GENUS MYCOBACTERIUM

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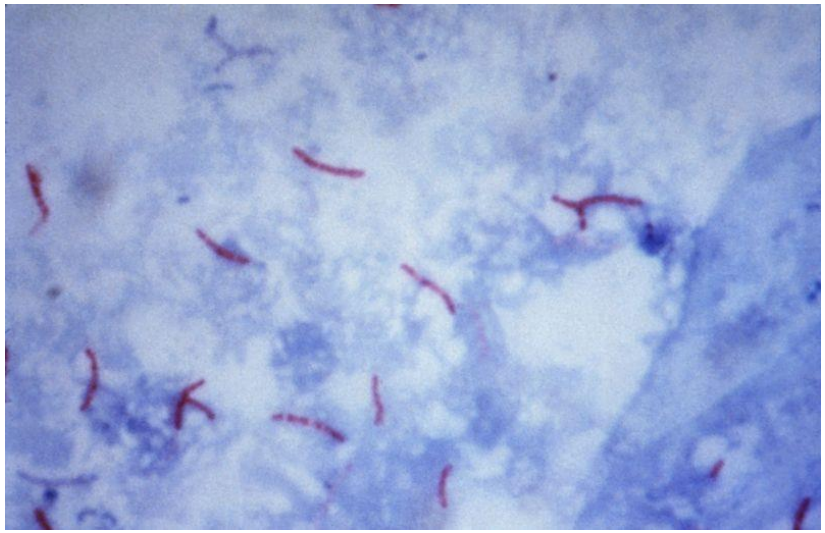
Mycobacterium

- Domain - Bacteria
- Phylum - Actinobacteria
- Class - Actinobacteria
- Order - Actinomycetales
- Family - Mycobacteriaceae
- Genus - Mycobacterium



History:

- Generic name mycobacterium (fungus bacterium) was proposed by Lehmann and Neumann (1896).
- First member of this genus - leprae bacillus discovered by Hansen (1868) – Hansen bacillus.
- Koch (1882) isolated the mammalian tubercle bacillus and proved its causative role in tuberculosis by satisfying Koch's postulates.
- Acid-fast property of Mycobacterium was discovered by Ehrlich (1882).
- Johne (1895) described Johne's bacillus - *Mycobacterium paratuberculosis*

Introduction

- Although mycobacteria are cytochemically Gram-positive, the high lipid, waxes and mycolic acid content of their cell walls prevents uptake of the dyes employed in the Gram stain.
- The cell wall lipids bind carbol fuchsin which is not removed by the acid-alcohol decolourizer used in the Ziehl-Neelsen (ZN) staining method.
- Bacilli, which stain red by this method, are called acid-fast or ZN-positive.
- Complex egg-enriched media required for growth of pathogenic species,
- Aerobic, non-motile, non-spore-forming
- Genus includes obligate pathogens, opportunistic pathogens and saprophytes

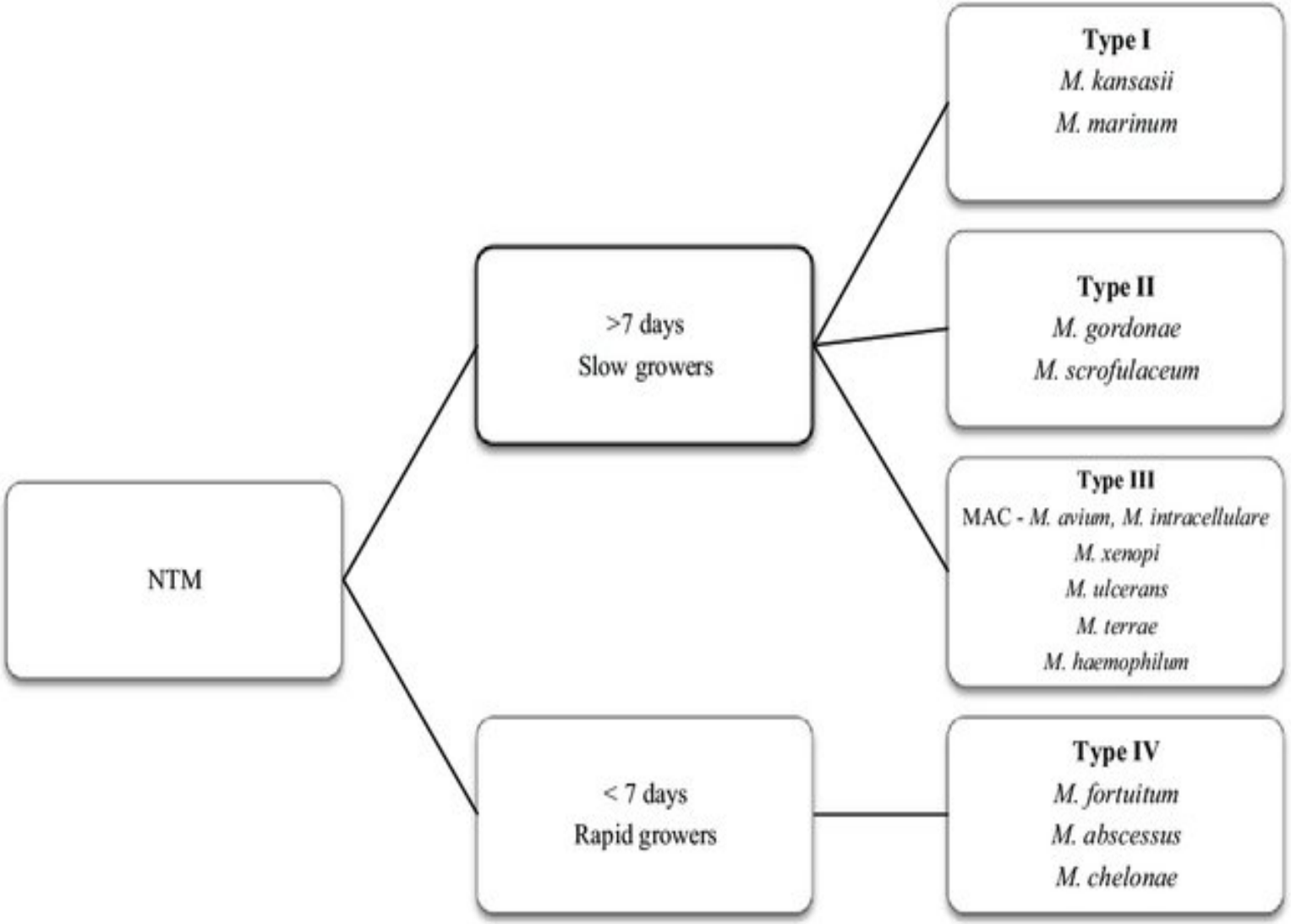
Introduction

- Pathogenic species grow slowly, colonies visible after several weeks
- Some mycobacteria produce carotenoid pigments
- Resistant to chemical disinfectants and environmental influences but susceptible to heat treatment.
- Multiply intracellulary and cause chronic, granulomatous infections
- Major diseases include tuberculosis, Johne's disease and feline leprosy.

