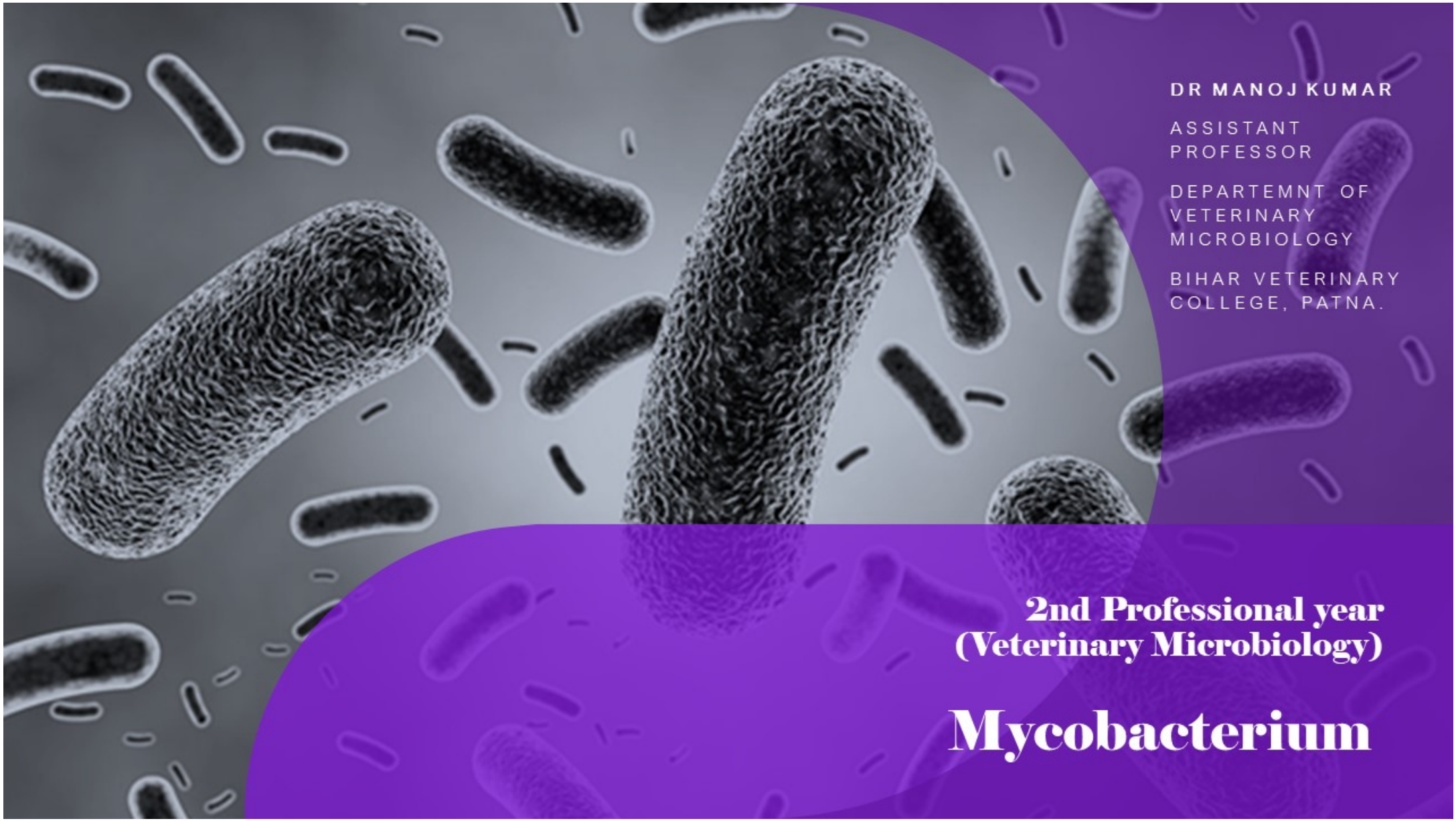

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Mycobacterium

Genus *Mycobacterium*

- Nonmotile, non-spore-forming aerobic bacilli
- **Ubiquitous presence,** 130 species; 7 species cause most infections
- **Pathogenic Mycobacteria**

