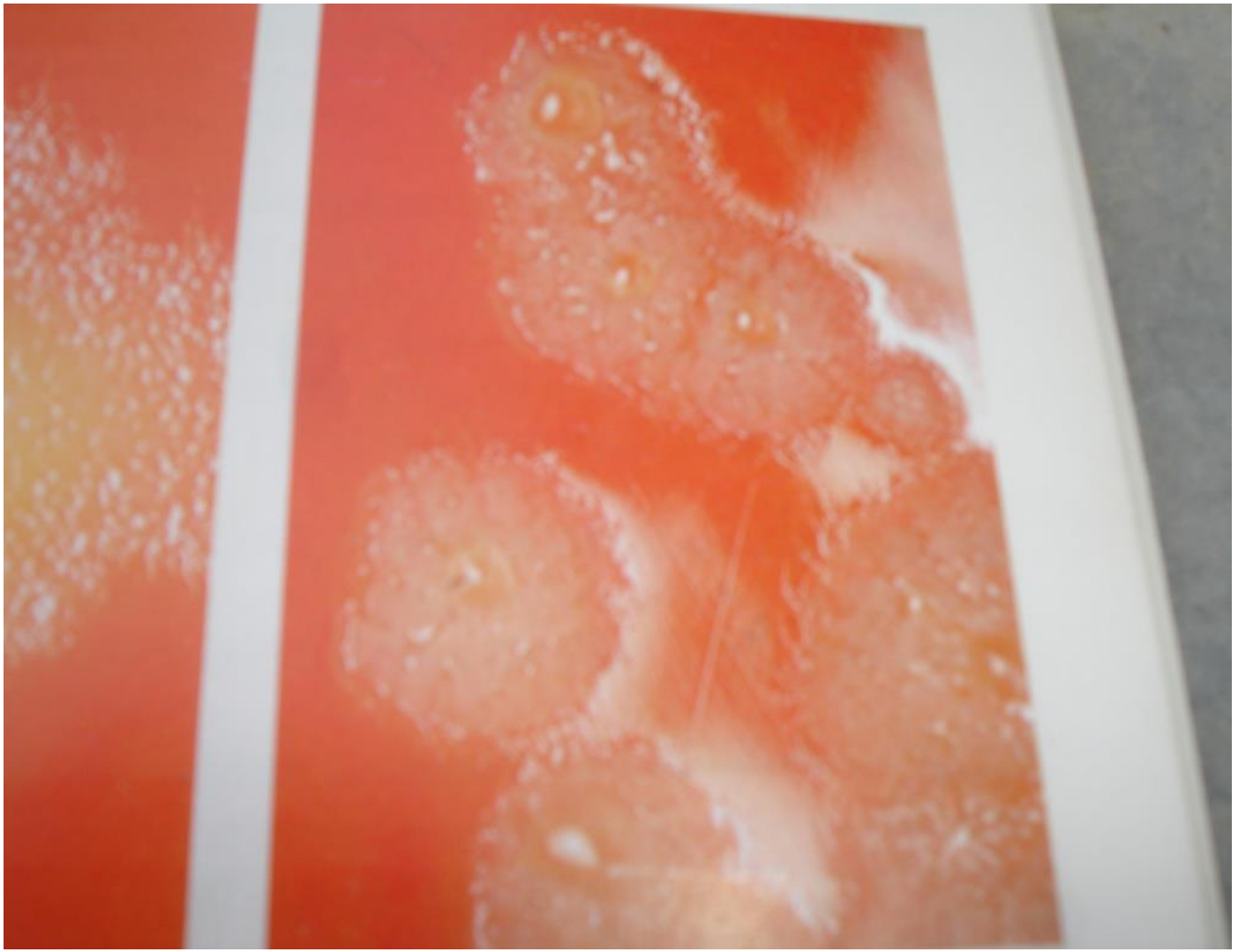


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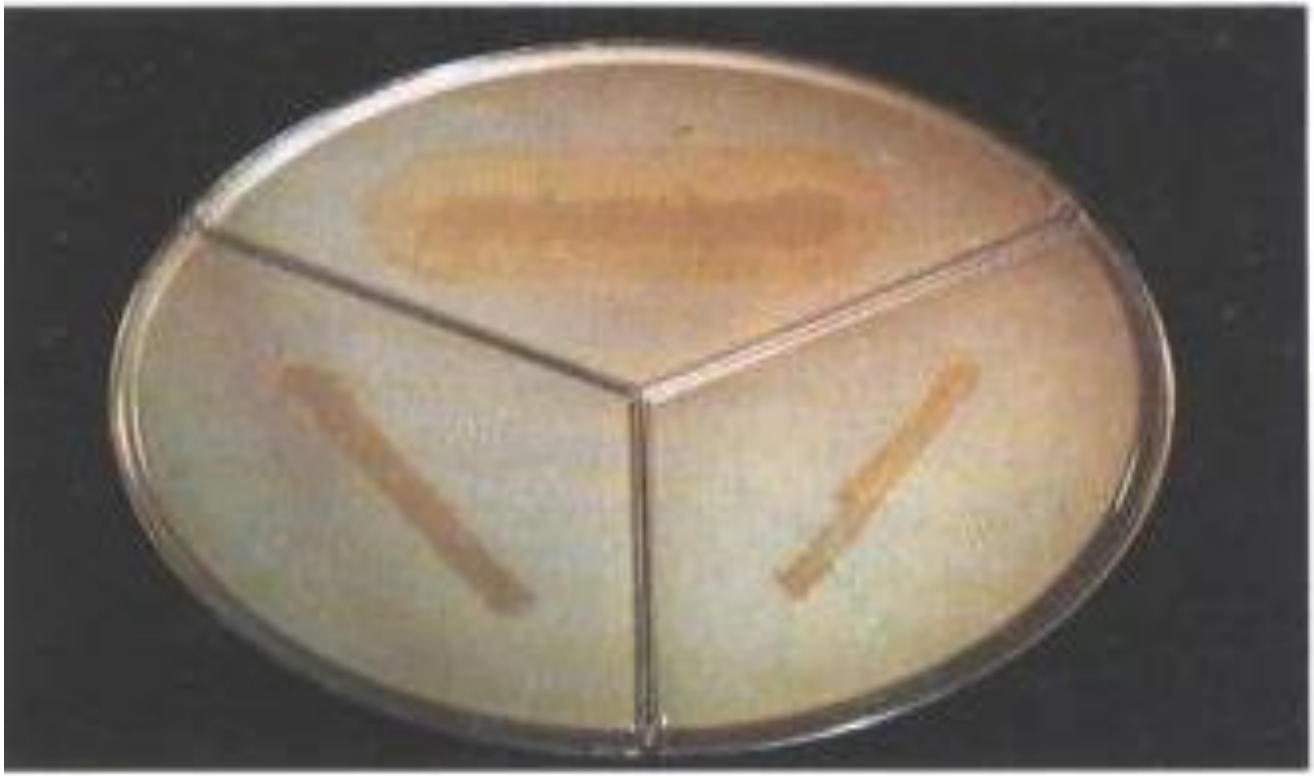
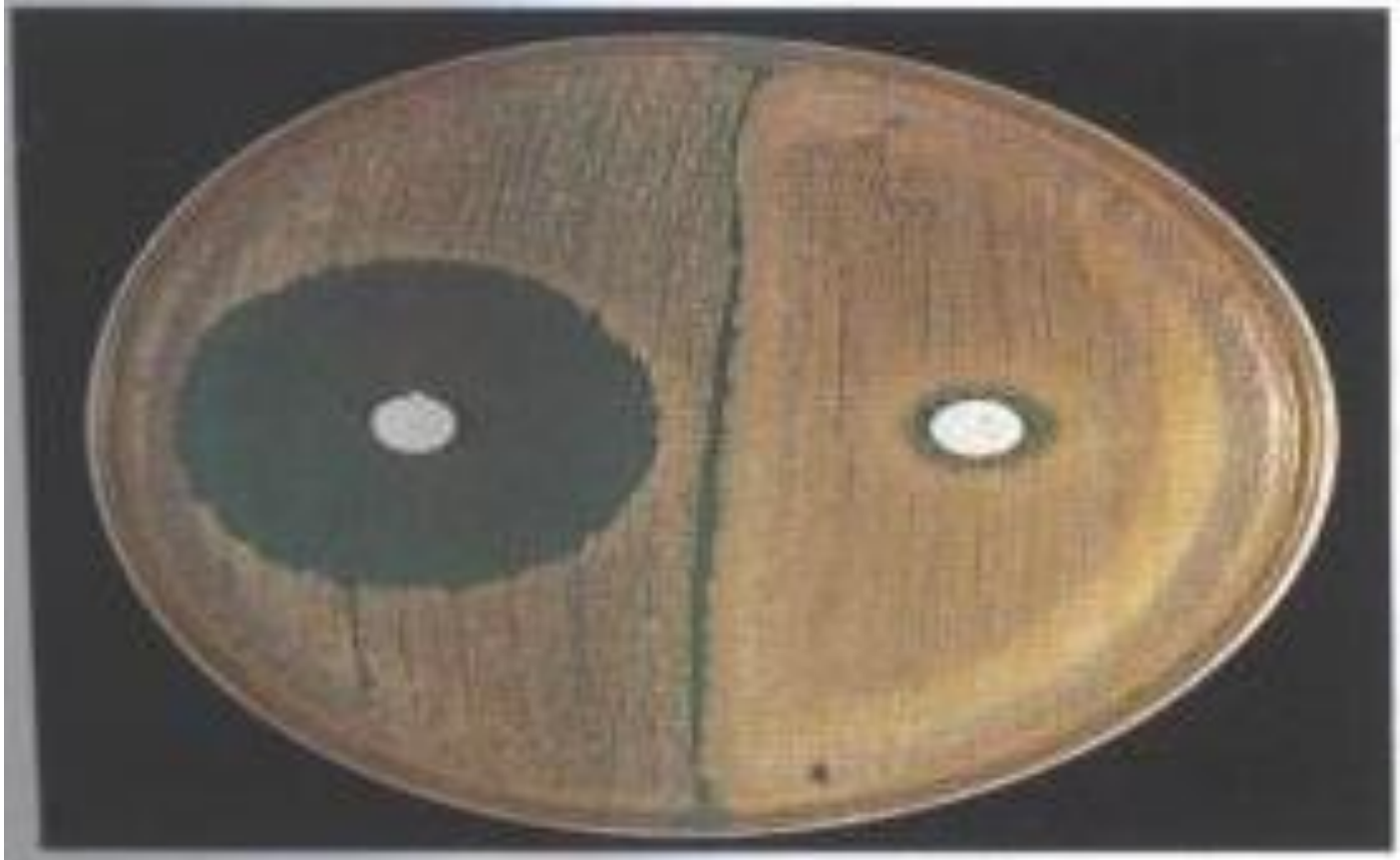
221 *B. anthracis* stab inoculated into nutrient gelatin giving the characteristic 'inverted fir-tree' type growth after 8 days at 25°C.

