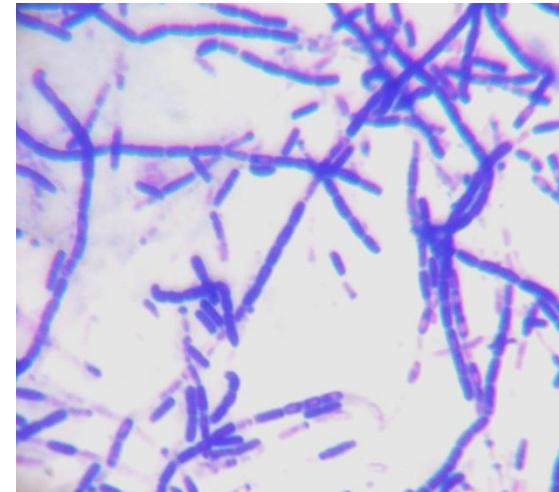


# BACILLUS

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# BACILLUS

- Kingdom -Prokaryotes
- Division -Firmicutes
- Class -Firmibacteria
- Family -Bacillaceae
- Genus -Bacillus
- Species - *B. anthracis*



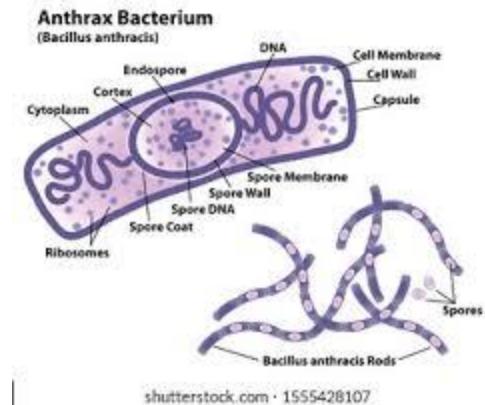
## HISTORY:

- Koch (1863) – first bacillus to be isolated in pure culture : *Bacillus anthracis*
- Pasteur (1881) – used for the preparation of attenuated vaccine.
- Wade (1980) – *B. anthracis* has received much consideration as a potential agent for use in biological warfare

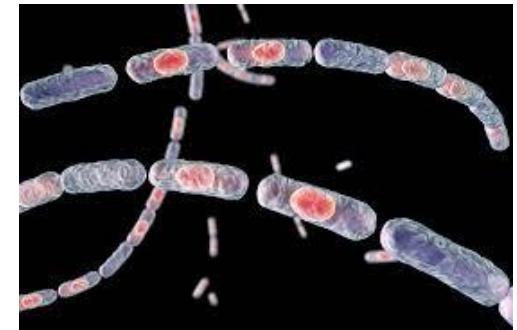


# INTRODUCTION

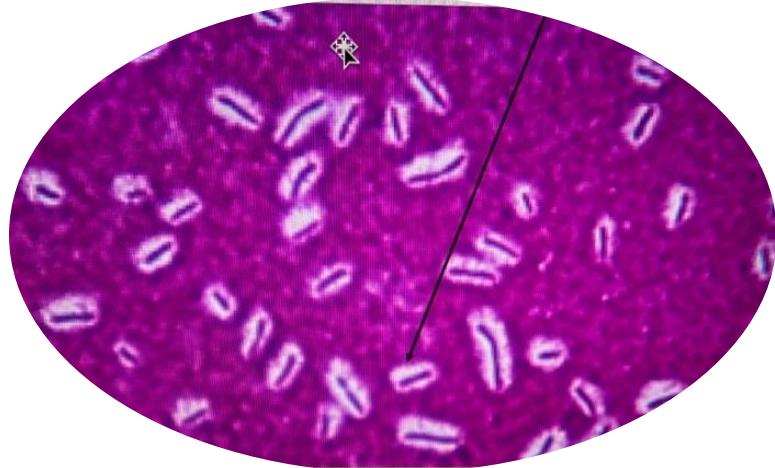
- Gram positive, Medium-to-large rods
- Arranged singly or in short chains
- Endospore forming - oval, centrally located endospores
- Aerobic (facultative anaerobic), Capsulated
- Catalase positive, Oxidase Negative
- Fermentative organisms
- Motile by peritrichous flagella except *Bacillus anthracis*.



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- Major pathogen is *B. anthracis* (*B. cereus* rarely produce disease in animals).
- Most species are saprophytes with no pathogenic potential.
- Some produce antibiotics (bacitracin, polymyxin & other polypeptides)



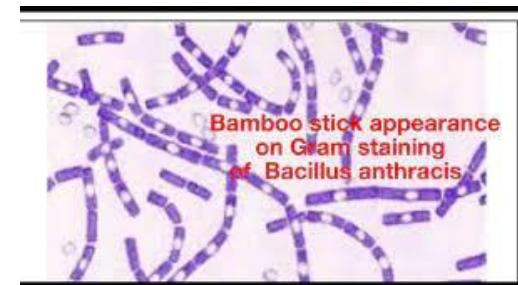
# NATURAL HABITAT

- Nearly worldwide distribution
- Ubiquitous in nature and are found in air, soil, dust, water
- *B. anthracis* natural reservoir is soil
- Anthrax zones – Soil rich in organic matter (pH< 6.0)
- Major naturally-occurring anthrax areas are tropical, subtropical
- *B. cereus* is found in dust, soil and spices
- They produce highly resistant endospores.
- In soil, endospores of *B. anthracis* can survive for more than 50 years.