Natural Habitat

- Lipid-rich walls render mycobactcria hydrophobic and resistant to adverse environmental influences.
- Environmental mycobacteria are found in soil, on vegetation and in water.
- *Mycobacterium bovis* is excreted in respiratory discharges, faeces, milk, urine and semen.
- *Mycobacterium avium* and *Mycobacterium paratuberculosis* are shed in faeces
- *Mycobacterium tuberculosis* mainly in respiratory discharges
- Obligate pathogens, shed by infected animals, can also survive in the environment for extended periods

Classification



Classification of non-tuberculous mycobacteria (NTM) (Adapted from Runyon 1959)

Classification

- The Runyon classification of nontuberculous mycobacteria based on the rate of growth, production of yellow pigment and whether this pigment was produced in the dark or only after exposure to light.
- It was introduced by Ernest Runyon in 1959.
- On these bases, the nontuberculous mycobacteria are divided into four Runyon groups.
- The first three groups (Runyon I, II, and III) are classified as slowly growing mycobacteria.
- Runyon IV organisms are rapid growing for mycobacteria

- Runyon I: Photochromogens

Runyon I organisms are slow growing, and produce a yellow-orange pigment when exposed to light.

M. intermedium & *M simiae*

- Runyon II: Scotochromogens

Runyon II organisms are slow-growing and produce a yellow-orange pigment regardless of whether they are grown in the dark or the light.

M. scrofulaceum and *M. szulgai*

- Runyon III: Nonchromogens

Runyon III organisms are slow-growing and never produce pigment, regardless of culture conditions.

M. africanum and *M. bovis*

- Runyon IV: Rapid Growers

Runyon IV organisms are rapid growing for mycobacteria (colonies in 5 days). They do not produce pigment. Some rapidly growing mycobacteria are considered "late-pigmenting“

M. abscessus and *M. chelonae*

Differentiation of mycobacteria

- The ZN staining method is used to differentiate mycobacteria from other bacteria.
- Differentiation of pathogenic mycobacteria relies on cultural characteristics, biochemical tests, animal inoculation, chromatographic analyses and molecular techniques.
- Pathogenic mycobacteria grow slowly and colonies are not evident until cultures have been incubated for at least three weeks.
- In contrast, the colonies of rapidly growing saprophytes are visible within days.

- *Mycobacterium bovis*, *M. tuberculosis* and *M. avium* subsp, paratuberculosis have an optimal incubation temperature of 37°C.
- *Mycobacteria* belonging to the *M. avium* complex grow in the temperature range of 37 to 43°C.
- Pathogenic species of mycobacteria can be distinguished by their colonial appearance on egg-based media.
- The influence of glycerol and sodium pyruvate on growth rate is used to differentiate pathogenic a species.
- Supplementation of media with mycobactin is required for *M. avium* subsp. paratuberculosis.

- Guinea-pig and rabbit inoculation was used in the past to differentiate *M. tuberculosis* from *M. bovis* and *M. avium*.
- Guinea-pigs are highly susceptible to infection with *M. tuberculosis* and *M. bovis*.
- Rabbits are highly susceptible to infections with *M. bovis* and *M. avium*.
- Chromatographic analyses of the lipid composition of some mycobacterial species are used in specialized laboratories.

- Pigment production and photo reactivity for opportunistic mycobacteria:
- ✓ Non-chromogens produce colonies devoid of orange, carotenoid pigments.
- ✓ Photochromogens, when cultured in the dark, produce non-pigmented colonies which become pigmented after a period of exposure to light.
- ✓ Scotochromogens produce pigment when cultured in the dark or in light.

- Molecular techniques:
  - DNA probes, complementary to species-specific sequences of rRNA, arc commercially available for the *M. tuberculosis* complex, the *M. avium* complex and *M. kansasii*.
  - Nucleic acid amplification procedures, including the polymerase chain reaction, are being developed as sensitive and rapid methods for the detection of mycobacteria in tissue samples.
  - DNA restriction endonuclease analyses (DNA fingerprinting) are used in epidemiological studies.

Table 17.1 Mycobacteria which are pathogenic for animals and man.

Mycobacterium species	Main hosts	Species occasionally infected	Disease
M. tuberculosis[a]	Man, captive primates	Dogs, cattle, psittacine birds, canaries	Tuberculosis (worldwide)
M. bovis	Cattle	Deer, badgers, possums, man, cats, other mammalian species	Tuberculosis
M. africanum	Man		Tuberculosis (regions in Africa)
M. avium complex[a]	Most avian species except psittacines	Pigs, cattle	Tuberculosis
M. microti	Voles	Occasionally other mammalian species	Tuberculosis
M. marinum	Fish	Man, aquatic mammals, amphibians	Tuberculosis
M. leprae	Man	Armadillos, chimpanzees	Leprosy
M. lepraemurium	Rats, mice	Cats	Rat leprosy, feline leprosy
M. avium subsp. *paratuberculosis*	Cattle, sheep, goats, deer	Other ruminants	Paratuberculosis (Johne's disease)
Unspecified acid-fast bacteria[a]	Cattle		Associated with skin tuberculosis
M. senegalense, M. farcinogenes	Cattle		Implicated in bovine farcy

Table 17.2 Clinical significance, growth characteristics and biochemical differentiation of pathogenic mycobacteria.