Biochemical test

- *Catalase-Positive*
- *Oxidase-Negative*
- *Egg yolk agar- Weak lecithinase activity*
- *Nitrates reduce Nitrites*
- *Susceptibility to Penicillin*



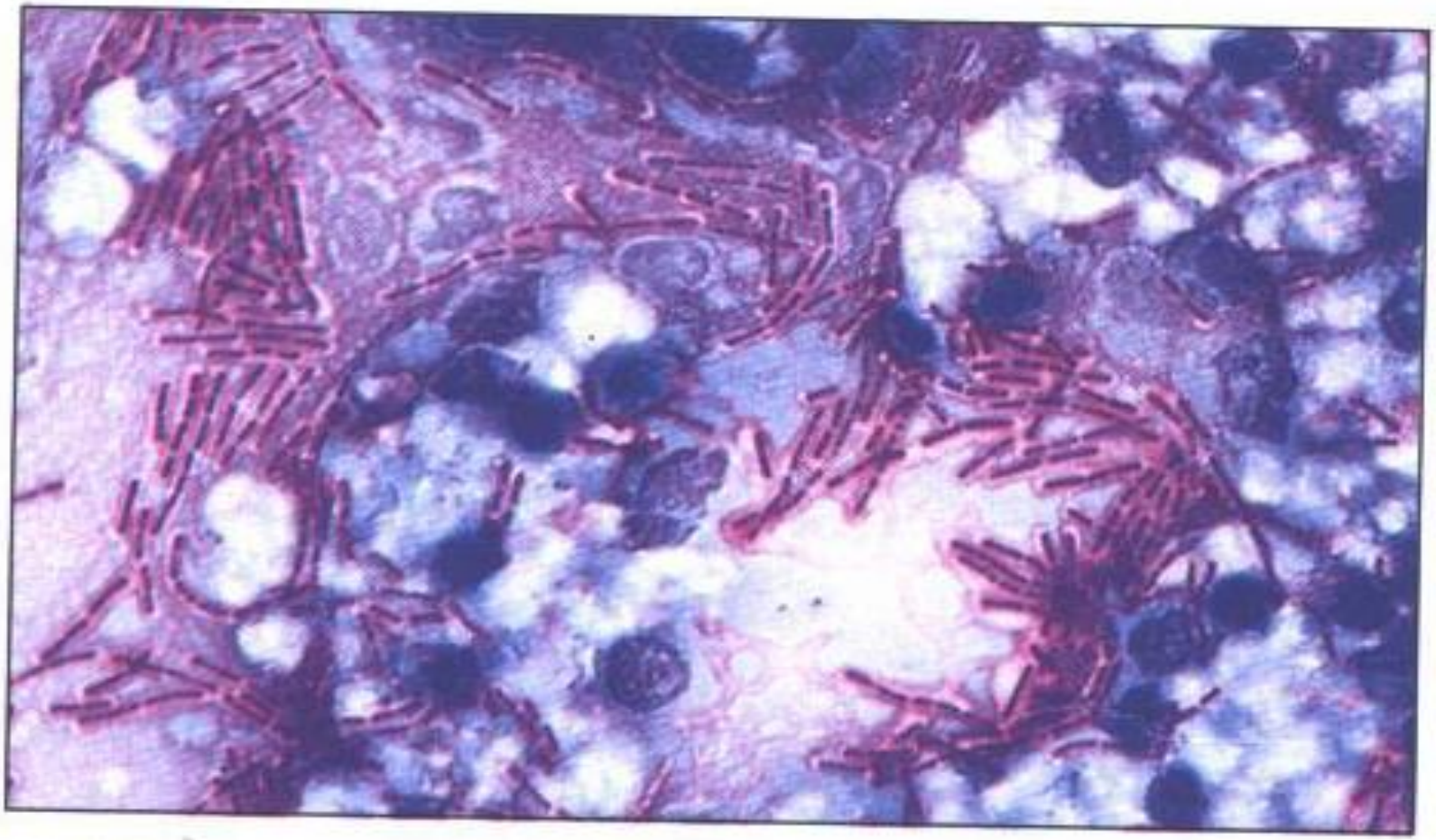
222 Strong lecithinase activity by *B. cereus* (top) on egg yolk agar after 24 hours' incubation. *B. anthracis* (left) gives a weak opaque zone after 48 hours and *B. licheniformis* (right) is unreactive on this medium.



