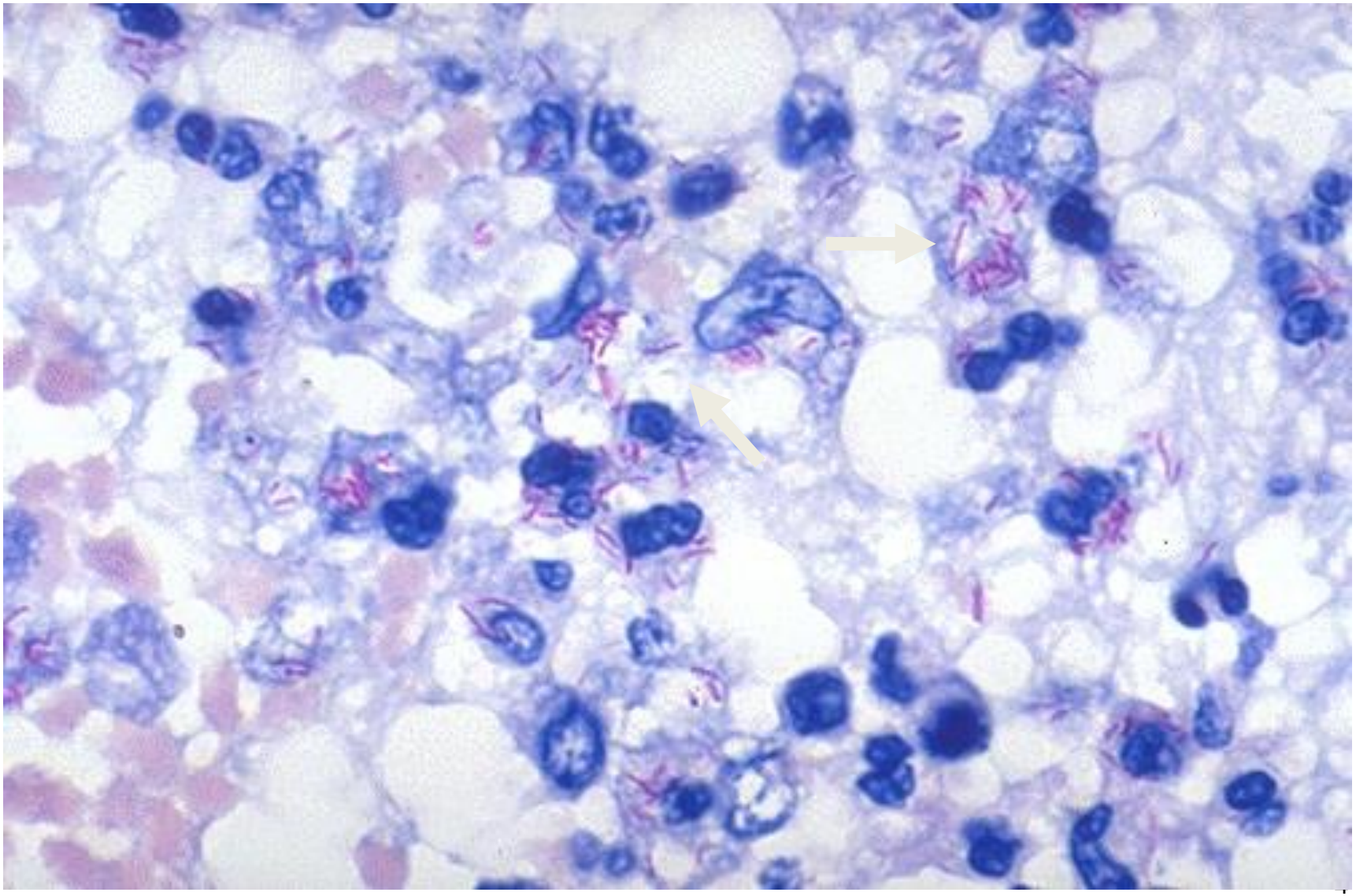
Mycobacterium

Mycobacteria

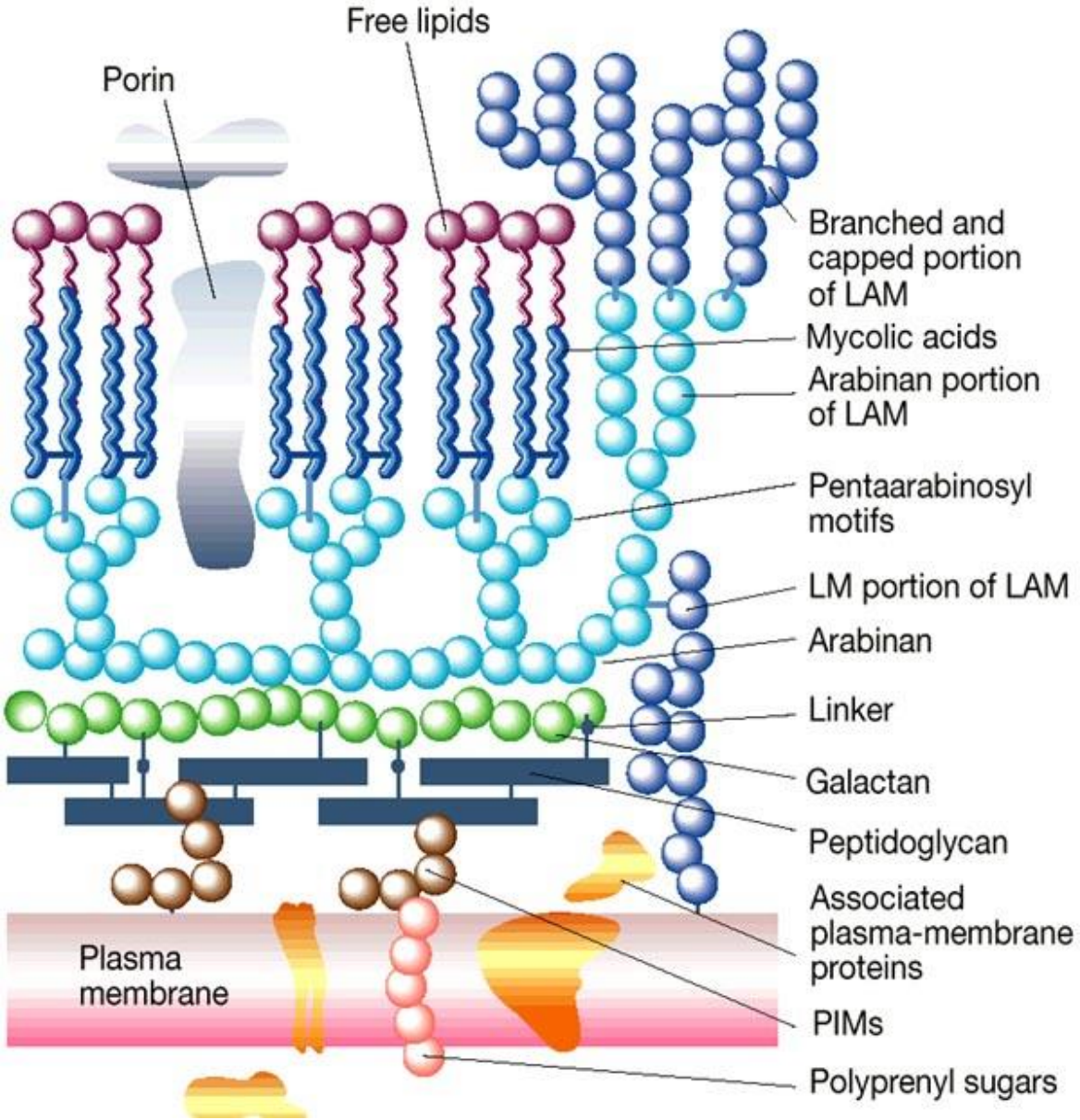
- **Aerobic**
- **Acid fast – bind phenol based dyes (carbol fuchsin) and resist acid alcohol decoloration (Ziehl-Neelsen stain).**
- **Non spore forming**
- **Non motile, rods with varying sizes (1-10µm)**
- **Gram positive – do not stain well with Gram stain**
- **Catalase positive**
- **Many produce pigments on culture**

- **Relatively simple growth requirements**
- **Rapid (<7 days) or slow growing (weeks or months)**
- **Most pathogens slow growing**
- **Unique cell walls – Lipid rich - acid fastness related to presence of peptidoglycan but particularly glycolipids**
- **Lipids in cell wall related to pathogenicity particularly survival in phagolysosome of macrophages, resists drying, extreme pH and other stresses**
- **Complex egg-enriched media required for growth of pathogenic species**
- **Resistant to chemical disinfectants and environmental influences but susceptible to heat treatment (pasteurization)**
- **Multiply intracellularly and cause chronic, granulomatous infections**
- **Major diseases include tuberculosis, Johne's disease, feline leprosy**

Acid fast (Ziehl-Neelsen) staining of Mycobacteria



Mycobacterial (acid-fast) cell wall



Virulence factors of Mycobacteria

Cell wall components

Mycolic acids – resist phagocytic digestion.