    M. tuberculosis: airborne

    M. leprae: close contact

    M. avium complex : water/soil

    M. kansasii : water/soil

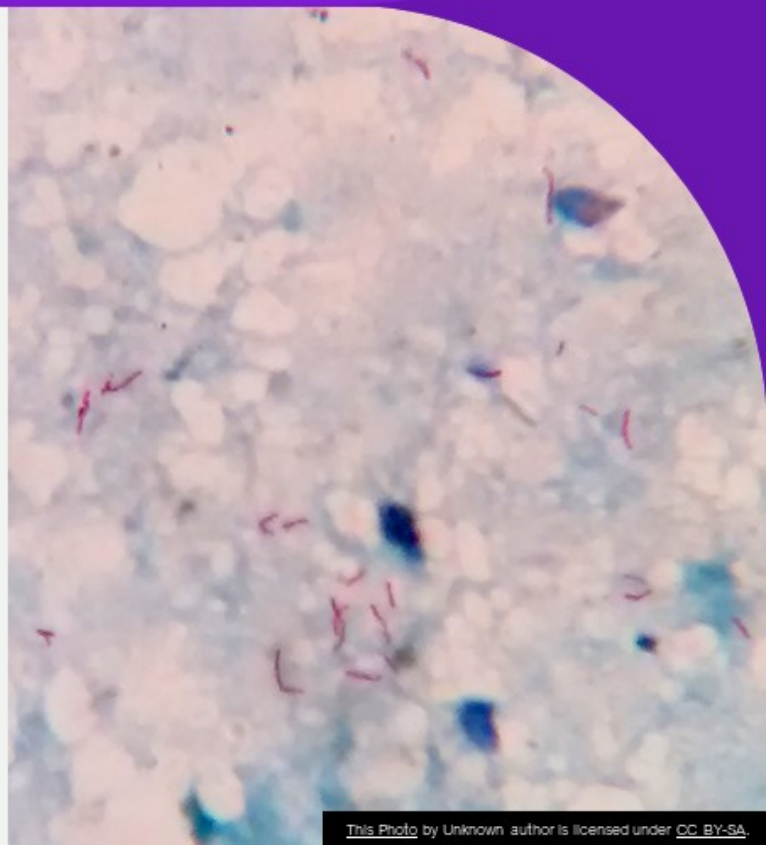
    M. fortuitum : water/soil

    M. chelonae : water/soil

    M. abscessus : water/soil

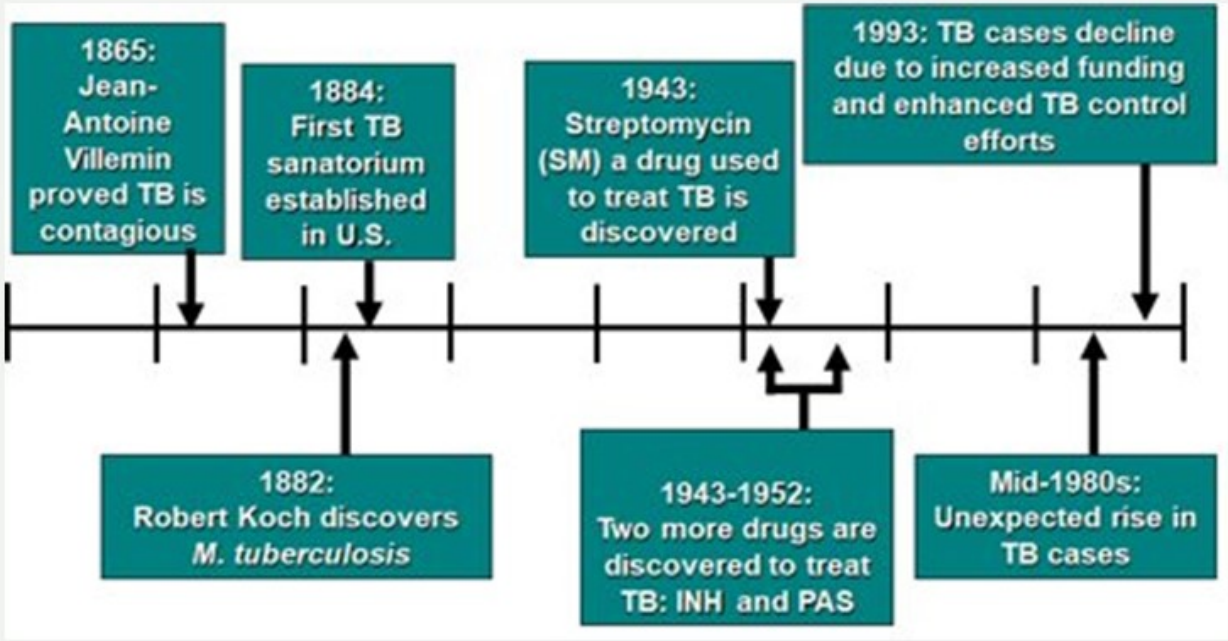
General Features of Mycobacteria –Acid Fast Bacilli (AFB)

- Closely related to the genera Nocardia, Corynebacterium, and Rhodococcus
- What does the term Acid Fast refer to?
- Once stained the rods resist decolorization with acid alcohol (HCl)
- Very beaded and faded on Gram stain
- Gram stain is NOT a good stain to detect AFB



Mycobacterium

- Unique bacteria
- Cell walls contain a lot of waxy material (mycolic acid)
  - inhibits the uptake of nutrients into the bacterial cell
  - causes the cell to clump
- These factors contribute to the slow growth rate
- Mycobacteria do not grow outside of a host except in cultured media
  - Slow growth rate
  - Multiply approximately once every 20 hours