220 *B. anthracis* (left) and *B. cereus* (right) on Isosensitest agar demonstrating the susceptibility of *B. anthracis* to penicillin (10 unit disc) compared to the resistance of *B. cereus*.

POLYCHROME METHYLENE BLUE STAIN (M'FADYEAN'S REACTION)

Polychrome methylene blue is methylene blue solution that has been allowed to oxidise by storing it exposed to the air (loosely plugged) for several months. A thin blood or exudate smear taken from a suspect case of anthrax is air dried, flame-fixed and flooded with the stain for 2–3 minutes. The stained smear is washed and dried. The rods of *B. anthracis* stain blue and the capsular material a pale pink colour (**17**). Any suspect anthrax material should be handled with care and the stained slides autoclaved after use. Viable spores may be present on the slide after staining.



17 Polychrome methylene blue-stained bovine blood smear showing capsulated *Bacillus anthracis*. Note the square-ended rods in short chains with pink capsule surrounding the blue cytoplasm. (×1000)

Antigenic characters:

- *Somatic cell wall Ag-O*
- *Capsular Ag-K (Only virulent strain)*
- *Flagellar Ag-H (Only motile anthracoid)*

Toxins & Virulence factor

- It produce exotoxin.

- which is protein in nature

  - Factor I: (Oedema factor),
  - Factor II: (Protective antigen)
  - Factor III: (lethal factor)

Production of the anthrax toxin is mediated by a temperature-sensitive plasmid termed as pX01.

- Factor III is known as the **lethal factor (LF)** because it is essential for the **lethal effects** of the anthrax toxin.

- Individual each factor lack of toxic activity in experimental animals

PA+LF combine to produce lethal activity
EF+PA produce edema
EF+LF is inactive
PA+LF+EF produces edema and necrosis and is lethal

Pathogenesis

- Organism / Spore enter in to body by ingestion, inhalation, Wound, Grazing

↓

Multiplication at the site of entry

↓

Enter into blood through lymphatic route

↓

Produce Septicaemia

↓

Produce clinical sign and symptoms

I.P- Hour to Day

Note:-Birds are highly resistant

Contt..

- Per acute-

➢ Sudden death without any symptom

➢ Splenomegaly

➢ Body cavity filled with uncloatted blood

❑ Acute-

➢ Increase body temp (108 F).

➢ Anorexia

➢ Blood oozing from natural orifice

(Nostrile, Anus, Mouth)

Contt..

- Sub acute-

  Edema in neck, brisket,Thorex and abdomen

- Chronic

- Incomplete Rigor Mortis

Tarry discharge from the nose, mouth or anus that continues to ooze after the animal dies

Horse:

- Is always acute.