	M. tuberculosis	*M. bovis*	*M. avium* complex	*M. avium* subsp. *paratuberculosis*
Significance of infection	Important in man and occasionally in dogs	Important in cattle and occasionally in other domestic animals and man	Important in free-range domestic poultry, opportunistic infections in man and domestic animals	Important in cattle and other ruminants
Cultural characteristics and requirements				
Growth rate	Slow (3-8 weeks)	Slow (3-8 weeks)	Slow (2-6 weeks)	Very slow (up to 16 weeks)
Optimal incubation temperature	37°C	37°C	37-43°C	37°C
Atmospheric requirements	Aerobic	Aerobic	Aerobic	Aerobic
Colonial features	Rough, buff, difficult to break apart	Cream-coloured, raised with central roughness, break apart easily	Sticky, off-white, break apart easily	Small, hemispherical; some pigmented
Essential growth supplement	None	None	None	Mycobactin
Effect of added glycerol	Enhanced growth (eugonic)	Growth inhibited (dysgonic)	Enhanced growth (eugonic)	
Effect of added sodium pyruvate	No effect	Enhanced growth	No effect	
Biochemical differentiation				
Niacin accumulation	+	−	−	
Pyrazinamidase production	+	−	+	
Nitrate reduction	+	−	−	
Susceptibility to TCH (10 μg/ml)[a]	Resistant	Susceptible	Resistant	

a TCH, Thiophen-2-carboxylic acid hydrazine

Bovine tuberculosis

- Bovine tuberculosis, caused by *M. bovis*, occurs worldwide.
- The virulence of *M. bovis* relates to its ability to survive and multiply in host macrophages.
- Specific toxic factors, contributing to virulence, have not been identified.
- The macrophage accumulation at the primary site of infection is initially a response to the foreign body effect of waxes and lipids in the mycobacterial cell wall.
- Survival within the cytoplasm of macrophages is promoted by interference with phagosome-lysosome fusion and failure of lysosomal digestion.

- Bacilli released from dead macrophages are engulfed by surrounding viable phagocytes.
- Migration of macrophages containing viable mycobacteria can disseminate infection.
- The complex lipid and waxy composition of the mycobacterial cell wall contributes not only to virulence but also, in association with tuberculo proteins, to the immunogenicity on which the development of the host responses and the lesions depends.

Steps in the development of tuberculosis

Inhalation of bacteria

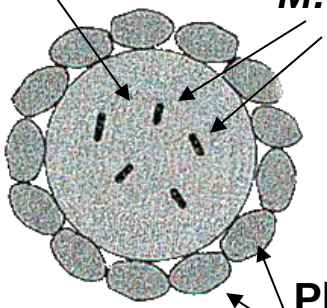
Bacteria reach lungs, enter macrophages

Bacteria reproduce in macrophages

Lesion begins to form (caseous necrosis)