Timeline – History of TB

Mycobacteria – General Characteristics

- Aerobic, no spores, slightly curved or straight rods, rarely branch, variable in length depending on the species.
- *M tuberculosis* is a straight or slightly curved rod, about 3 X 0.3 µm in size, occurring singly, in pairs or as small clumps. *M bovis* is usually straighter, shorter and stouter.
- Hardy in the environment for months and most grow on simple substrates
- Mycobacteria include obligate pathogens, opportunists and saprophytic species
- High amount of mycolic acids and free lipids in cell wall which give many properties to this genus including the AFB staining property

Cell Wall of Mycobacteria

- Inner cytoplasmic membrane

  Anchor : proteins, phosphotidylinositol mannosides, lipoarabinomannan (LAM)

- Thick peptidoglycan layer

  Attach arabinogalactan (a branched polysaccharide) + **mycolic acid** (70-90 carbon)

- No outer membrane

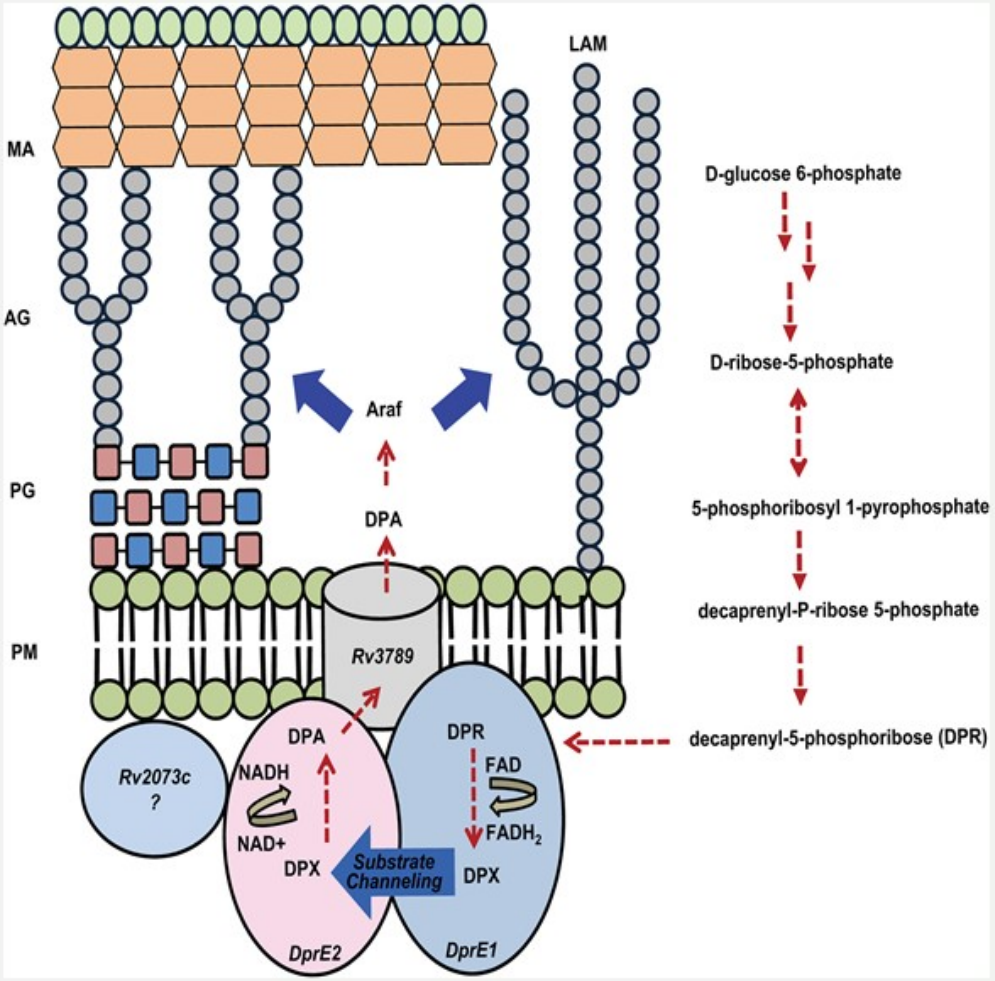
- **Rich in lipids:** responsible for many characteristic properties (acid-fastness, resistant to disinfectants and antibiotics, antigenicity, slow growth, clumping), 60% of dry weight.

  Acid-fastness: mycolic acid -resistant to common laboratory stain. Once stained, cannot be decolorized with acid solutions

- **Polypeptides:** transport proteins and porins; 15% dry weight; **PPD** (purified protein derivatives) - **induce cell-mediated immunity**.