- When infection is by ingestion there is septicemia with enteritis & colic with the passage of bloody faeces

- When infection is by insect transmission, hot painful, edematous, subcutaneous swellings appear at the throat, lower neck, floor of the thorax and abdomen, prepuce & mammary gland

- There is high fever and severe depression & dyspnoea due to swelling of the throat or colic due to intestinal irritation. The course is usually 48-96 hours. Rapid pulse & respiration

- Hyperaemic or cyanotic mucous membrane

Sheep & Goat:

- Peracute form more or less like cattle.
- There is sudden death which is characteristic in nature
- Some time the death is preceded by staggering gait, trembling of the body vertigo, grinding of teeth & laboured breathing
- There is frothy hemorrhagic discharge from the mouth & other natural orifices
- The animal shows increased temperature, anorexia, depression and drooping of ears

Pig:

- Acute or sub acute in nature
- Characterized by edema of neck and pharynx
- Lymph node of neck region is swollen, interferes with respiratory exchange
- Hemorrhagic races are found on skin
- Death usually takes place within 2 to 3 days following infection

Man:

- **Zoonotic disease.**

- Food animals & their products constitute potential danger to these persons whose occupation necessitates handling of animal products on contact with animals

- Usually butchers, skinners, meat retailers, meat inspectors, tanners & veterinarians are the worst victim

- The chief complains in man are the formation of malignant carbuncles & pneumonia due to involvement of skin & lungs

- Occasionally diarrhoea may develop due to ingestion of infected meat

- Cutaneous form of anthrax is the main manifestation in man

Zoonotic aspect

- In human beings, the infection occurs as pulmonary form or as malignant carbuncle or pustule (wool sorter's disease).

- Intestinal anthrax is also reported. In humans, anthrax is fairly rare; the risk of infection is about 1/100,000.

- The most common form of the disease in humans is **cutaneous anthrax**, which is usually acquired via injured skin or mucous membranes.

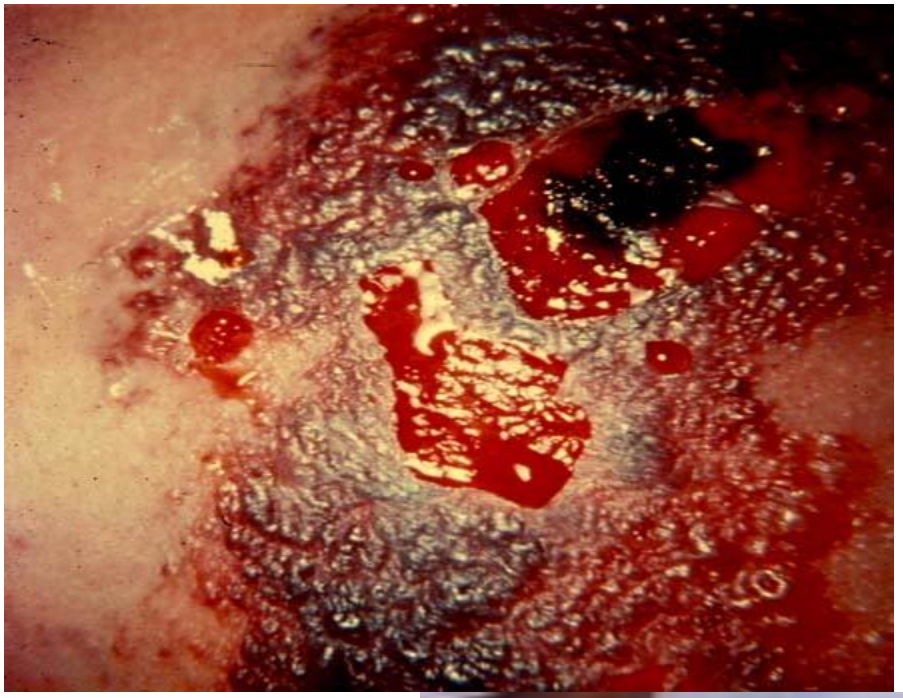
- A minor scratch or abrasion, usually on an exposed area of the face or neck or arms, is inoculated by spores from the soil or a contaminated animal or carcass.

- The spores germinate, vegetative cells multiply, and a characteristic gelatinous edema develops at the site. This develops into papule within 12-36 hours after infection. The papule changes rapidly to a vesicle, then a pustule **(malignant pustule)**, and finally into a necrotic ulcer from which infection may disseminate, giving rise to septicemia.

- Lymphatic swelling also occurs within seven days. In severe cases, where the blood stream is eventually invaded, the disease is frequently fatal.