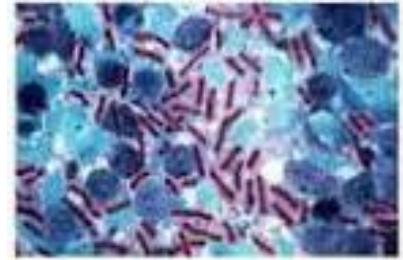


# MORPHOLOGICAL CHARACTERISTICS

- *B. anthracis* is rod shaped organism with truncated ends - presents a bamboo stick appearance.
- Anthracoid organisms are rod shaped with rounded ends
- Gram's stain: gram positive
- Arrangement: Forms long filaments in culture media, but filaments are never seen in tissues they are in pairs or short chains
- Spore formation: Forms oval, centrally located endospores
- Motility: Motile by peritrichous flagella except *B. anthracis*.



*Bacillus anthracis* :MacFedyean reaction(polychrome methylene blue)



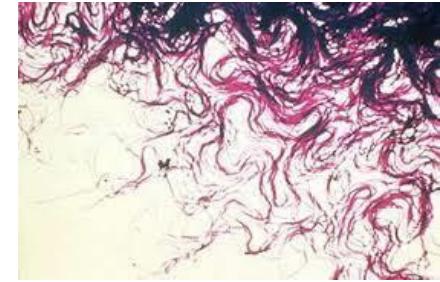
## *Bacillus anthracis* – capsule

- Capsule is polypeptide in nature, being composed of a polymer of d-glutamic acid.
- When blood films are stained with polychrome methylene blue , an amorphous purplish material is noticed around the bacilli
- Represents the capsular material and is characteristic of the anthrax bacilli - called as **McFaydean reaction**.

# *Bacillus anthracis* – Endospore

- Sporulation requires
  - Presence of oxygen
  - Poor nutrient conditions
- 1 spore present per cell
- Highly resistant to heat, cold, chemical disinfectants, dry periods
- Protoplast carries the material for future vegetative cell
- Cortex provides heat and radiation resistance
- Spore coat- made up of keratin, 50% of volume of bacterial spore, provides protection from chemicals & enzymes
- Sporulation occurs readily outside the body in the presence of oxygen.
- Because of lack of air or sufficient oxygen, spores are not formed in the blood and internal organs
- Sporulation takes place at an opt. temp. of 25-30° C and in atmosphere containing low partial pressure of oxygen.
- Sporulation is inhibited by anaerobic conditions and by CaCl<sub>2</sub>.

# CULTURAL CHARACTERISTICS



- Aerobic and facultative anaerobic.
- Grow well in ordinary laboratory medium and enrichment with blood or serum enhances the growth.
- On agar plates *B. anthracis* produce irregular, round, raised, dull, opaque, greyish white, 2-3mm in diameter frosted glass appearance colonies
- Under the low power microscope, the slightly serrated edge of the colony is composed of long, interlacing chains of bacilli, resembling locks of matted hair.
- Referred as medusa head or judges wig or women's curling hair type of growth.

# CULTURAL CHARACTERISTICS

- Selective medium for isolation is the **PLET** medium (Knisely 1966) consisting of polymyxin, lysozyme, EDTA and thallous acetate added to heart infusion agar.
- It is useful for isolate *B. anthracis* from mixtures containing other spore-bearing bacilli
- Virulent capsulated strains form rough colonies, while avirulent attenuated strains form smooth colonies
- In gelatin stab - Inverted fir tree appearance
- On blood agar *B. anthracis* produces slight haemolysis compared with anthracoid organisms.
- *B. cereus* produce a wide zone of complete haemolysis around the colonies.
- Laboratory animals of choice for cultivation of the organisms are guinea pigs and mice

## CULTURE

### GELATIN STAB

- CHARACTERISTIC INVERTERED FIR TREE APPEARANCE





PLET medium (Knisely 1966) consisting of polymyxin, lysozyme, EDTA and thallous acetate added to heart infusion agar.



# RESISTANCE

- Growing forms of the anthrax bacillus are only slightly resistant.
- Killed by ordinary disinfectants, by pasteurization.
- Under aerobic conditions spores are readily formed.
- If the carcass is opened, the tissues are exposed to air, and then the organisms are able to sporulate.
- Spores resist dying for long periods of time.
- Spores surviving for 60 or more years have been recorded.
- Heat fixation of smears does not kill spores
- Resist dry heat at  $140^{\circ}\text{C}$  for 2-3 hrs and boiling for 10 mts.
- Killed at  $120^{\circ}\text{C}$  for 10 min and 4%  $\text{KMnO}_4$  treatment for 15 mts



# VIRULENCE FACTORS

## ○ Poly D glutamic acid capsule

Plasmid mediated. Protects against phagocytosis, lytic antibodies, and complement activity.

Have two plasmids PX 01 and PX 02

- It secretes **two toxins** which are composed of three proteins component
  - The lethal toxin (PA+LF)
  - The edema toxin (PA+EF)
- a) Oedema factor (EF)
- b) Protective antigen (PA)
- c) Lethal factor (LF)
- Work in combination and have little or no toxic action as single entities
- Although protective antigen induces antibodies which confer partial immunity.
- Protective antigen acts as the binding moiety for both oedema factor and lethal factor.

