Bacillus Anthracis

HISTORICAL IMPORTANCE

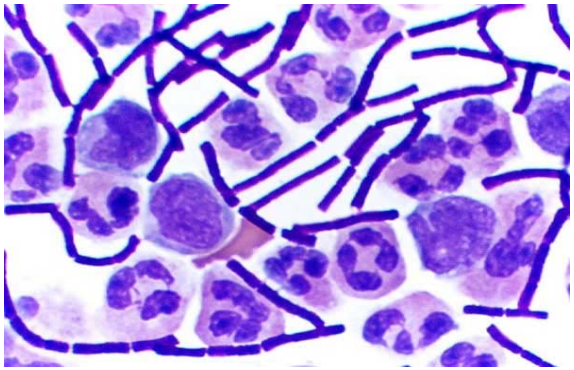
- First pathogenic bacteria to be seen under microscope (Pollender 1849)
- First demonstration of blood borne transmission (Davaine 1850)
- The first bacterium shown to be the cause of a disease- **Koch's Postulate**
- In 1877, Robert Koch grew the organism in pure culture, demonstrated its ability to form endospores
- First effective vaccine (Pasteur 1881)

Robert Koch

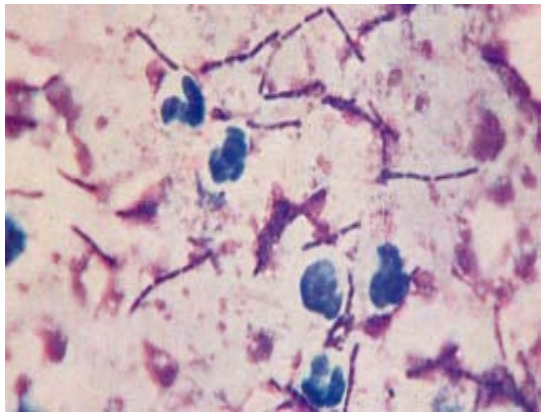
Louis Pasteur

AGENT

- ***Bacillus anthracis***
- Gram positive rods
  - 3-10 micron by 1-1.6 micron

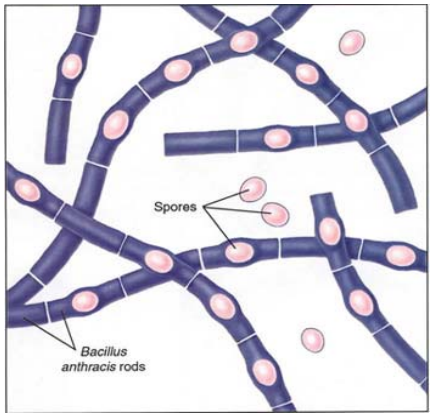


- Aerobic bacilli forming heat-resistant spores
- Non acid fast, Non-motile

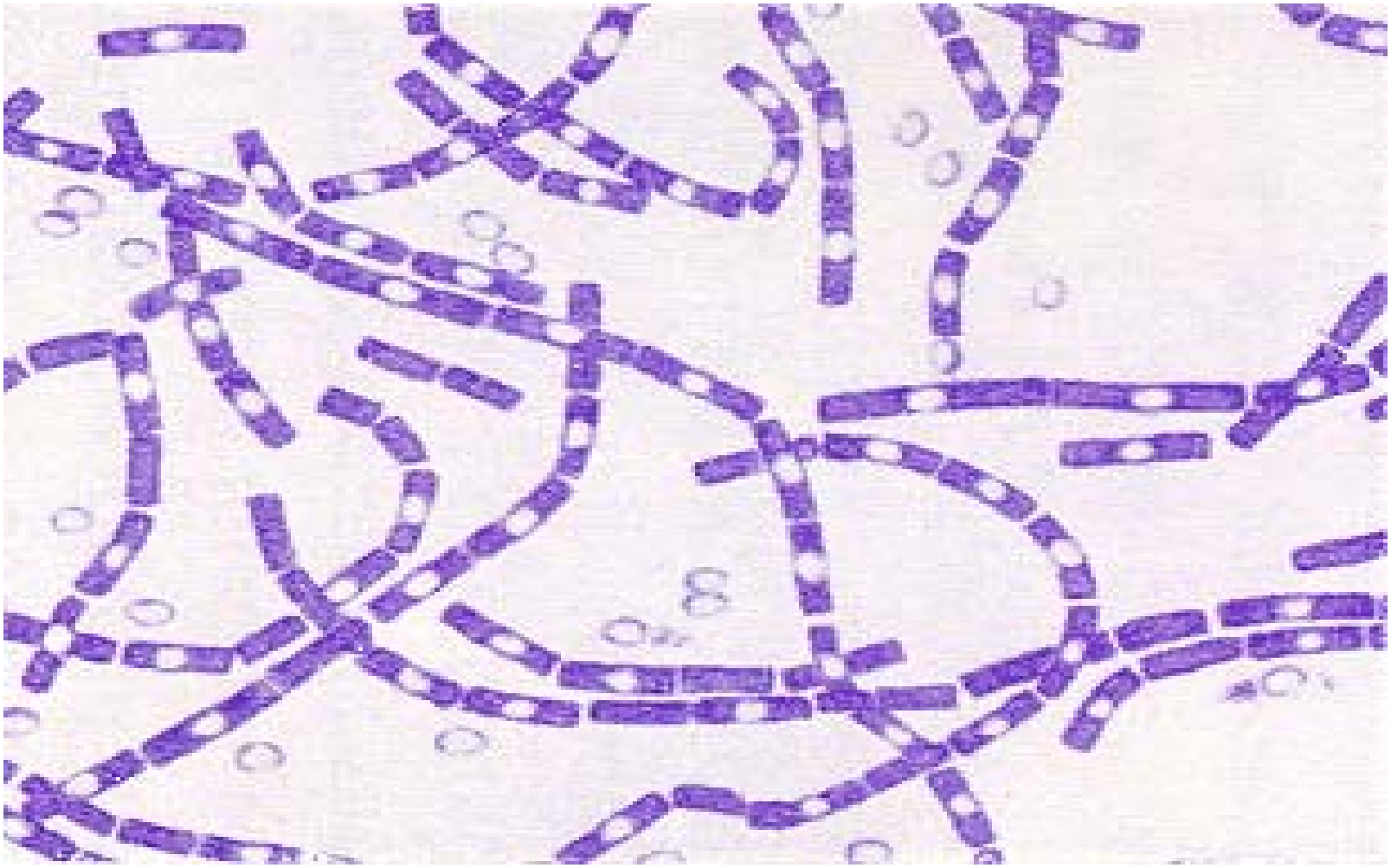


- Capsule (poly d-glutamate-protein)
  - (10-25% CO_2 bicarbonate, serum albumin charcoal, starch

Spores



- Aerobic bacilli forming heat-resistant spores
  - Formed in culture/soil
  - survive in soil for decades
  - Never formed in animal body
  - Occurs under un-favourable conditions
  - Encouraged by DW, 2% NaCl, oxalated agar
  - Inhibited by calcium chloride
  - central or subterminal, oval
  - Of same width as the bacillary body-no bulging
  - resistant to drying/UV/gamma rays/heat



***Bacillus anthracis*. Gram stain. The cells have characteristic squared ends. The endospores are ellipsoidal shaped and located centrally in the sporangium. The spores are highly refractile to light and resistant to staining.**

AGENT (contd...)

- Cultural characteristics
  - 35-37° C
  - Optimum for sporulation- 25-30°C
  - Good growth occurs on ordinary media
  - grey white (sheep blood agar)
  - Irregular, non-haemolytic
  - Tenacious, rough

LPF : **Medusa head colony**

- Edge of a colony is composed of long, interlacing chains of bacilli resembling locks of matted hair

PLET medium

SPORES SURVIVE FOR MANY YEARS (DRY STATE AND SOIL)