Dead phagocytes, necrosis

M. tuberculosis



Phagocytes, T cells, and B cells trying to kill bacteria

Activated macrophages

Bacteria cease to grow; lesion calcifies

Immune suppression

Reactivation

Lesion liquefies

Bacteria coughed up in sputum

Spread to blood organs

Death

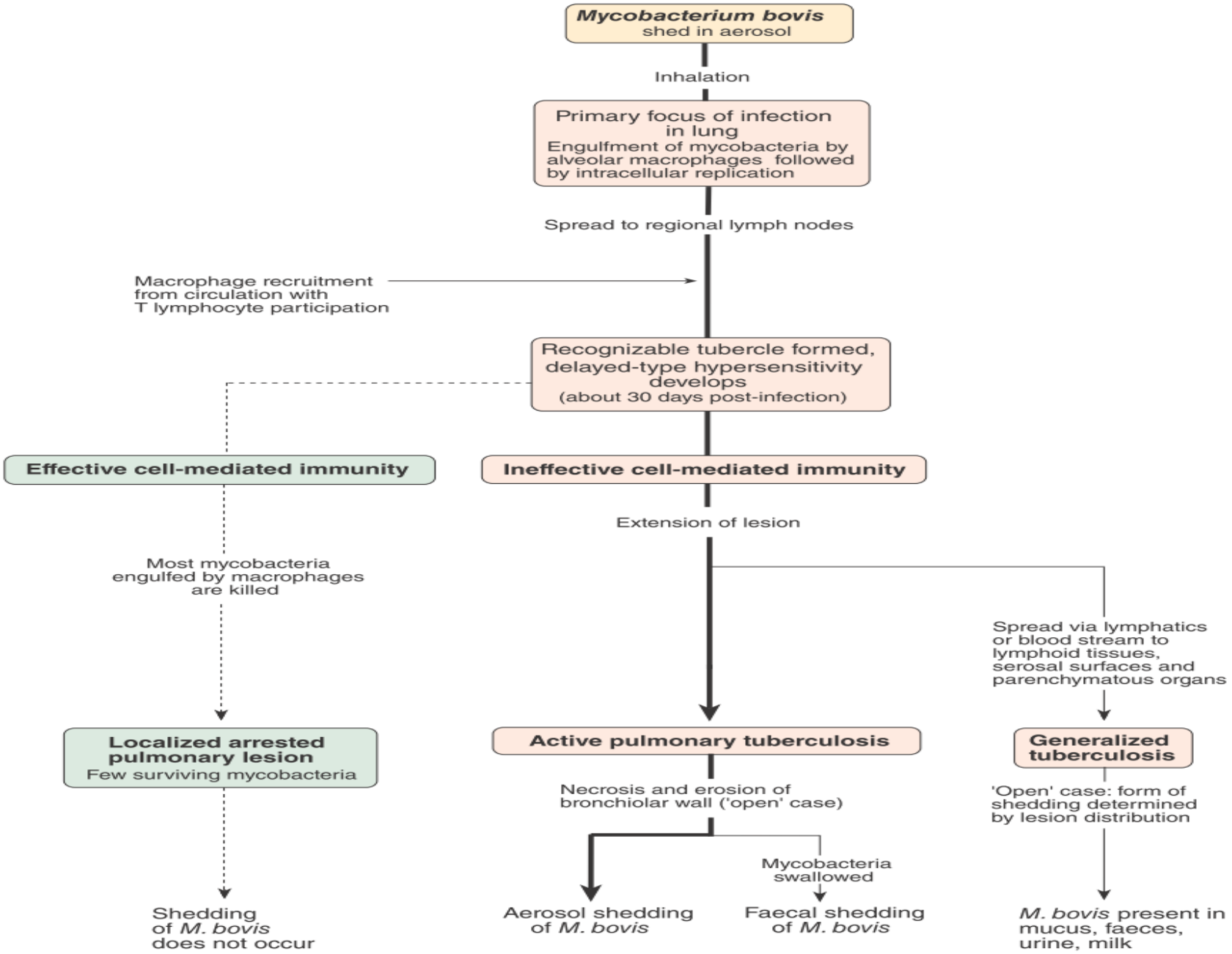
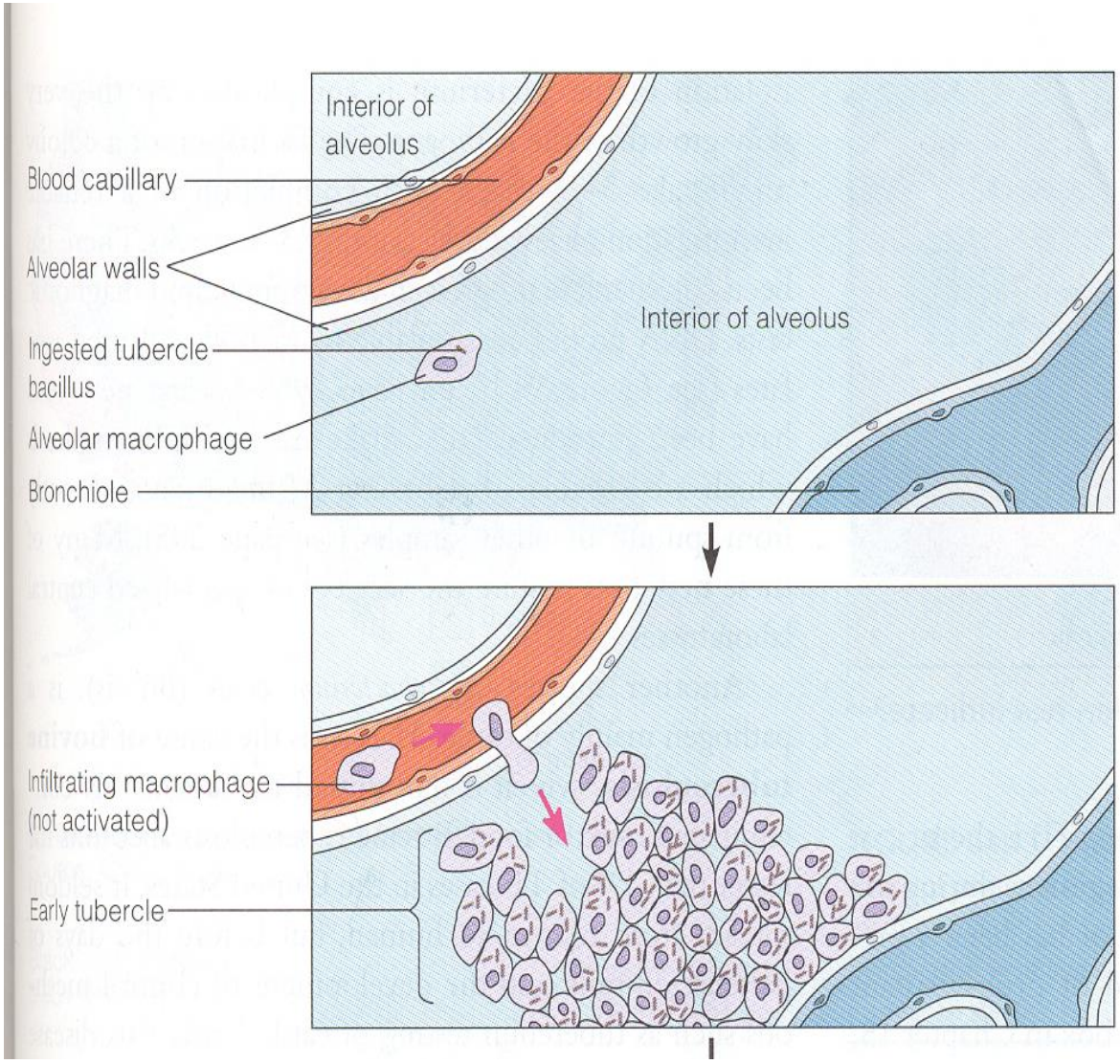


Figure 23.1 The possible consequences of *Mycobacterium bovis* infection in cattle, acquired via aerosols.