- Anthrax toxin is an A/B toxin.
- Each individual anthrax toxin protein is nontoxic.
- Toxic symptoms are not observed when these proteins are injected individually into laboratory animals.
- The co-injection of PA and EF causes edema, and the co-injection of PA and LF is lethal.
- Anthrax toxin is *A / B* paradigm. The *A* component is enzymatically active, and the *B* component is the cell binding component.
- Anthrax toxin is of the form A<sub>2</sub>B, where the two enzymes, EF and LF, are the A components and PA is the B component.



# ENZYME FUNCTION OF LF AND EF

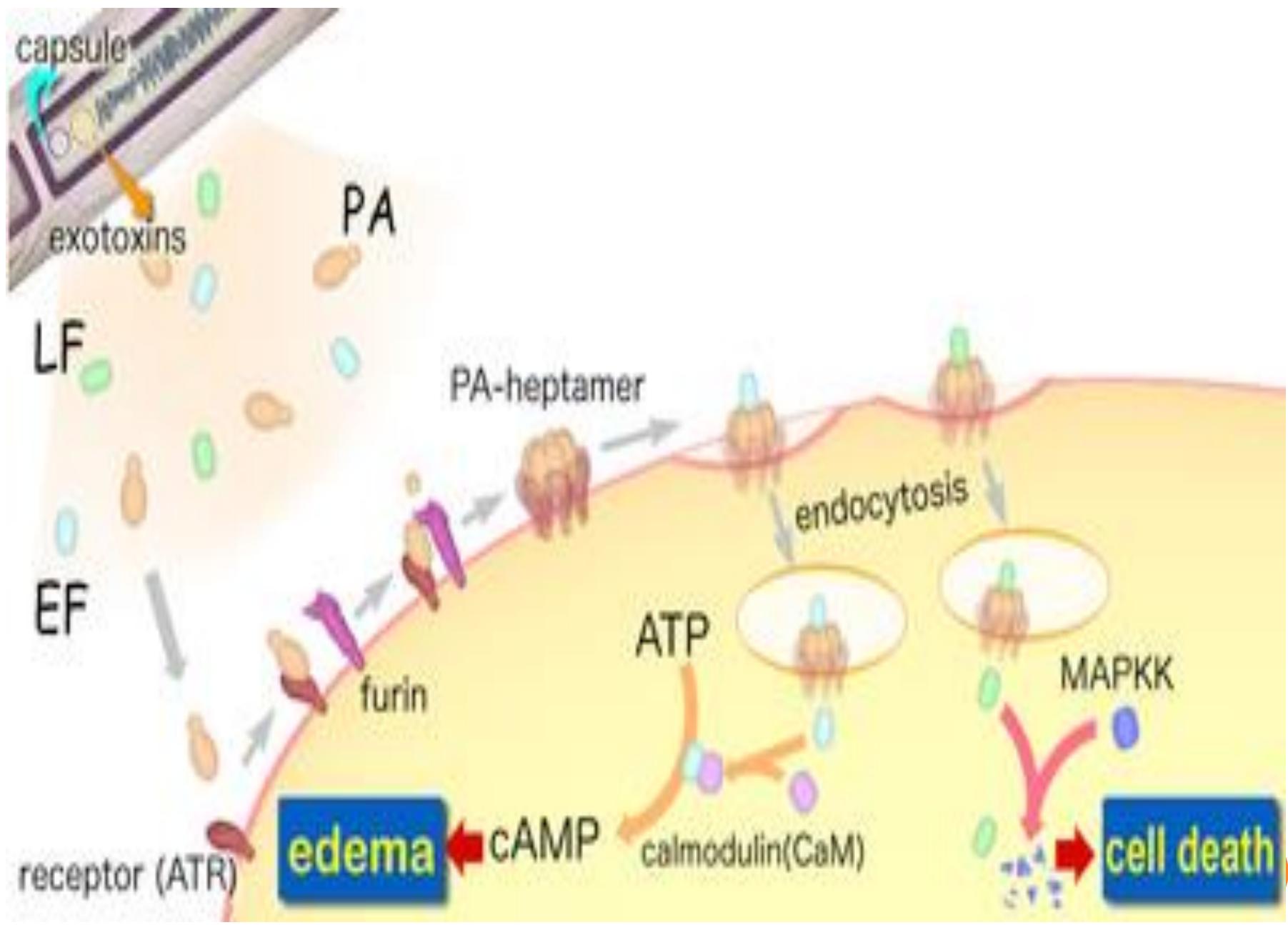
- Once in the cytosol, the EF and LF then carry out their respective damage-inducing processes
- EF acts as a  $\text{Ca}^{2+}$  and calmodulin dependent adenylate cyclase that greatly increases the level of cAMP in the cell.
- This increase in cAMP upsets water homeostasis, severely imbalances the intracellular signalling pathways, and impairs macrophage function, allowing the bacteria to further evade the immune system.