The various cell wall components

MA (Mycolic Acids)
AG (Arabinogalactan)
PG (Peptidoglycan)
PM (Plasma Membrane)
LAM (Lipoarabinomannan)

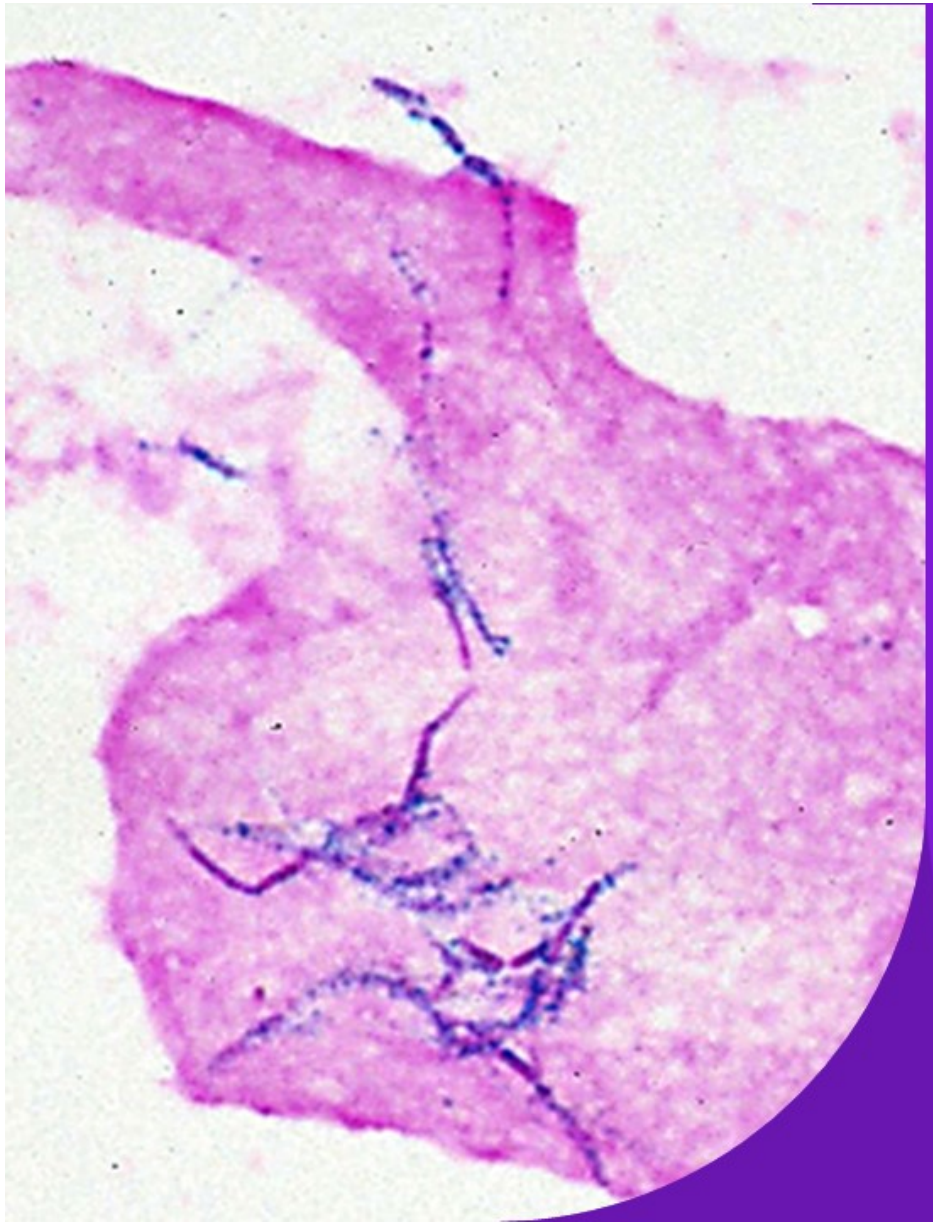


Culture characteristics

- The bacilli grow and invitro time for generation is 14-15 hours.
- Optimum temperature - 37°C and growth does not occur below 25°C or above 40°C.
- Optimum pH is 6.4-7.0.
- *M tuberculosis* is an obligate aerobe while *M bovis* is microaerophilic on primary isolation.
- *M tuberculosis* grows luxuriantly in culture as compared to *M bovis* which grows sparsely.

Identification of the Mycobacteria

- For decades the identification was based on the production of pigment in the light and dark and biochemical reactions
- With expanding taxonomy, biochemical reactions are not able to separate and identify some of the newly recognized species
- New methods have evolved for identification:
- HPLC – high-performance liquid chromatography to identify mycolic acids, good but not the best for definitive speciation
- Genetic probes – RNA/DNA hybridization probes
- MALDI-TOF Mass Spectrometry to analyze proteins
- Sequencing 16 sRNA for genetic sequence identification



Morphology

- *Mycobacterium* species appear as beaded gram-positive short to long rods.
- In clinical material, beaded rods may be observed singly or in small clumps of organisms.