Sulfatides – prevent phagocyte activation and phagosome-lysosome fusion.

Trehalose di-mycolate (cord factor) – Inhibits phagocyte chemotaxis, activation, phagosome-lysosome fusions and digesion.

Lipoarabinomannan (LAM) – prevents phagocyte activation and digestion within the phagocyte.

Mycosides – prevent intracellular killing and digestion

Cell wall antigens in general induce DTH

Other factors include **SOD (superoxide dismutase)** and **heat shock proteins**.

Table 36. Mycobacteria capable of causing disease in animals.

Species	Host(s)	Significance
TUBERCULOSIS-GROUP: slow-growing		
M. africanum	Humans	Human tuberculosis (Africa)
M. tuberculosis	Humans, dogs, canaries and psittacine birds	Human tuberculosis (worldwide)
M. bovis	Many animal species and humans	Bovine tuberculosis
M. microti	Voles	Vole tuberculosis. Localised lesions seen in rabbits, calves and guinea-pigs
RUNYON'S GROUPS		
I. PHOTOCHROMOGENS: slow-growing (over 7 days' incubation) saprophytes but rare disease in man and animals.		
M. kansasii	Deer, pigs and cattle	Tuberculosis-like disease. Isolated from lungs and lymph nodes
M. simiae	Humans (monkeys)	Isolated from lymph nodes of healthy monkeys. Pulmonary disease in man
M. marinum	Marine fish, aquatic mammals and amphibians	Fish tuberculosis: granulomatous and disseminated disease
M. vaccae	Saprophytic	Non-pathogenic
II. SCOTOCHROMOGENS: slow-growing, ubiquitous saprophytes found commonly in grasslands. Occasional disease in animals and humans		
M. scrofulaceum	Domestic and wild pigs, cattle and buffaloes	Tuberculous lesions in cervical and intestinal lymph nodes.
III. NON-CHROMOGENS: (slow-growing)		
M. avium	Poultry and wild birds	Avian tuberculosis. Generalised form rare in mammals
	Pigs	Lesions in cervical lymph nodes
	Horses, pigs and others	Intestinal lesions (rare)
M. intracellulare (Battey bacillus)	Poultry and wild birds	Avian tuberculosis. Saprophyte in soil and water
	Pigs and cattle	Can be present in intestinal lymph nodes
	Non-human primates	Granulomatous enteritis (resembles Johne's disease)
M. ulcerans	Cats	Nodulo-ulcerative skin lesions
M. xenopi	Cats	Nodulo-ulcerative skin lesions
	Pigs	Tuberculous lesions in lymph nodes of the alimentary tract
IV. RAPID-GROWING MYCOBACTERIA: need less than 7 days' incubation. Pigmentation variable. Saprophytes in soil, water and on plants. They are found regularly in intestines of pigs, ruminants and other animals. Occasionally pathogenic for animals		
M. chelonae	Fish	Disseminated granulomatous lesions
	Turtles	Tuberculosis-like lesions in lungs
	Cattle	Granulomatous lesions in lymph nodes
	Manatees, cats and pigs	Abscesses and nodulo-ulcerative lesions in various tissues
	Monkeys	Abscesses in lymph nodes or disseminated disease
M. fortuitum	Cattle	Granulomatous lesions in lymph nodes and mammary glands
	Cats	Ulcerative, pyogranulomatous lesions of skin
	Dogs	Granulomatous lesions in skin and lungs
	Pigs	Granulomas in lymph nodes, joints and lungs
M. phlei	Cats	Nodulo-ulcerative lesions of skin (rare)
M. smegmatis	Cattle	Granulomatous mastitis
	Cats	Ulcerative skin lesions
OTHER MYCOBACTERIA		
M. paratuberculosis	Cattle, sheep, goats and other ruminants	Paratuberculosis (Johne's disease). Chronic, progressive, intestinal, wasting disease
M. lepraemurium	Cats and rodents	Feline and murine leprosy (respectively). Not yet isolated on conventional media
M. leprae	Humans and 9-banded armadillo	Leprosy in humans. Replication in armadillos. Not isolated *in vitro*
Unidentified acid-fast bacterium	Cattle	Skin tuberculosis (lymphangitis)

Natural Habitat

- Source of pathogenic mycobacteria is usually infected animal
- M.bovis - Respiratory discharges, faeces, milk, urine, and semen
- M. avium and M. paratuberculosis - Faeces
- M. tuberculosis – mainly in Respiratory discharges

Pathogenesis

- No toxins and enzymes
- **Histological signs (i.e., granuloma) are host immune responses** to infection (DTH response)
- **Immunopathology** results in tissue necrosis (cytokine toxicity, complement activation, ischemia, etc.)