# ENZYME FUNCTION OF LF AND EF

- LF also helps the bacteria evade the immune system through killing macrophages.
- Once in these cells, LF acts as a Zn<sup>2+</sup>-dependent endoprotease that snips off the N-terminus of mitogen-activated protein kinase kinases (MAPKK).
- This inhibits these kinases by not allowing them to efficiently bind to their substrates, which leads to altered signaling pathways and ultimately to apoptosis.





# VIRULENCE FACTORS

- The resultant upset in water homeostasis causes the fluid accumulation seen in clinical disease.
- Neutrophils are the principal target of oedema factor which severely inhibits their function.
- Lethal toxin consists of lethal factor, a zinc metallo-protease and protective antigen which acts as the binding domain as for oedema factor.
- In naturally-occurring disease, local effects of the complex toxin include swelling and darkening of tissues due to oedema and necrosis.
- When septicaemia occurs, increased vascular permeability and extensive haemorrhage lead to shock and death



- Kills phagocytes & Inhibits complement.
- Capillary thrombosis occurs
- Increases capillary permeability
- Blood pressure falls
- Increase in WBC, Damages clotting mechanism
- Decreased oxygen consumption by tissues
- Suppresses CNS- myocardial hypoxia and death.
- Net effect of the toxic complex on the animal is to produce hemorrhage, edema, shock, and death.

# DIFFERENTIATION OF BACILLUS SPECIES

- The ability to grow aerobically and to produce catalase distinguishes *Bacillus* species from the Clostridia which are also Gram-positive, endospore-forming rods.
- Colonial characteristics of *Bacillus* species
- *B. anthracis* colonies are up to 5 mm in diameter, flat, dry, greyish and with a 'ground glass' appearance after incubation for 48 hours.
- At low magnification, curled outgrowths from the edge of the colony impart a characteristic, 'Medusa head' appearance. Rarely, isolates are weakly haemolytic.



- *Bacillus cereus* colonies are similar to those of *B. anthracis* but are slightly larger with a greenish tinge.
- The majority of strains produce a wide zone of complete haemolysis around the colonies. Because they have some similar characteristics, *B. anthracis* and *B. cereus* require careful differentiation.
- *Bacillus licheniformis* colonies are dull, rough, wrinkled and strongly adherent to the agar.
- Characteristic hair-like outgrowths are produced from streaks of the organisms on agar media.
- Colonies become brown with age. The name of this species derives from the similarity of its colonies to lichen.
- The name *Clostridium piliforme* has been proposed for *Bacillus piliformis*, the agent of Tyzzer's disease

**Table 15.1** Differentiating features of *Bacillus anthracis* and *B. cereus*.

| Feature   | <i>B. anthracis</i>     | <i>B. cereus</i> |
|---|-------------------------|------------------|
| Motility  | Non-motile              | Motile           |
| Appearance on sheep blood agar  | Non-haemolytic          | Haemolytic       |
| Susceptibility to penicillin (10 unit disc)                                     | Susceptible             | Resistant        |
| Lecithinase activity on egg yolk agar   | Weak and slow           | Strong and rapid |
| Effect of gamma phage   | Lysis                   | Lysis rare       |
| Pathogenicity for animals (application to scarified area at tail base of mouse) | Death in 24 to 48 hours | No effect        |

## Difference between *B. anthracis* & Anthracoid organisms

| <i>B. anthracis</i>             | Anthracoid organisms  |
|---------------------------------|-----------------------|
| Non motile                      | Generally motile      |
| Capsulated                      | Non capsulated        |
| Grows in long chains            | Grows in short chains |
| No turbidity in broth           | Turbidity in broth    |
| Inverted fir tree in gelatin    | Atypical or absent    |
| Methylene blue reduced weakly   | Reduced strongly      |
| Haemolysis weak / Absent        | Strong                |
| Liquefaction of gelatin is slow | Rapid                 |
| Lecithinase reaction is weak    | Rapid                 |
| Ferments salicin slowly         | Rapid                 |
| Pathogenic to G.pigs & mice     | Non pathogenic        |
| Susceptible to gamma phage      | Not susceptible       |

**Table 44.** Main diseases and hosts of the *Bacillus* species.

| Bacillus species                                 | Host(s)  | Disease   |
|--|--|---|
| <i>B. anthracis</i>                              | Cattle and sheep                               | Septicaemic form of anthrax. Usually sudden death   |
|  | Pigs   | Subacute anthrax with oedematous swelling in pharyngeal tissues and regional lymphadenitis or intestinal form with a higher mortality           |
|  | Horses   | Oral route: septicaemia with colic and enteritis.<br>Wound infections: localised oedema and lymphadenitis                                       |
|  | Carnivores<br>(including mink)                 | Comparatively resistant. Disease pattern similar to that in pigs. A massive dose from eating anthrax-infected carcasses can lead to septicaemia |
|  | Humans   | Skin form: 'malignant pustule'.<br>Pulmonary ('wool-sorters' disease') and intestinal forms are often fatal                                     |
| <i>B. cereus</i>                                 | Humans   | Food poisoning  |
|  | Cattle   | Rare cases of mastitis  |
| <i>B. licheniformis</i>                          | Cattle and sheep                               | Reported as a cause of abortion   |
| ' <i>B. piliformis</i> '<br>(taxonomy uncertain) | Laboratory mice,<br>foals and other<br>animals | Tyzzer's disease. An acute fatal infection causing hepatitis,<br>enteritis and colitis  |

# ANTHRAX – DISEASE

- Synonyms: Malignant Pustule, Wool sorters' Disease, Hide porter's disease, Spleenic Fever
- Anthrax is an acute febrile disease of mammals caused by *B. anthracis* and characterized by an enlarged, black, soft spleen, edema and hemorrhage of subcutaneous and sub serous tissues
- *Bacillus anthracis* is pathogenic for cattle, sheep (except Algerian), mules, horses etc.
- Characterized by sudden onset and a rapidly fatal course.
- Some animals in a herd are found dead without having previously shown any evidence of a disease.