Mycobacteria Taxonomy

- TB and genetically related organisms that are separated taxonomically from the other species
- TB complex include:
  - Mycobacterium tuberculosis
  - M. bovis
  - M. africanum
  - Other vary rare species
- Other Mycobacteria in the TB complex are grouped into “MOTT” Mycobacteria other than TB
- The Runyon System is used to classify those species not in the TB complex (MOTT)- divided into four groups: Pigment when exposed to light in Light Test
  - Pigment in both light and dark in the Light Test
  - No pigment produced in light or dark in the Light Test
  - Growth rate (<= 7 days) – Rapid grower

Taxonomic hierarchy

Domain: *Bacteria*

Division: *Actinobacteria*

Class: *Actinobacteria*

Subclass: *Actinobacteridae*

Order: *Actinomycetales*

Suborder: *Corynebacterineae*

Family: *Mycobacteriaceae*

M tuberculosis complex (mammalian tubercle bacilli) includes:

- *M. tuberculosis*
- *M. canettii*
- *M. bovis*
- *M. caprae*
- *M. pinnipedii*
- *M. microti*
- *M. mungi*
- *M. africanum*

M. avium complex includes:

- *M avium avium* (avian tubercle bacilli)
- *M avium hominissuis* (isolated from human, swine, and other mammals)
- *M intracellulare*

Subspecies of Mycobacterium

Mycobacterium bovis, *species*.

- *Mycobacterium bovis* subsp. *bovis*
- *Mycobacterium bovis* subsp. *caprae*
- *Mycobacterium bovis* subsp. *Bacillus Calmette-Guerin*

Mycobacterium avium, ***species***

- *Mycobacterium avium* subsp. *avium*
- *Mycobacterium avium* subsp. *paratuberculosis*
- *Mycobacterium avium* subsp. *silvaticum*

Light Test – does it produce a yellow pigment after being exposed to light ?

Group I	Group II	Group III
Photochromogen Turn yellow after light exposure	**Scotochromogen-** Always has yellow pigment– light or no light exposure	**Nonphotochromogen** – never has pigment

Runyon Classification System – Groups determined by results of the light test

- **Group I** - Photochromogen – turns yellow when exposed to light, no color in the dark
  - M. kansasii
  - M. simiae
  - M. szulgai when incubated at 25˚C
  - M. marinum

Runyon Classification cont'd

- **Group II** - Scotochromogen – yellow pigment in dark or exposure to light
  - M. gordonae
  - M. scrofulaceum
  - M. szulgai when incubated at 37*C

Runyon Classification cont'd

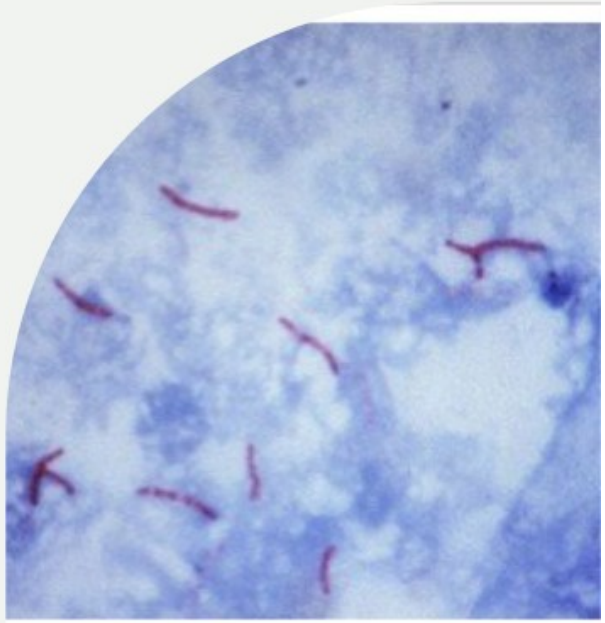
- **Group III** – Non-photochromogen – No pigment produced in the light or dark
  - M. avium-intracellulare
  - M. haemophilum
- **Group IV** – Rapid growers – grow in 7 days or less
  - M. fortuitum group
  - M. abscessus
  - M. chelonae
  - M. mucogenicum
  - >= 20 spec

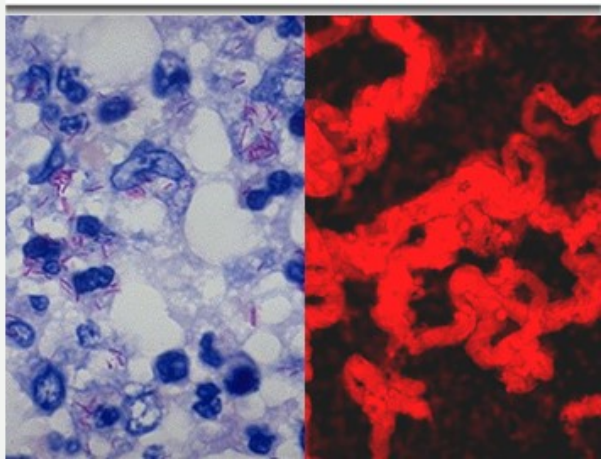
Mycobacterium tuberculosis

- Optimal Temp 37° C, Grows in 12 –25 days
- Buff colored, dry cauliflower-like colony
- Manual tests for identification
  - Niacin Positive - accumulation of niacin, a product produced form growth on this egg containing medium (LJ medium)- must be performed on culture growing on LJ medium
  - Nitrate reduction – Reducing nitrate to nitrite = Positive
  - Confirmation of TB vs M Bovis
    - M bovis = nitrate negative
    - M. bovis does not grow in Thiophene-2-carboxylic hydrazide (T2H)
- Molecular identification:
  - Gen-Probe AccuProbe DNA/RNA hybridization identifies TB complex organisms with excellent accuracy