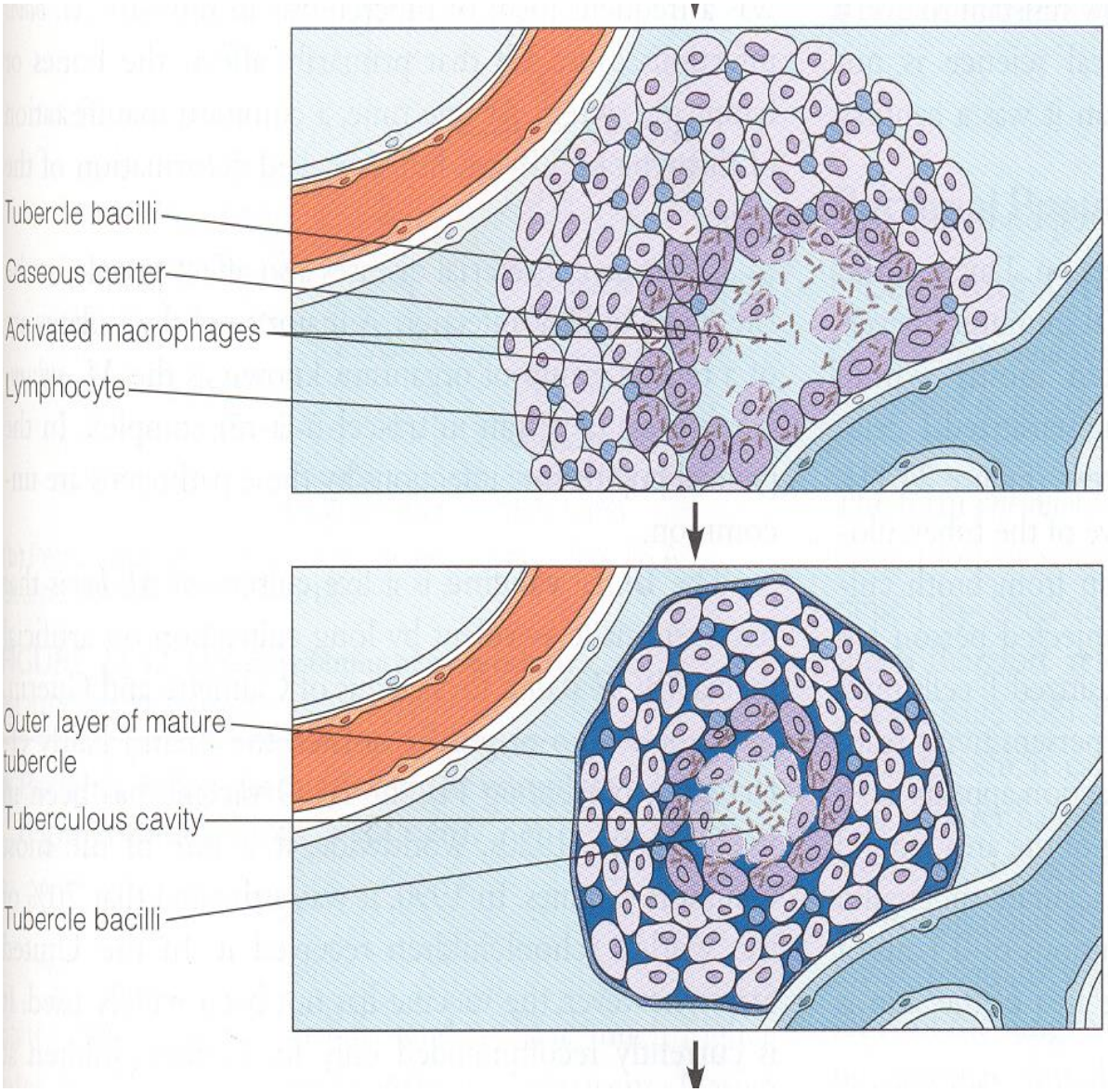
Progression of tuberculosis



1. Tubercle bacilli that reach the alveoli of the lung (Figure 24.2) are ingested by macrophages, but some often survive.

2. Tubercle bacilli multiplying in macrophages cause a chemotactic response that brings additional macrophages and other defensive cells to the area. These form a surrounding layer and, in turn, an early tubercle. Most of the surrounding macrophages are not successful in destroying bacteria but release enzymes and cytokines that cause a lung-damaging inflammation.

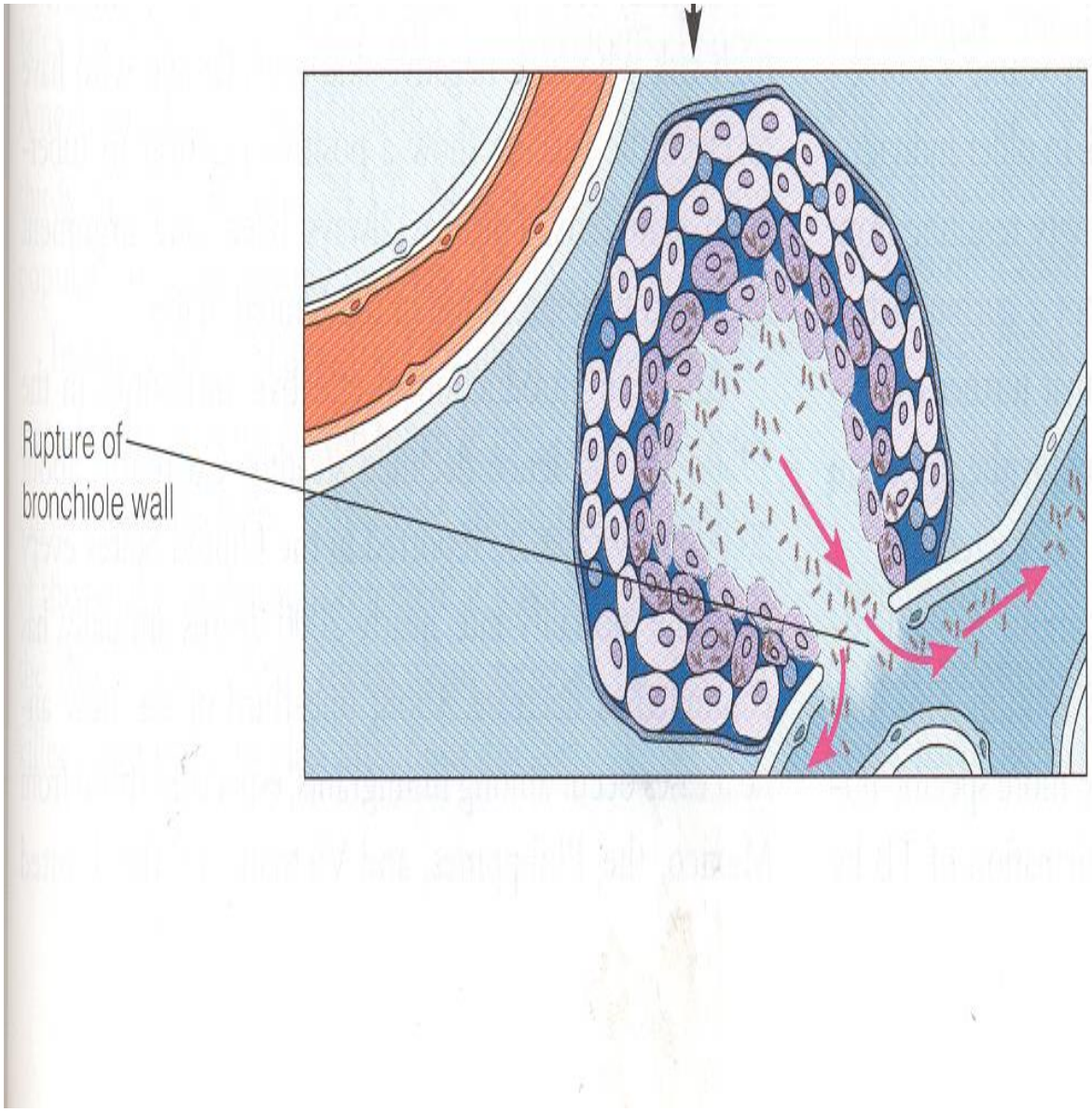
Progression of tuberculosis



3 After a few weeks, many of the macrophages die, releasing tubercle bacilli and forming a *caseous center* in the tubercle. The aerobic tubercle bacilli do not grow well in this location. However, many remain dormant and serve as a basis for later reactivation of the disease. The disease may be arrested at this stage, and the lesions become calcified.