Pathogenesis

- **Small antigenic burden + protective immunity:** activated macrophages can penetrate small granulomas (< 3 mm) and kill all bacteria with minimal tissue damage.
- **But if many bacilli are present, cellular immune response (over-reactive, impaired)** results in formation of large, necrotic or caseous granulomas encapsulated with fibrin, which protect bacteria from macrophage killing (latent), thus may be reactivated years later when patients' immunologic responsiveness wanes.

Special mechanisms for cell entry

- mycobacterium can bind directly to mannose receptors on macrophages via the cell wall-associated mannosylated glycolipid, LAM, or indirectly via certain complement receptors or Fc receptors.

Intracellular growth

This is an effective means of evading the immune system. In particular, antibodies and complement are ineffective. it can inhibit phagosome-lysosome fusion by secretion of a protein that modifies the phagosome membrane & find a protected environment for growth in the macrophage

Virulence factors of Mycobacteria

Cell wall components

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Cell wall antigens in general induce DTH

Other factors include **SOD (superoxide dismutase)** and **heat shock proteins**.

Slow generation time

The immune system may not readily recognize the bacteria or may not be triggered sufficiently to eliminate them.

High lipid concentration in cell wall

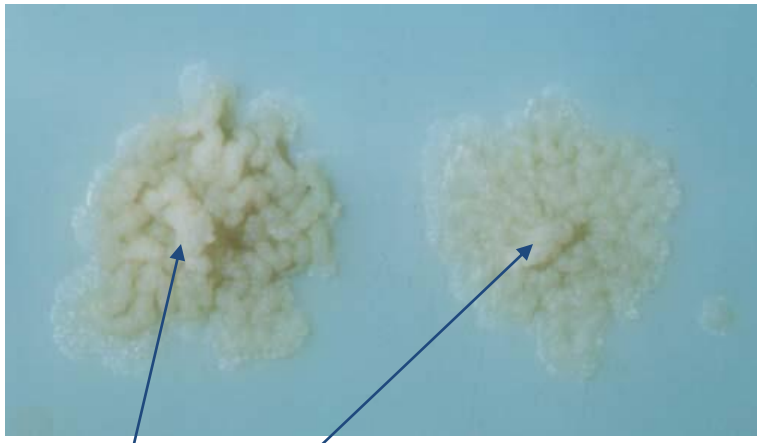
Impermeability and resistance to antimicrobial agents, resistance to killing by acidic and alkaline compounds in both the intracellular and extracellular environment, resistance to osmotic lysis via complement deposition and attack by lysozyme.

Cord factor (trehalose 6, 6' dimycolate)

destroy mitochondria

cause chronic granulomatosis

suppress WBC wandering



Cord factor (absent in M. avium)