# CATTLE, SHEEP AND GOAT

- **Per acute form:** may sometimes occur in herbivores; may terminate fatally in 1-2 hours, sick animals are rarely seen.
- Rapidly-developing cerebral anoxia, pulmonary edema
- **Acute form:** is fatal in less than 24 hours, the first sign is a rise in temp. to about 104-108°F.
- Excitement stage is followed by depression, respiratory distress, trembling staggers, convulsions, and death.
- Rumen stasis and a great reduction in the amount of milk produced.
- One of the most important features in fatal cases is the bloody discharge from the natural openings, particularly the anus.
- Organism found in the excretion or the blood in fairly large numbers at the time of death.

# SWINE AND HORSES

- Anthrax usually assumes a localized form in swine and horses. These animals are infected only by eating heavily contaminated feed, either the raw meat of animals which have died of anthrax, or in the case of swine, infected bone or meat meal given as a feed supplement.
- Horses: course acute-to-subacute (survive  $\leq 96$  h)
- Symptoms: colic, edematous swellings of the throat, neck, shoulders
- Transmission: insect bite: similar to human cutaneous anthrax
- Local subcutaneous edema affects throat, ventral thorax, abdomen



# HUMANS:

("Woolsorters' disease“ )- fatal, cutaneous – carbuncle & intestinal meningitis.

## **Inhalation Anthrax (Wool sorter's Disease)**

- Dust particles contaminated with spores are inhaled,
- deposit in terminal alveoli
- Spores engulfed by macrophages, transported to regional LN
- Germinate, vegetative cells produce toxin
- Extensive necrotic hemorrhage, Multiple organs
- Involved, rapid death frequently results



# HUMANS:

## **Gastrointestinal Anthrax**

- Results from ingestion of contaminated meat
- Organisms or spores penetrate oropharynx / intestinal mucosa
- Deposited in sub mucosal tissue, multiply and produce toxin
- Usually extends to regional Lymph node, systemic symptoms develop