4 In some individuals, a mature tubercle is formed. The disease progresses as the caseous center enlarges in the process termed *liquefaction*. The caseous center now enlarges and forms an air-filled *tuberculous cavity* in which the aerobic bacilli multiply outside macrophages.

Progression of tuberculosis



5 Liquefaction continues until the tubercle ruptures, allowing bacilli to spill into a bronchiole (see Figure 24.2) and thus be disseminated throughout the lungs and then to the circulatory and lymphatic systems.

Clinical signs

- Clinical signs are evident only in advanced disease, and cattle with extensive lesions can appear to be in good health.
- Loss of condition may become evident as the disease progresses.
- In advanced pulmonary tuberculosis, animals may eventually develop a cough and intermittent pyrexia.
- In older lesions, fibroplasia produces early capsule formation and there is an area of central caseous necrosis, with the appearance and consistency of soft cheese. The characteristic histological appearance of a typical tubercle

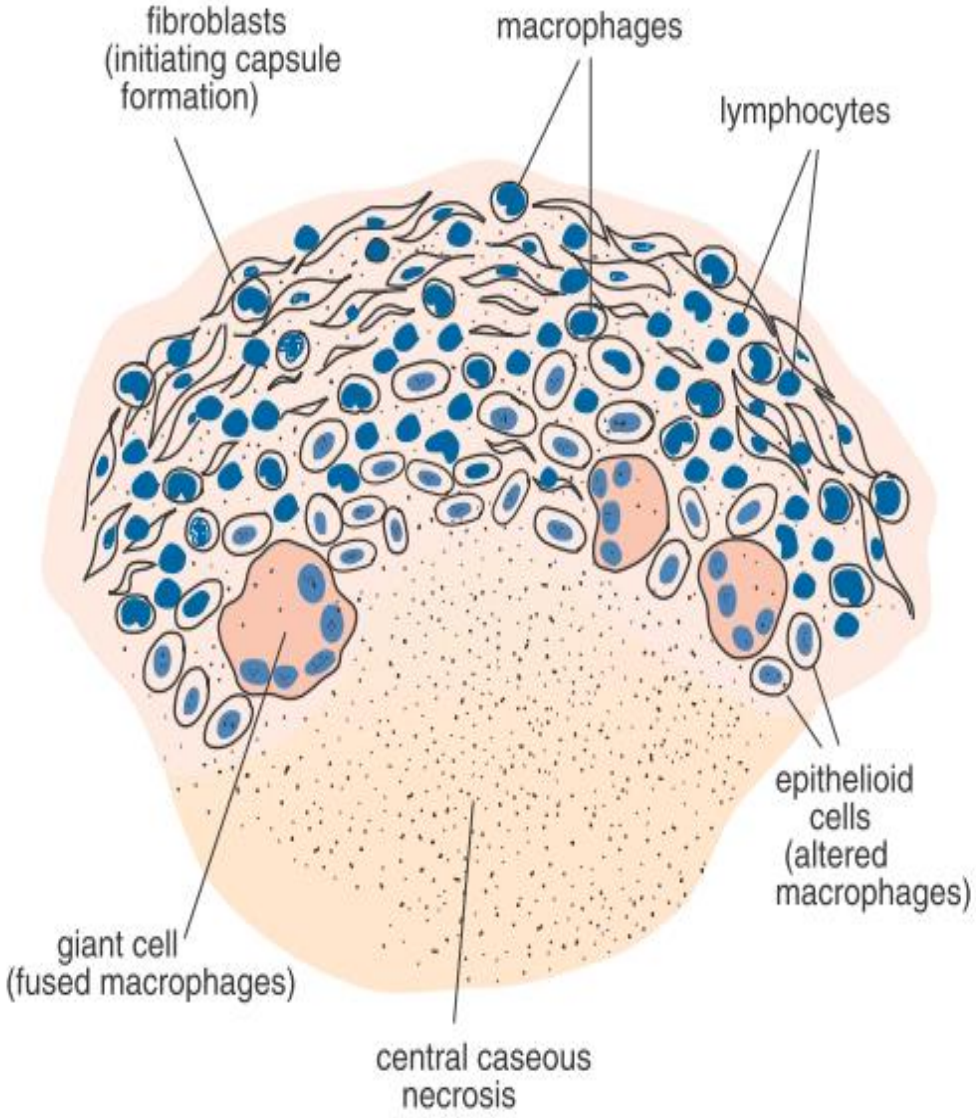


Figure 23.2 Microscopic appearance of part of a typical bovine tuberculous lesion. The tubercle consists of a peripheral zone of mononuclear cells, fibroblasts and giant cells with central caseous necrosis.

Diagnostic procedures

- The tuberculin test, based on a delayed - type hyper-sensitivity to mycobacterial tuberculin, is the standard ante-mortem test in cattle.
- Tuberculin, pre-pared from mycobacteria and called purified protein derivative (PPD), is injected intradermally to detect sensitization.
  - In the single intradermal (caudal fold) test
  - Comparative intradermal test

- ELISA for detecting circulating antibodies.

These tests may be most useful in countries with a high prevalence of bovine TB where there are a large number of animals with chronic disease, and which require low - cost methods of detection

- Gamma interferon assay

This test identifies animals at a slightly earlier stage of infection than the tuberculin test and is approved as a supplementary test for cattle.