M. tuberculosis on Lowenstein-Jensen medium

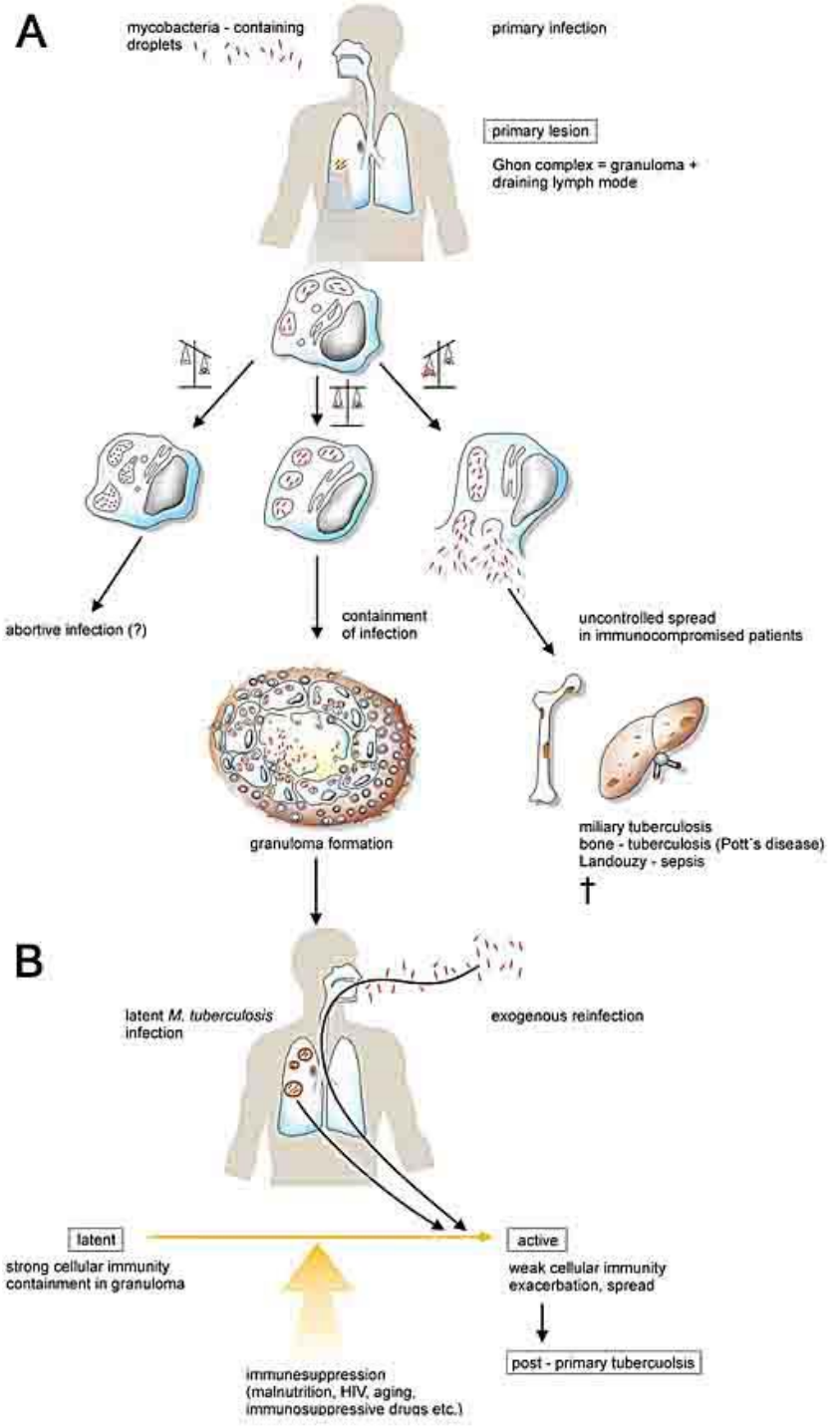
19-kDa protein

The 19-kDa *Mycobacterium* protein induces macrophage apoptosis through Toll-Like Receptor-2

Mycobacteria (TB)

The ability to mount an effective activated macrophage response determines the outcome of an encounter with *M. tuberculosis*. <u>Less than 10% of those infected develop disease</u>

- **Infects , killed by immune response, no disease**
- **Infects, lies dormant for many years, no disease (infection contained) (Most common)**
- **Infects, lies dormant for many years, <u>re-activates causes acute disease</u>**
- **Infects, causes rapid <u>acute</u> disease, may disseminate (children, immunocompromised, HIV)**



Immune responses to Mycobacterial infections

- Humoral response irrelevant to protection. A bias towards a Th2 response exacerbates the condition. Th1 (CMI) required to limit the disease and provide protection
- Immune status of the animal important. Active response results in lymphocyte infiltration, central necrosis in the lesion, tubercule maybe limited by a fibrin capsule. Response may be strong enough to kill the bacteria but often the response is only able to restrict the disease. Reactivation occurs with stress/immunosuppression.
- IFN gamma from CD4 lymphocytes activates macrophages to kill intracellular mycobacteria. CD8 lymphocytes become cytotoxic killing mycobacterial infected cells. CD1 restricted T cells recognise glycolipids
- Exposure to environmental Mycobacteria provides some cross-protection which may limit the disease caused by virulent species (also complicates hypersensitivity testing).

Bovine Tuberculosis

Mycobacterium bovis: control measures have led to a greatly reduced prevalence in Europe. Spread is promoted by high densities of animals and immune suppression.