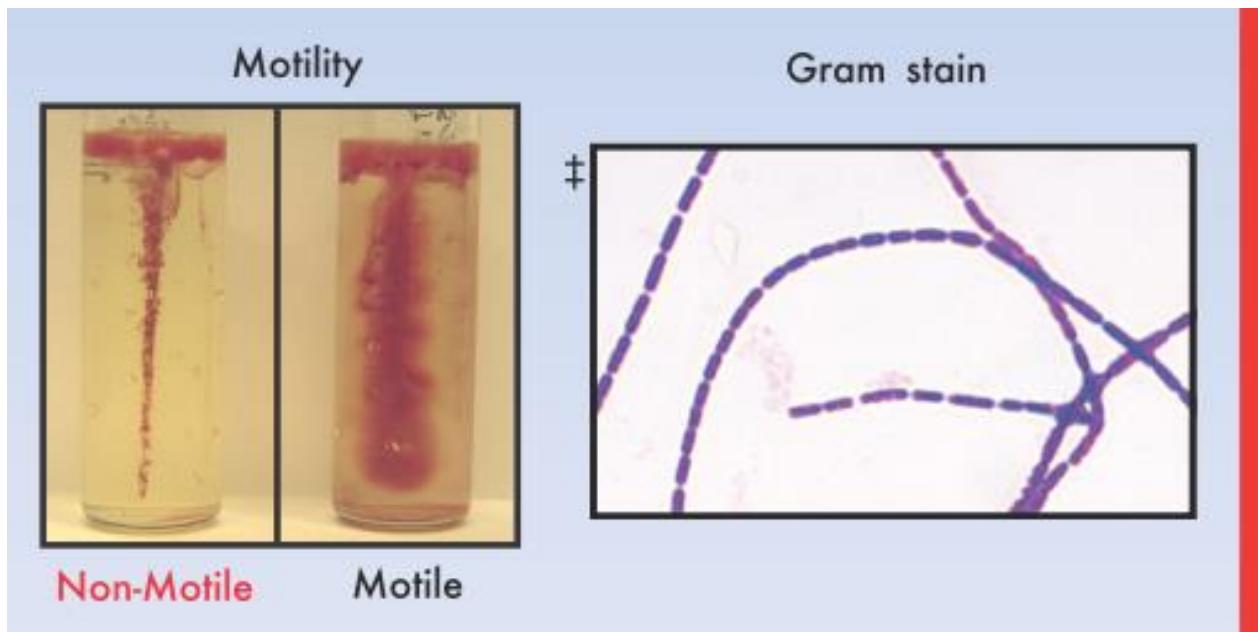
## **Cutaneous Anthrax**

- Malignant carbuncle folliculitis



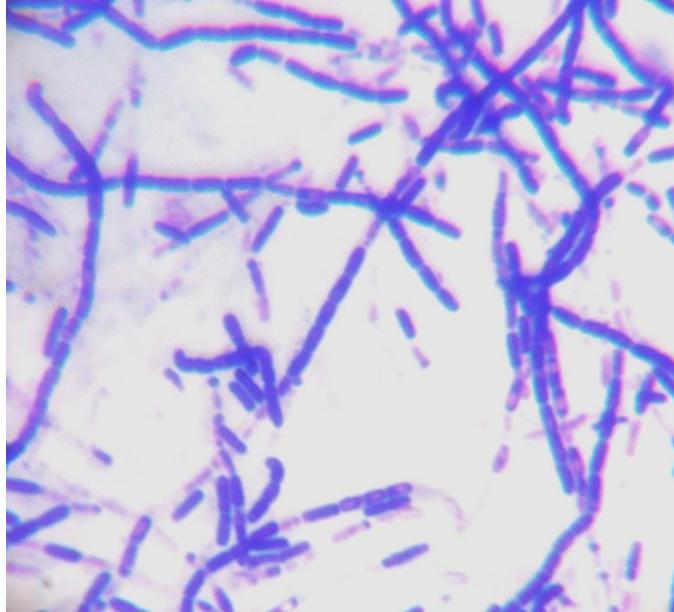
# DIAGNOSIS

- Clinical symptoms
- Microscopical examination of peripheral Blood films from dead animals
- Cultural examination
- Bacteriological examination of hair, wool, hide, bone, bone meal & others



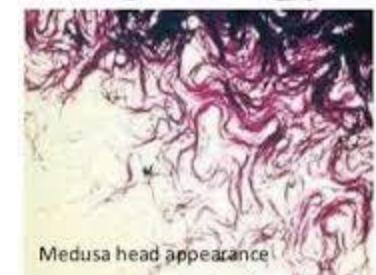
# MICROSCOPY

- Gram staining: Anthrax is an aerobic, Gram positive, rod-shaped bacillus occurring in chains with a propensity to form spores under unfavourable conditions
- in high power microscopy it gives boxcar appearance. (Also called bamboo-stick appearance)