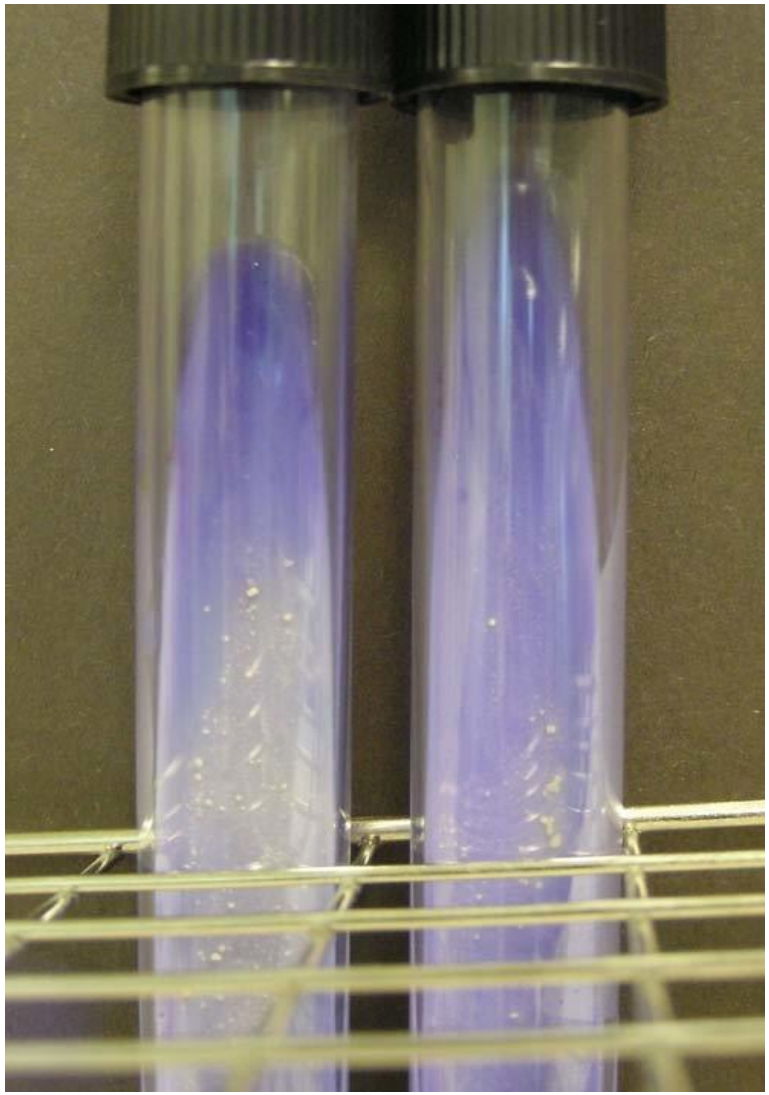
- Tissue sections usually reveal typical patterns of tubercle formation

Isolation

- **Egg based medium (Lowenstein-Jensen)**
- **Agar and broth based medium (Middlebrook)**
- **Stonebrinks medium**

Lowenstein-Jensen granular ,rough, dry colonies. & yellow-orange colonies.

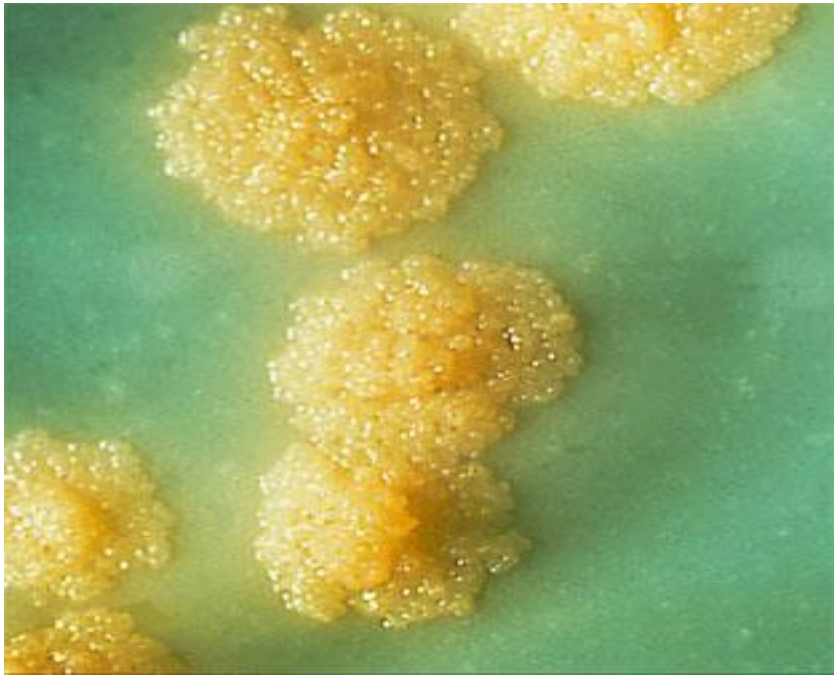
M. bovis Lowenstein-Jensen



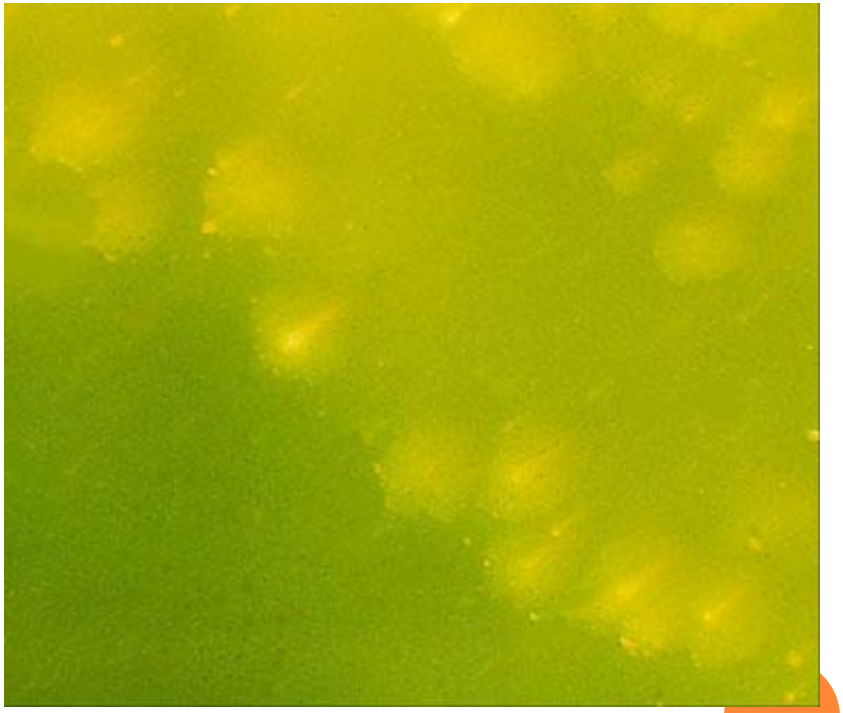
Mycobacterium bovis growth on Stonebrink Medium

Colonial morphology-

- Rough, tough & buff known as Eugonic
- Smooth, crystal glass appearance known as Dysgonic



EUGONIC GROWTH 14 DAYS



DYSGONIC GROWTH 14 DAYS

Tuberculosis in poultry and other avian species

- Avian tuberculosis, which occurs worldwide, is usually caused by members of the *M. avium* complex, serotypes 1 to 3.
- The disease is encountered most often in free-range adult birds.
- Bacilli, excreted in the faeces of birds with advanced lesions, can survive for long periods in soil.