Mycobacterium tuberculosis

- Demonstrates Cord factor – due to high lipid content in TB organisms they stick together and can develop long ropes – unique to TB





M. bovis

- M. bovis produces disease in cattle and other animals
  - Spread to humans by milk ingestion
  - Most common disease symptoms like that of TB
- M. bovis can cause bladder infections in patients treated with BCG [Bacille Calmette-Guerin] used as an immune adjuvant to treat bladder cancer
  - BCG is an attenuated strain of M. bovis
  - It can become "active" and cause infection in the bladder Is it TB – or is it M Bovis?
  - M bovis = nitrate negative, M TB = nitrate positive
  - M. bovis does not grow in Thiophene-2-carboxylic hydrazide
  - TB grows in this compound
  - M. bovis does not produce Pyrazinamidase enzyme
  - TB produces PYRZ enzyme

- Cattle are considered to be the definitive hosts of *M. bovis*
- Most *M. bovis* infected cattle appear normal.
- They carry and may shed the organism for years without showing signs of disease.
- Clinical signs occur only in the advanced stages of the disease

Transmission & infection

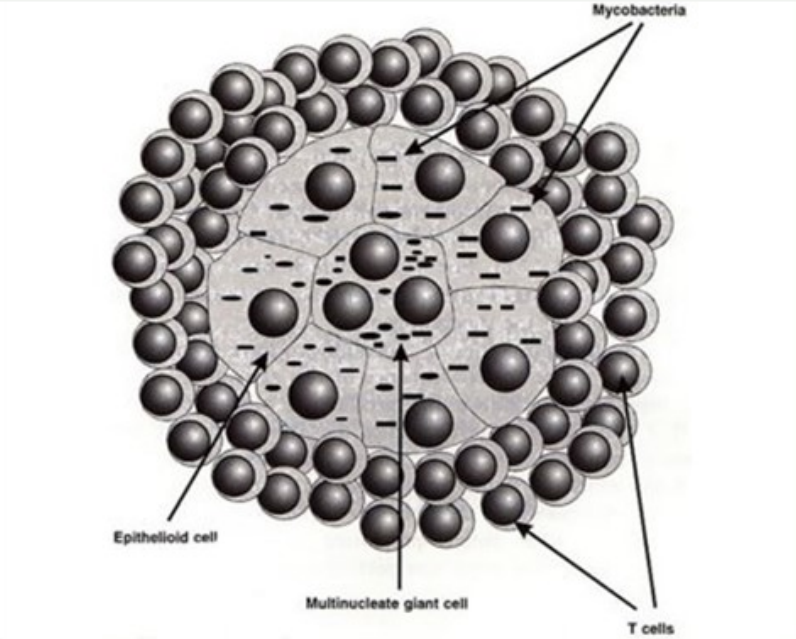
- Bacterium usually enters by inhalation, multiplies locally, and causes primary lesions in the lungs and the lymph nodes that drain the respiratory tract
- Infection also occurs through ingestion of contaminated milk, water, or feed, resulting in primary lesions in the lymph nodes associated with the alimentary tract and the intestine.
- Calves may become infected with *M. bovis* in utero or by ingestion of milk from a tuberculous udder.

Pathogenesis

- Inhaled or ingested *M. Bovis* enter the tissue through breeches in the epithelium lining the respiratory or alimentary tracts or through specialized transporting epithelial cells, called M cells.
- Bacteria in tissue are engulfed by neutrophils and macrophages.
- Some of the infected macrophages migrate carrying the infection to the regional lymph nodes.
- Occasionally, infected macrophages migrate into the blood and the infection is disseminated throughout the body.
- *M. bovis* can infect multiple organ systems or become localized in any organ in the body.