Characteristic, 'Medusa head' appearance on Blood Agar



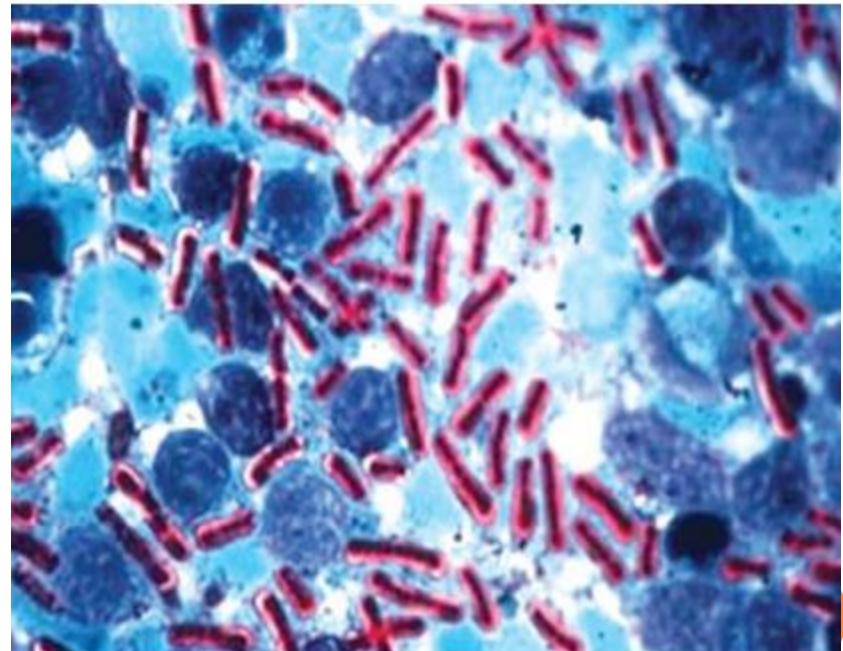
Medusa head appearance



‘Ground glass’ appearance colony

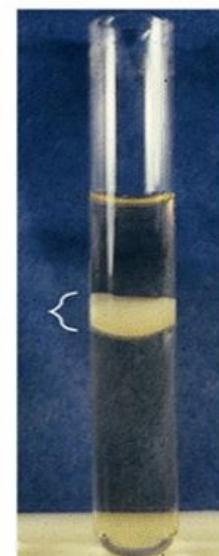
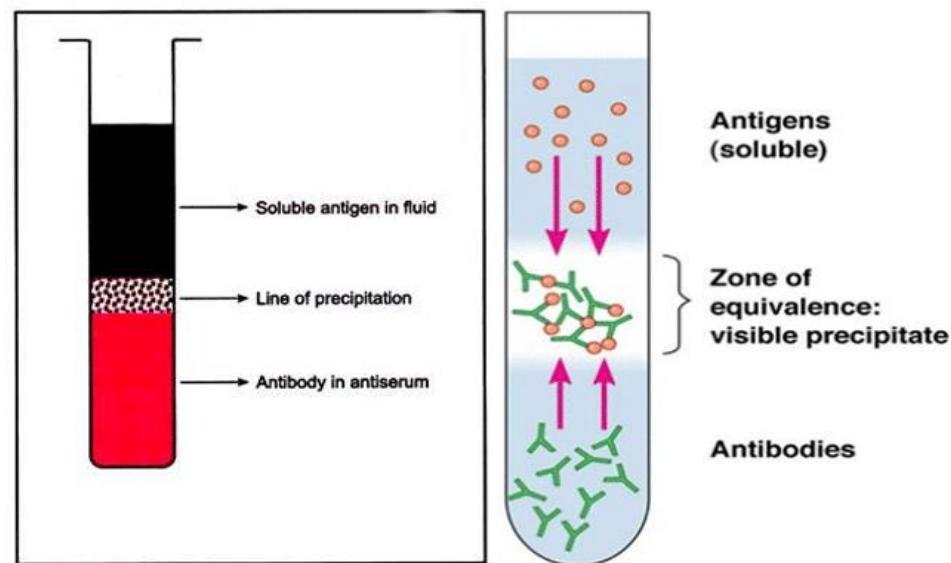


- **Mc'Fadyean reaction:** Giemsa or Polychrome methylene blue stains are used to demonstrate the capsule which is of diagnostic importance. The capsular material is more abundant if the blood smear has been taken from a recently dead animal.
- Polychrome methylene blue-stained smears reveal square-ended, blue rods in short chains surrounded by pink capsular material and is characteristic for *B. anthracis*



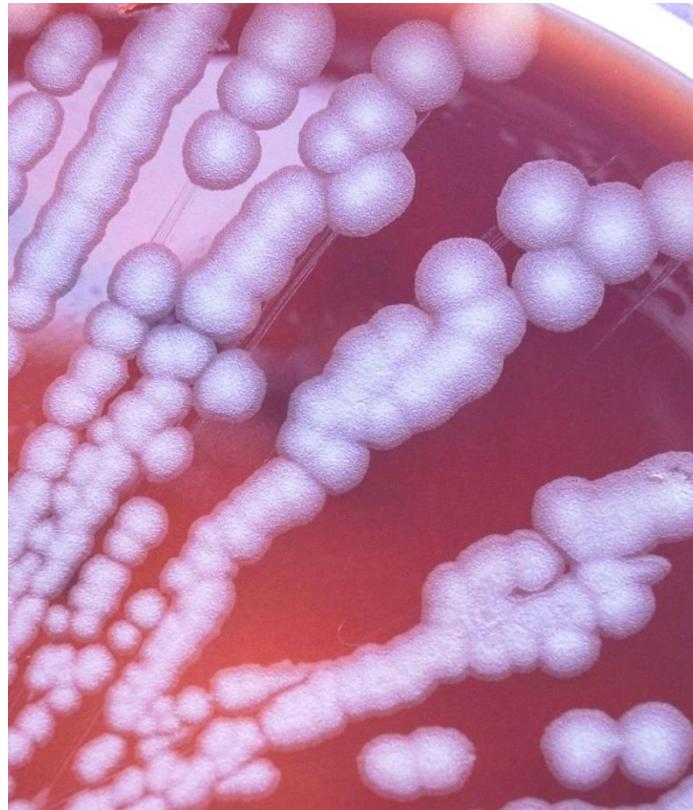
# ASCOLI'S PRECIPITATION TEST

- This thermoprecipitation test is used if viable *B. anthracis* can no longer be demonstrated in tissues.
- Boil a piece of tissue (ear piece) in 5 ml of acidified saline (with 1/1000 acetic acid) for 5 minutes.
- Filter the fluid and this will serve as the source of antigen.
- Take 0.5 ml of anthrax antiserum in a narrow tube and add 0.5 ml of the filtrate.
- Development of a distinct ring of precipitate at the interface within 15 minutes indicates positive reaction.