- DUCKERING: 2% Formaldehyde kills spores at 30-40°C for 20 mins for disinfection of wool and as 0.25% at 60°C for 6 hrs for animal hair and bristles

- 4% $KMnO_4$ kills spores in 15 mins

PATHOGENICITY

HOST

- In nature, primarily an infection of :
  - herbivores :cattle, horse, goat, sheep, - from soil they graze,
  - wild animals-carnivores
- Farmers
- Animal product handlers :meat, hide, wool, hair, bones etc.
- Healthcare workers
- Lab personel
- ? Common man : threat of bioterrorism

ENVIRONMENT

- Favorable factors for spore formation & survival
  - abundant rainfall following a period of drought
  - soil pH >6 , abundant organic matter
  - improper disposal of animal carcasses (ideally incineration or rendering needs to be done)

- Human-animal interaction

- Asymmetric warfare

VIRULENCE FACTORS

Anthrax Toxin – Complex of proteins (all the components thermolabile)

- **Edema factor (Factor I)**
- **Protective factor (Factor II)**
- **Lethal Factor (Factor III)**

Protein capsule – Poly D Glutamic acid capsule
- Inhibits phagocytosis (Unencapsulated strains – nonpathogenic)

Anthrax Toxin

↓

Protective antigen : Binds plasma membrane of target cells

↓

Cleaved to 2 fragments (cellular trypsin or proteases)

↓

Larger fragment is attached to cell surface – binding domain for LF & EF

↓

Specific receptor mediated endocytosis of LF & EF

EDEMA FACTOR

(Edema Factor + Protective Ag = Edema toxin)

Calmodulin dependent adenyl cyclase

Increased cellular cAMP ⟶ Edema ⟶ Impaired Neutrophil function

Depletes ATP from Macrophages

LETHAL FACTOR

(Lethal Factor + Protective Ag = Lethal toxin)

Zinc metallo proteases that inactivates protein kinases

Stimulates Macrophages –TNF alpha and IL – 1 beta – Shock & Death

Death due to oxygen depletion, secondary shock, increased vascular permeability, respiratory failure and cardiac failure.