Formation of tubercle

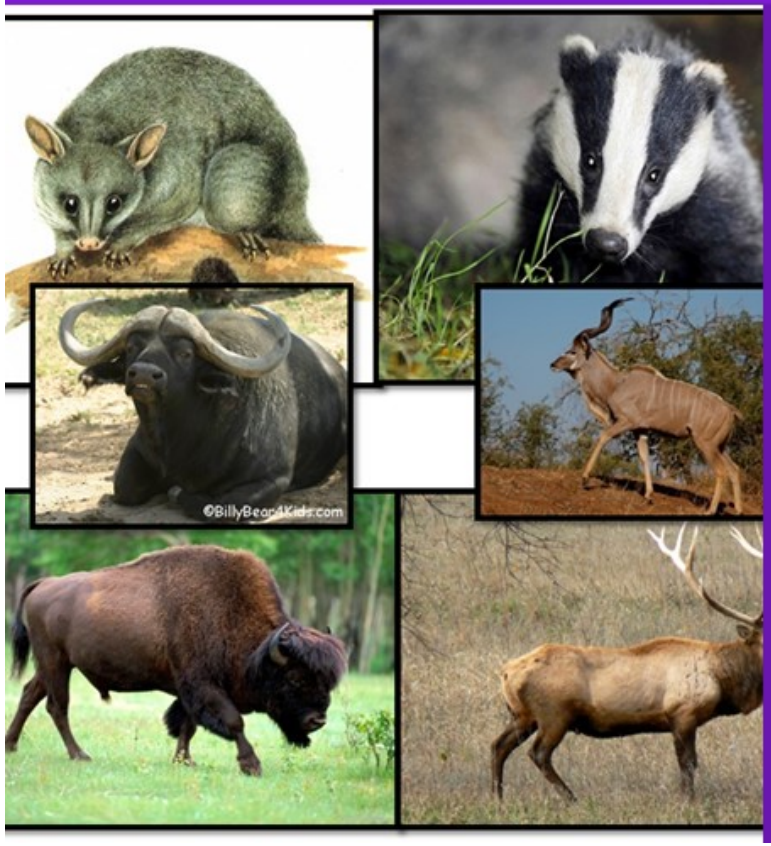
- *Mycobacterium bovis* survives and ultimately destroys the neutrophils and macrophages that engulf it .
- Growth of *M. bovis* in generations of macrophages accounts for the chronic progressive course of the disease.
- The characteristic lesion (tubercle or granuloma) contains debris from necrotic host cells and possibly free *M. bovis* cells at its core.
- The core is surrounded by *M. bovis* infected macrophages (epithelioid and giant cells), which are in turn surrounded by fibrous connective tissue and lymphocytes.
- Resolved or inactive lesions may be walled off by an extensive connective tissue capsule, have calcified debris at the core, and contain few if any viable *M. bovis* organisms.



Clinical signs

- Signs include:
  - Emaciation
  - Fever
  - Coughing
  - Laboured breathing infrequently
  - Diarrhoea
  - Superficial lymph nodes may be swollen, or rupture and drain to the outside.

Maintenance host



- Brush tailed opossums
- Badger
- Bison
- Elk
- African Buffalo
- Kudu

Tuberculosis in domestic animals

- **Tuberculosis in Sheep and Goats :** Lesions caused by *Mycobacterium bovis* in the lungs and lymph nodes of sheep and goats are similar to those seen in cattle.
- **Tuberculosis in Horses:** Relatively resistant ; tuberculosis caused by *Mycobacterium tuberculosis*; they are also susceptible to *M bovis.*
- **Tuberculosis in Elephants:** Causd by *Mycobacterium tuberculosis* . Lesions most often involve the lung and associated lymph nodes.
- **Tuberculosis in Pigs:** Pigs are susceptible to *M tuberculosis*, *M bovis*, and *M avium* complex. *M avium avium* and *M avium hominissuis* .
- **Tuberculosis in Dogs:** Dogs may be infected with *Mycobacterium tuberculosis*, *M bovis*, and occasionally with *M avium* complex or *M fortuitum.*
- **Tuberculosis in Cats:** Cats are quite resistant to infection with *Mycobacterium tuberculosis* but are susceptible to *M bovis*, *M avium* complex, or *M microti*. *M lepraemurium*

Diagnostic methods

- Clinical examination
- Microscopic examination - Ziehl-Neelsen technique
- Macroscopic examination
- Histopathology
  - Rapid diagnosis
  - Good correlation with culture (94%)
  - 85% of lesions diagnosed by histopathology
- Intradermal Tuberculin test (“herd tests”)
- Lymphocyte proliferation test
- Gamma-interferon test
- Animal inoculation

Serological tests

- ELISA (γ-IFN assay)
- Agglutination and precipitation tests
- Complement fixation tests
- Hemagglutination
- Latex agglutination
- Bentonite flocculation
- Indirect fluorescent antibody
- Radioimmunoassay procedures

Diagnostic methods

- Confirmatory diagnosis
- Culture
  - Solid media (Stonebrinks + LJ)
  - Liquid media (MGIT 960)
  - Blood agar
- Polymerase chain reaction (PCR)
- multiplex-PCR (m-PCR)
- Restriction fragment length polymorphism (RFLP)
- Variable number tandem repeat (VNTR)