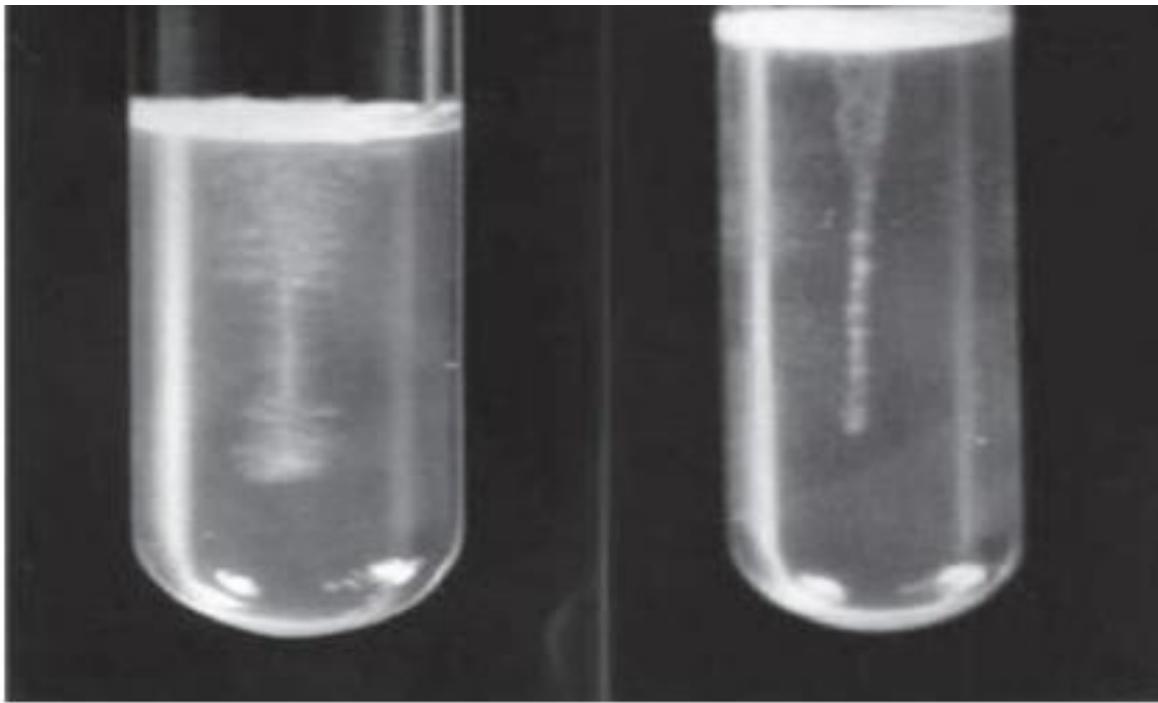
# STRING OF PEARL'S TEST

- When *Bacillus anthracis* is grown for 2-3 hours on solid media containing 0.05 to 0.5 IU penicillin / ml, due to impairment of cell wall, the bacilli become spherical in appearance resembling a string of pearls. This test is an adjunct to the rapid recognition of anthrax bacilli.



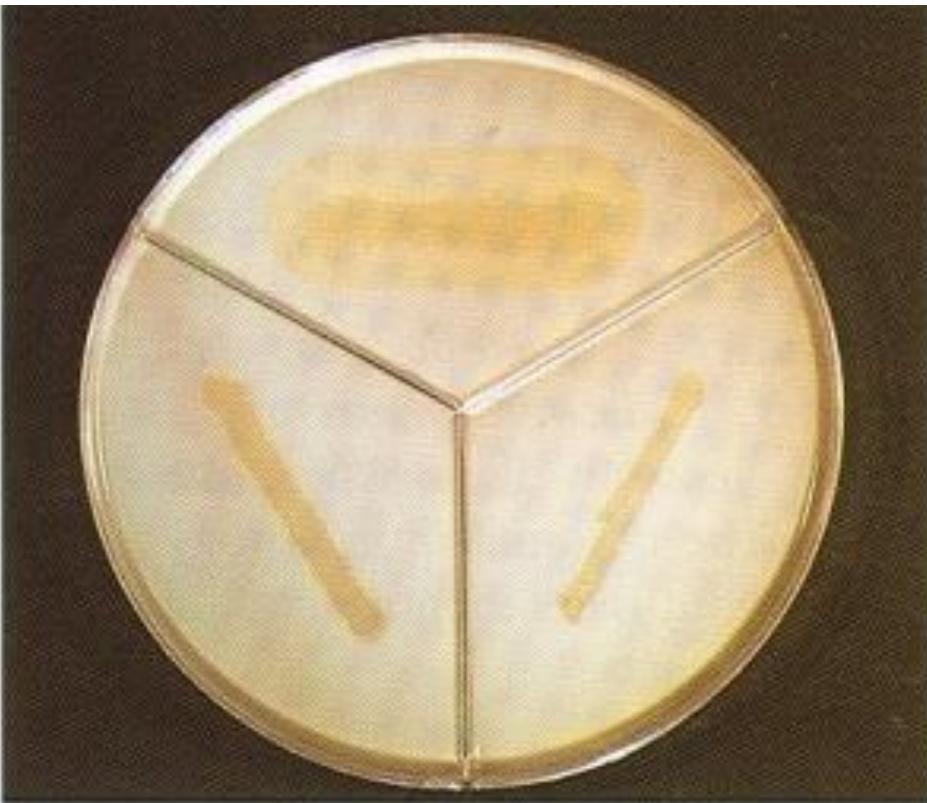
## GELATINE STAB CULTURE

- It forms a characteristic ‘inverted fir tree’ appearance with slow liquefaction commencing from the top.



**FIG. 28-2.** Inverted fir tree appearance of colony of *Bacillus anthracis* in a gelatin stab.

# LECITHINASE ACTIVITY TEST



**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.

# ANIMAL INOCULATION TEST

- Sub-cutaneous inoculation of suspected material into guinea pig or mouse result in death within 48 hours with lesions like gelatinous haemorrhagic oedema at the inoculation site, congested viscera, dark red blood and enlarged darkened spleen.
- Smears from splenic pulp if stained by Gram's method will reveal typical Gram positive bacilli.

## PHAGE LYSIS

- Anthrax bacillus is highly susceptible to gamma bacteriophage. This property is used to distinguish anthrax bacillus from other anthracoid bacilli.

## FURTHER READINGS

- Clinical Veterinary Microbiology 2nd Edition 2013 By Bryan Markey
- Veterinary Microbiology and Microbial Disease



thank you