Sudden and unexpected.

Clinically three forms of Human anthrax occur

A. Cutaneous anthrax-Hide-porters disease
A. Pulmonary anthrax-Woo-Isorters disease
B. Intestinal anthrax

Broadly can be classified into

Non Industrial/Agricultural (Through infected animals):

Cutaneous anthrax
Rarely intestinal anthrax

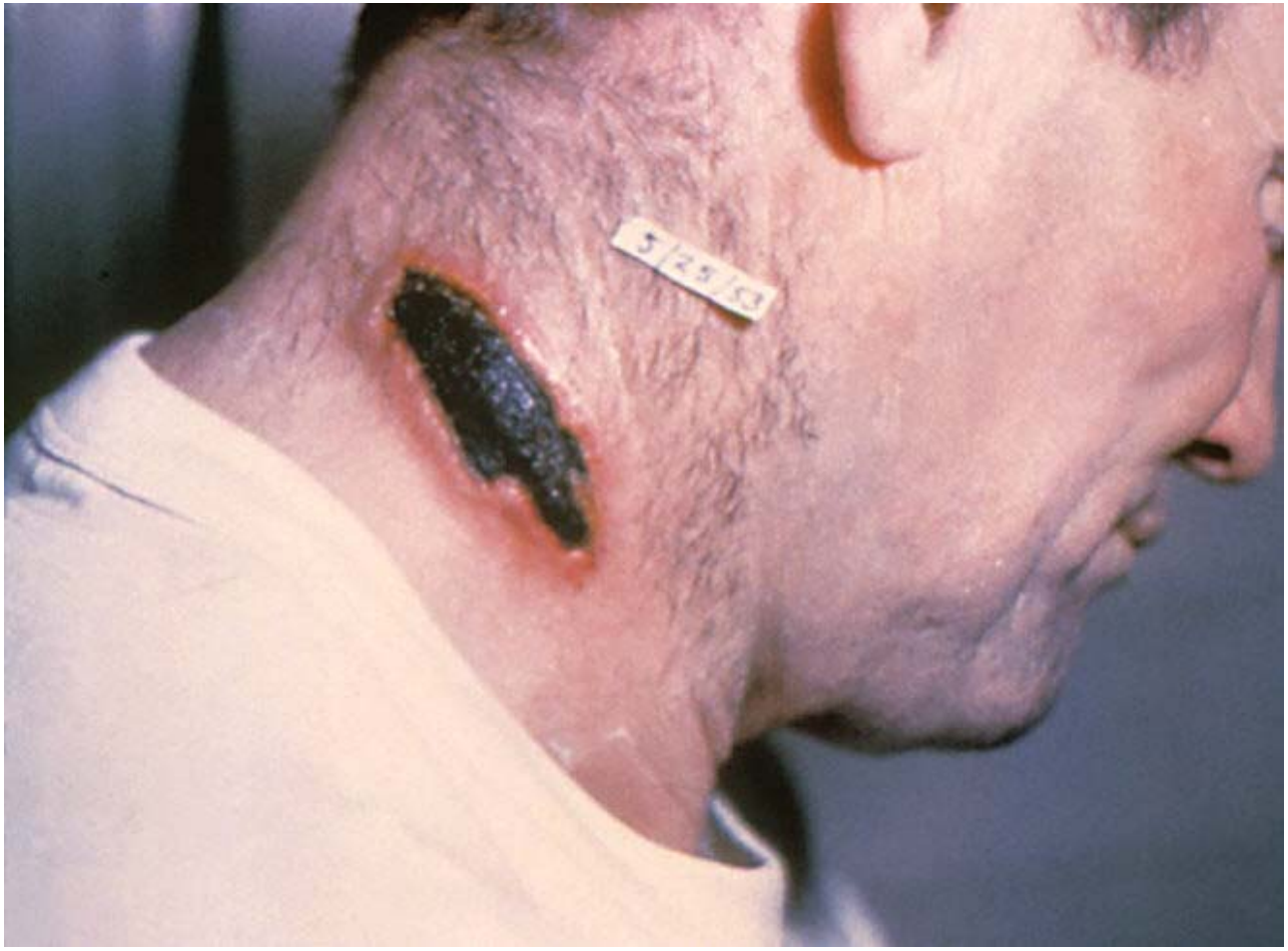
Industrial Anthrax (Through animal products):

Mostly through animal products(wools, hair, hides, bones)
Likely to develop Cutaneous and pulmonary anthrax (inhalation)