- Another form of the disease, inhalation anthrax (woolsorters' disease), results most commonly from inhalation of spore-containing dust where animal hair or hides are being handled. The disease begins abruptly with high fever and chest pain. It progresses rapidly to a systemic hemorrhagic pathology and is often fatal if treatment cannot stop the invasive aspect of the infection

Cutaneous anthrax in humans



Diagnosis

1.Necropsy finding/ Sign / Symptome

- If suspected for anthrax, then the carcass should not be opened

- Absence of rigor mortis

➢Blood oozing from natural orifice

Sample collection

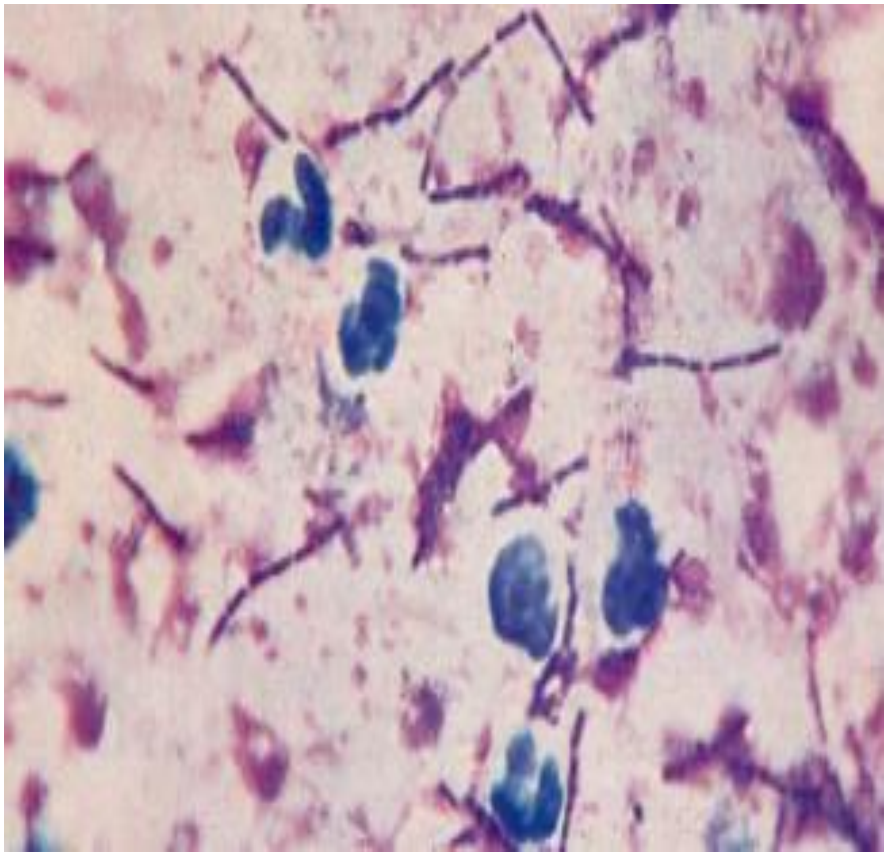
- Piece of ear, muscle for Ascoli's test
- Blood
- Tissues for histopathology in 10% formalin