Paratuberculosis (Johne's disease)

- Paratuberculosis is a chronic, contagious, invariably fatal enteritis which can affect domestic and wild ruminants.
- The aetiological agent, *M. avium* subsp. *paratuberculosis*, is an acid-fast organism formerly referred to as *Mycobacterium johnei*.
- Infection is acquired by calves at an early age through ingestion of organisms shed in the faeces of infected animals.
- Shedding of *M. avium* subsp. *paratuberculosis* in colostrum and milk has been recorded

- There are three major subtypes of *M. avium* subsp. *paratuberculosis*, bovine (type I), ovine (type II) and intermediate (type III) strains: ovine strains have been isolated from cattle and bovine strains from sheep.
- In addition, *M. avium* subsp. *paratuberculosis* has been isolated from many wildlife species, including rabbits, deer, ferrets and mice.
- Molecular typing techniques show that strains infecting wildlife and domestic animals sharing the same environment are frequently indistinguishable.
- The degree to which *M. avium* subsp. *paratuberculosis* strains exhibit host specificity or host preference is unclear at present.

Pathogenesis and pathogenicity

- *Mycobacterium avium* subsp. *paratuberculosis* is an intracellular pathogen and cell-mediated reactions are mainly responsible for the enteric lesions.
- Ingested mycobacteria are taken up by M cells over Peyer's patches.
- Uptake is through the interaction of fibronectin attachment proteins with fibronectin, followed by binding to integrins on the surface of the M cells.
- The organisms cross the intestinal epithelial layer and are engulfed by macrophages in which they survive and replicate.

- Interference with maturation of the phagosome and prevention of phagosome–lysosome fusion appears to be important for intracellular survival of *M. avium* subsp. *paratuberculosis* as is the case for *M. bovis*.
- As the disease progresses, an immune-mediated granulomatous reaction develops, with marked lymphocyte and macrophage accumulation in the lamina propria and submucosa.
- The resulting enteropathy leads to loss of plasma proteins and malabsorption of nutrients and water.
- The macrophages in the intestinal wall and in the regional lymph nodes contain large numbers of mycobacteria.

- Two types of lesion are recognized, multibacillary (lepromatous) and paucibacillary (tuberculoid), which appear to be correlated with host immune response.
- High levels of IL-10 gene expression were detected in cattle with extensive pathological changes and high numbers of bacteria, whereas up-regulation of IFN-γ was recorded in the intestinal tissues of cows with subclinical disease

Clinical signs

- Affected cattle are usually more than 2 years of age when signs are first observed. The disease is clinically evident only in mature sheep and goats.
- The main clinical feature in cattle is diarrhoea, initially intermittent but becoming persistent and profuse.
- Progressive weight loss results without loss of appetite, and affected animals seldom survive for more than a year after initial detection.
- In sheep and goats, diarrhoea is less marked and may be absent.

- In cattle, the mucosa of affected areas of the terminal small intestine and the large intestine is usually thickened and folded into transverse corrugations.
- The mesenteric and ileocaecal lymph nodes are enlarged and oedematous.

Diagnosis

- Specimens for direct microscopy from live animals include scrapings or pinch biopsies from the rectum.
- Faeces may be submitted for culture and serum for serological tests.
- Post-mortem specimens for histopathological examination from cattle include tissue from affected regions of the intestines and from regional lymph nodes.
- Specimens for microscopical examination should be stained by the ZN technique

- Isolation of *M. avium* subsp. *paratuberculosis* from faeces or tissues is a sensitive diagnostic procedure but it is difficult and time-consuming.
- After decontamination of the specimen with 0.3% benzalkonium chloride and concentration by centrifugation, slants of Herrold's egg-yolk medium with and without mycobactin are inoculated with the deposit.
- Slants are incubated aerobically at 37°C for up to 16 weeks and examined weekly for evidence of growth.
- Medium containing mycobactin supports growth.

- Serological tests:
  - Complement fixation tests
  - Several ELISA tests have been developed for the detection of antibodies to *M. avium* subsp. *paratuberculosis*
- Cell-mediated responses
  - Johnin, the counterpart of tuberculin PPD, may be used as a field test.
  - The gamma interferon assay is widely used for early detection of infected animals.
- DNA probes and several real-time PCR methods

  are highly sensitive, are being used to detect *M. avium* subsp. *paratuberculosis* in faeces.

Virulence Factors

- Do not produce any exotoxins or endotoxins.
- Virulence appears to reside in the lipids of the cell wall.
- Cell wall of the mycobacterium is composed of peptidoglycan, arabinogalactan and mycolic acid
- Outer layer of the cell wall has wide range of lipids mycosides (Peptido glycolipids or Phenolic glycolipids).
- Mycosides are responsible for the control of cellular permeability, resistance to action of water-soluble enzymes, antibiotics and disinfectants.
- Cord factor (Trehalose –6,6' dimycolate) and Wax D- inhibits chemotaxis, leukotoxic, responsible for delayed hypersensitivity
- Sulfatides- sulfur containing glycolipids inhibits phagolysosome formation and avoiding exposure to hydrolytic enzymes present in the lysozomes.
- Mycosides, phospholipids and sulpholipids are protecting the tubercle bacilli against Phagocytosis

Vaccination

- The French bacteriologists Albert C. Calmette and Guerin notified a loss of virulence of *M. bovis* when cultured in bile containing media.
- A live attenuated vaccine (BCG) is the current vaccine for tuberculosis. It was first used in 1921.

Further Readings

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