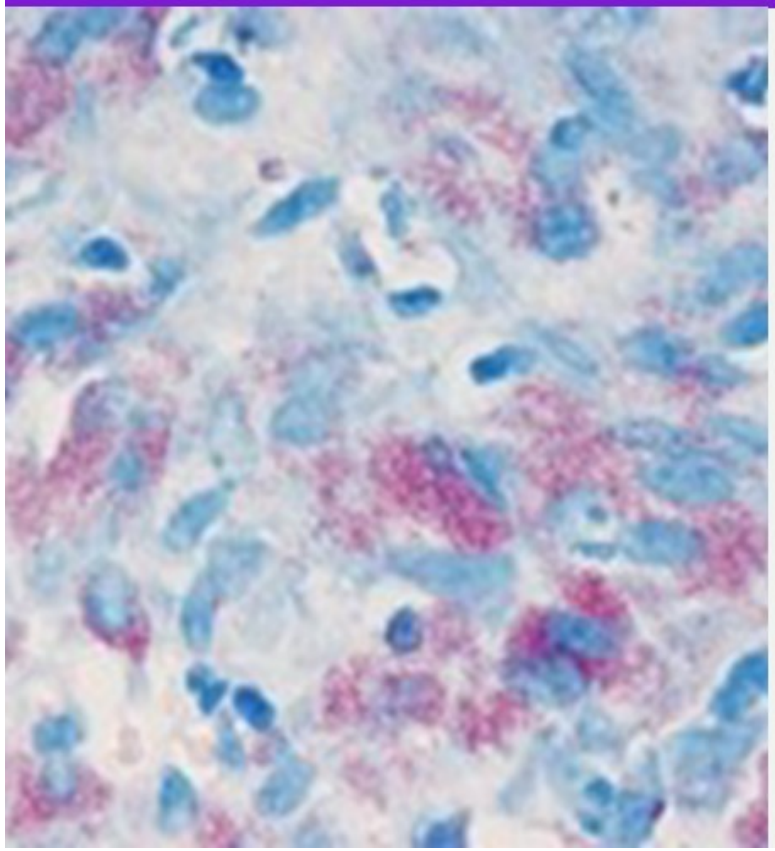
DNA typing methods

- Restriction fragment length polymorphism (RFLP) analysis
- Spoligotyping - PCR based method
- Variable number tandem repeat (VNTR)

Control

- Three principal approaches to the control of TB are:
  - test and slaughter
  - test and segregation
  - chemotherapy
- Routine hygienic measures
  - Cleaning and disinfecting contaminated food, water troughs, etc.
  - Cleaning infected premises, especially feed troughs and waterers, followed by disinfection with 5 percent phenol or equivalent cresol is recommended

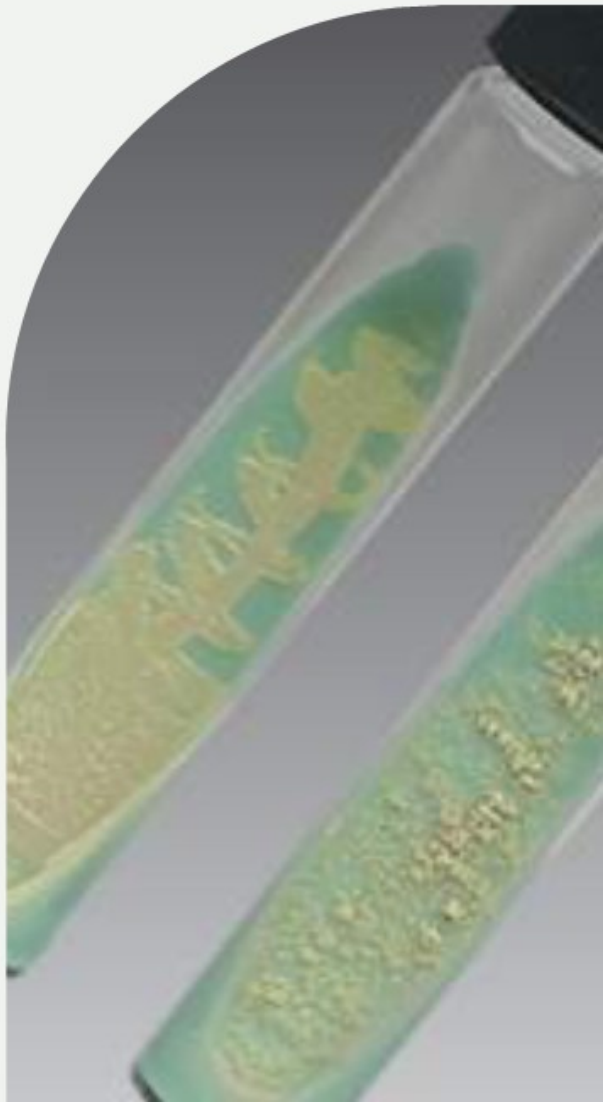
M. avium-intracellulare complex



- M avium and M intracellulare
  - Biochemically and genetically similar
  - difficult to distinguish the species
- Opportunistic infection
  - High organism load can be seen in AFB stain in intestine, liver and spleen
  - Can be seen in bone marrow
  - Organisms variable in size but mostly short
  - Smaller than TB
  - Do not have cord factor
- Positive blood cultures are common
- Involvement of GI tract can cause diarrhea
  - Positive AFB smears in stool
- Pathology - Necrotizing rather than granulomatous inflammation

M. avium-intracellulare

- Laboratory –
  - Growth at 37 °C / 7 – 21 days
  - Non-photochromogen
  - Smooth colony
  - Inert in biochemicals
  - Identify using
    - GenProbe (AccuProbe) molecular identification
    - MALDI-TOF
    - Genetic 16s rRNA Sequencing



Thank you

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