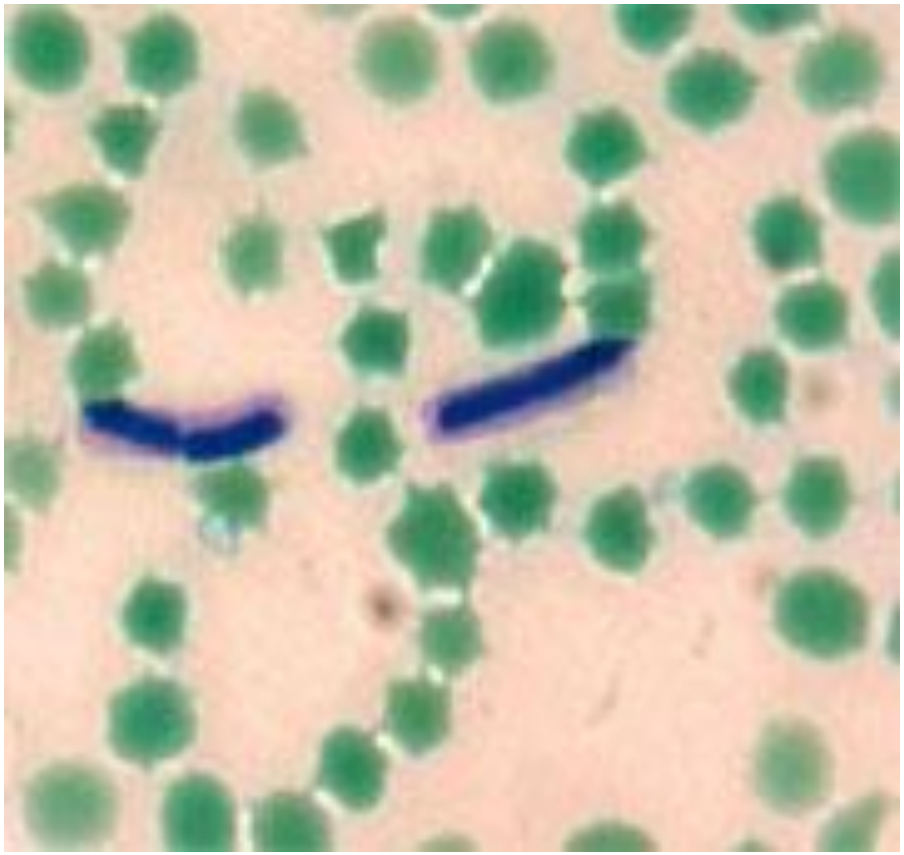
2. Direct smear examination

- Blood smear is stained with Leishman stain, Geimsa's stain, show the presence of bacilli in blue colour

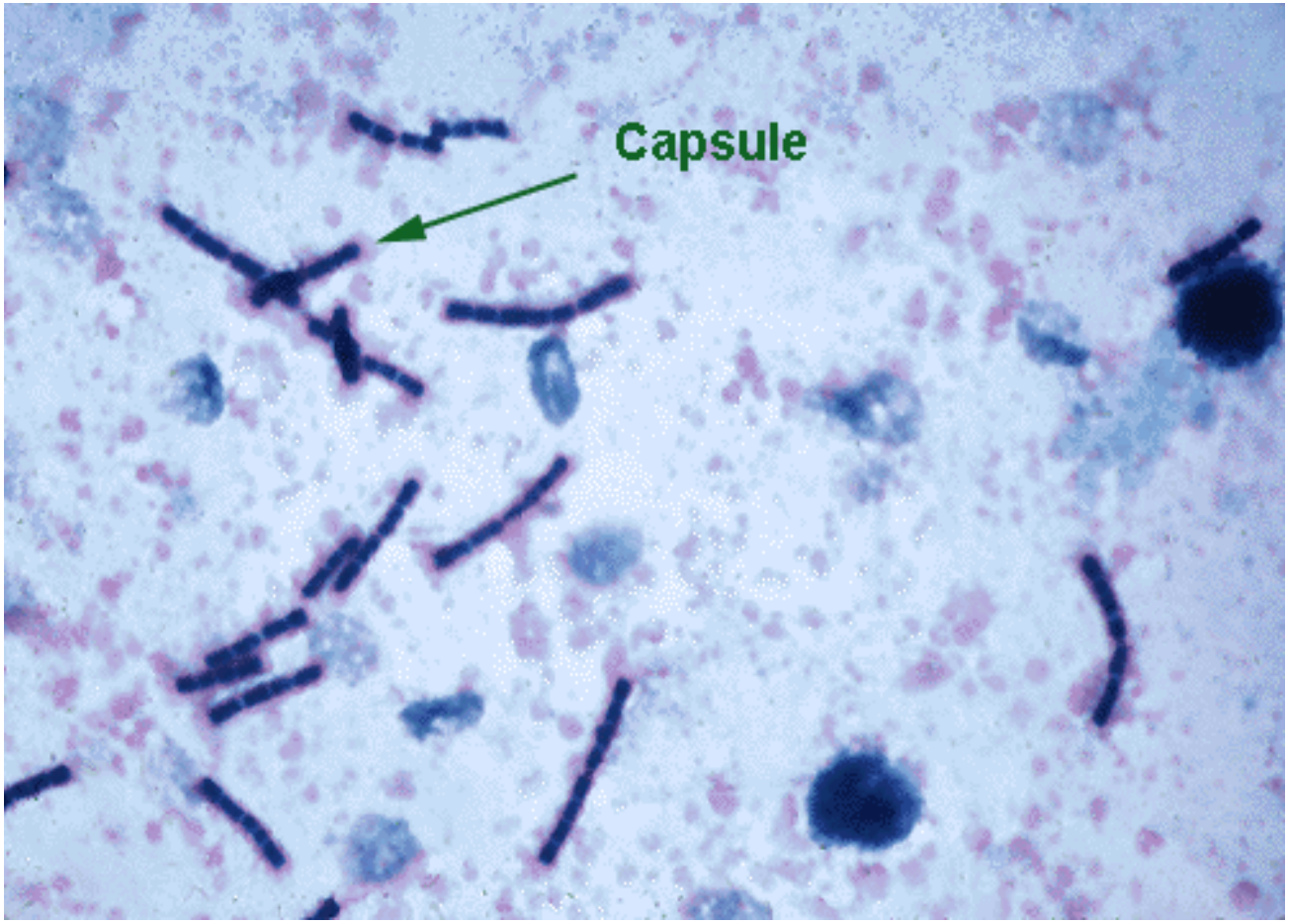
- Blood smear stain with polychrome methylen blue stain organism seen in blue colour and capsule pink colour is called as "Mc Fadyean's reaction"