Cutaneous Anthrax

- Mainly in professionals(Veterinarian, butcher)
- Spores infect skin- a characteristic gelatinous edema develops at the site (Papule- Vesicle-Malignant Pustule- Necrotic ulcer)
- Hide porter's disease
- IP – 1 to 12 days, 2000 cases annually
- 95-99% of all human anthrax occur as cutaneous anthrax
- Mortality – 20% untreated , 1% treated (edema/septicaemia)
- 80-90% heal spontaneously (2-6wks)
- 0-20% progressive disease – develop septicemia

Cutaneous Anthrax



Pulmonary Anthrax

- Wool sorter's disease
- Most common source of exposure – industrial exposure to spores specially tanneries, wool handlers
  - Hemorrhagic pneumonia, Haemorrhagic mediastinitis
  - Progress to septicemia very rapidly
  - Hemorrhagic meningitis –complication
- Acquired through inhalation of spores (Bioterrorism - aerosol)
- Present with symptoms of severe respiratory infection(High fever & Chest pain)
- Mortality rate is very high > 95%

- Intentional contamination of mails since 2001
- October 2001 letter associated Anthrax outbreak
- 22 cases
  - 11 Inhalational (5 deaths)
  - 11 Cutaneous (No deaths)
    - *Very different distribution compared to naturally occurring disease*
- Mortality – historically: >95%

Gastrointestinal Anthrax

- Rare –primitive communities who eat carcasses of animals dying of anthrax
- Nausea, anorexia, vomiting, fever
- Progresses to severe abdominal pain and bloody emesis and diarrhea
- Death 2 to 5 days after onset of symptoms
- Very difficult to diagnose

LAB DIAGNOSIS

- Samples:

  -Cutaneous:- vesicular fluid on swabs

  - full thickness punch biopsy in 10% buffered formalin

  -Gastrointestinal:- stool, blood, peritoneal fluid, splenic & node aspirate

  -Inhalational:- pleural fluid

  - blood/paired sera

  -Meningeal:- CSF

LAB DIAGNOSIS

- Disposable gloves, aprons, boots, face shields, respirators (autoclaved)
- Indisposable –

  10% formaldehyde

  5% glutaraldehyde

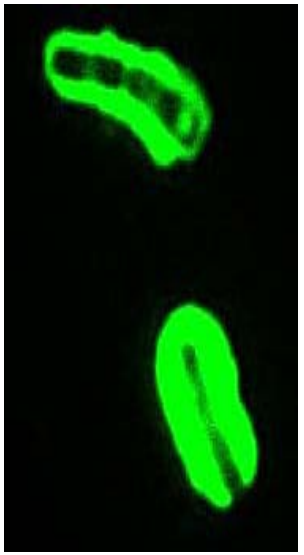
  fumigation
- Aerosol tight rotors
- Biosafety cabinet
- Level 2 / 3 practices

- **Microscopy**

Stains

  - Gram stain

- **<u>Carcasses 1 or 2 day old</u>**
  - **Aspirate blood - MacFadyean stain for bacilli**
  - **Direct demonstration by IFA**
  - Spore stain



- **Culture**:
  - SBA
  - Gelatin stab culture
  - Polymyxin lysozyme EDTA thallous acetate (PLET agar)

- **ELISA:** based on anthrax toxin (PA, LF and EF) for routine confirmation of antibodies

- **Molecular techniques** (Only in the referral laboratories):
  - RFLP
  - PCR Fingerprinting

- **Animal Inoculation:** Guinea pig and mice inoculation

TREATMENT

- Antibiotics therapy is effective in human cases but rarely succeeds in animals –not started sufficiently early
- Antibiotic treatment is effective in cutaneous anthrax
- Penicillin, tetracyclines, erythromycin and fluoroquinolones are effective
- Inhalation anthrax can be effectively treated with antibiotics administered prior to lymphatic spread or septicemia

Prophylaxis

- Hygiene-
  - improvement of factory environment
  - Proper sterilisation of animal products
  - Carcasses- buried deep in quicklime or cremated to prevent soil contamination
- Immunisation:
- Prevention of anthrax in animals-
  - Original Pasteur's anthrax vaccine
  - Anthrax bacillus attenuated by growth at 42-43°C
- Spore vaccines:
  - Sterne vaccine-avirulent, mutant strain
  - Mazzucchi vaccine-spores of stable Carbazoo strain in 2% saponin

DIAGNOSIS(contd....)

Characteristic	*B. anthracis*	*B. cereus and B.thuringiensis*
growth requirement for thiamin	+	-
hemolysis on sheep blood agar	-	+
glutamyl-polypeptide capsule	+	-
lysis by gamma phage	+	-
motility	-	+
growth on chloralhydrate agar	-	+
string-of-pearls test	+	-