Mac Faydean's reaction





Capsule will be stained pink while the bacilli blue in colour



- 3. Cultural examination

Collect sample from suspected animal and culture on BHI, Blood agar

Incubate at 37 C for 24 hr and examine colony character

- 3. Biochemical test

- *Catalase-Positive*
- *Egg yolk agar- Weak lecithinase activity*
- *Nitrates reduce Nitrites*

Ferment-Glucose, Sucrose, Maltose, Fructose and produce acid

4. Isolation in lab animals:

- *Guinea pig and mice use for isolation*

Collect sample (Blood/Culture/Tissue)

↓

Injected-0.5 ml S/C in G.pig

0.1 ml S/C in Mice

↓

The animals will die from 24 hours

↓

Then collect sample (bllood, spleen, tissue) from G.pig and mice and culture, microscopic examination

- *anthrax organisms as large capsulated rods can be seen in blood smear and spleen.*

5. Serological test:

- *Ascoli's test (Thermo*precipitation test)*:* *This test is used to detect anthrax in decomposed carcasses.*
- *Procedure*

Collect infected material (Ear, Hair, Tissue, Muscle)

Boiled in N.S for 15 min then filter it

in test tube 0.5 ml Anti anthrax serum & 0.5 ml filtered sample

Incubate for 15 min at room temp

If precipitation ring B/W two layer indicate positive

•**String of pearls test:**

- This test is based on the principle of impairment of cell wall development by penicillin.

- When anthrax bacilli is grown in a medium containing penicillin, due to cell wall impairment it will produce colonies which will resemble string of pearls.

•**Phage typing:** Gamma phages that specifically lyses *B.anthracis* are used to identify anthrax bacilli.

•**Fluorescent antibody test:** This test is used to identify anthrax organisms in tissue smears. Either direct or indirect test may be employed.

•ELISA, PCR, DNA prob

•Virulence associated plasmid-PXO1 & PXO2

TREATMENT

- Anthrax bacilli are sensitive to penicillin & other broad spectrum antibiotics
- Penicillin 10,000 units per Kg body weight twice daily parent rally has been proved to be effective
- Oxytetracycline 5mg/Kg , streptomycin 8-10 , erythromycin, chloramphenicol or sulphonamide have been advocated against anthrax
- Antianthrax serum 100-200 ml through intravenous route along with a course of penicillin may be given

CONTROL

- Control in area free from the disease should be achieved at by preventing the introduction of infected animals
- Isolate the infected animal
- The fodder, water of infected animal not be given to other animal.
- The disease should be brought under the notice of the regulatory officials in case of an outbreak.
- Care should be taken to destroy the dead body by deep burial with quicklime
- Strictly prohibited Post mortem (Do not open carcass)

CONTROL

- The adjacent area of & infected animal should be thoroughly disinfected by applying

  3% per acetic acid or

  10% caustic soda or

  5-10% formalin.

- 3% per acetic acid is considered as an efficient sporicide & thus may be applied to the soil for effective sterilization with a dose of 8 liters per square meter

- Carcasses should not be opened
- Persons handling the anthrax infected animals should adopt adequate sanitary measures for their own safety
- Animal clinicians should take care while making blood smear from dead animals & they should think seriously before undergoing rectal exploration of dull & febrile
- Is a zoonotic disease & thus has public health significance. Anthrax bacilli or spore may **produce cutaneous abscess known as 'hide porter's disease, pneumonia known as 'wool sorter's disease or dysentery in man**

IMMUNIZATION

- Anthrax vaccine should be executed annually.
- Pasteur vaccine
- Sterne Spore live vaccine/ Anthrax spore live vaccine

1. Raksha Anthrax- Live spore of attenuated non capsulated strain of *B.anthracis*- *Indian Immunologicals*,

2. Sterne Vaccine-Live spore of highly antigenic non capsulated avirulant Sterne strain 34 F2 of *B.anthracis*

- Dose: 1 ml s/c in cattle, buffalo, sheep, goat, camel & horse
- Revaccination annually.

Difference B/W Anthrax & Anthracoid

THANKS