Generally a primary respiratory infection leads to **tubercules** in the lung and associated lymph nodes (bronchial and retropharyngeal).

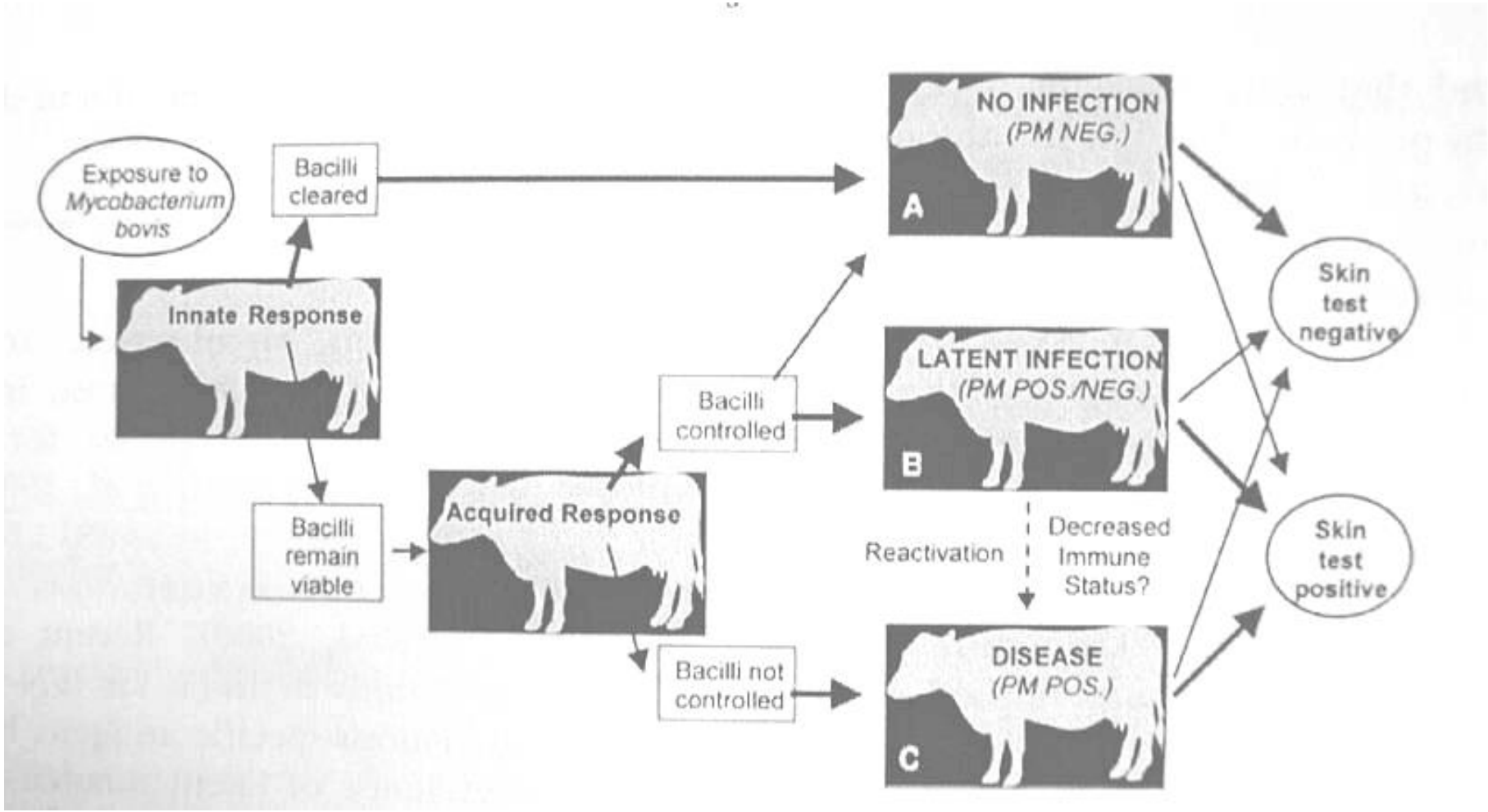
Closed or open lesions

Spread to intestine (via sputum) and serosal surfaces.
Pleural lesions (Pearls disease).

Further spread (usually haematogenous) to liver, spleen, kidney, brain etc.
Vertical transmission is possible after spread to mammary glands and uterus.

Antibiotic treatments are long term and very expensive for animals.
Consequently **tuberculin testing** and culling of exposed animals.

Prevent cattle movement

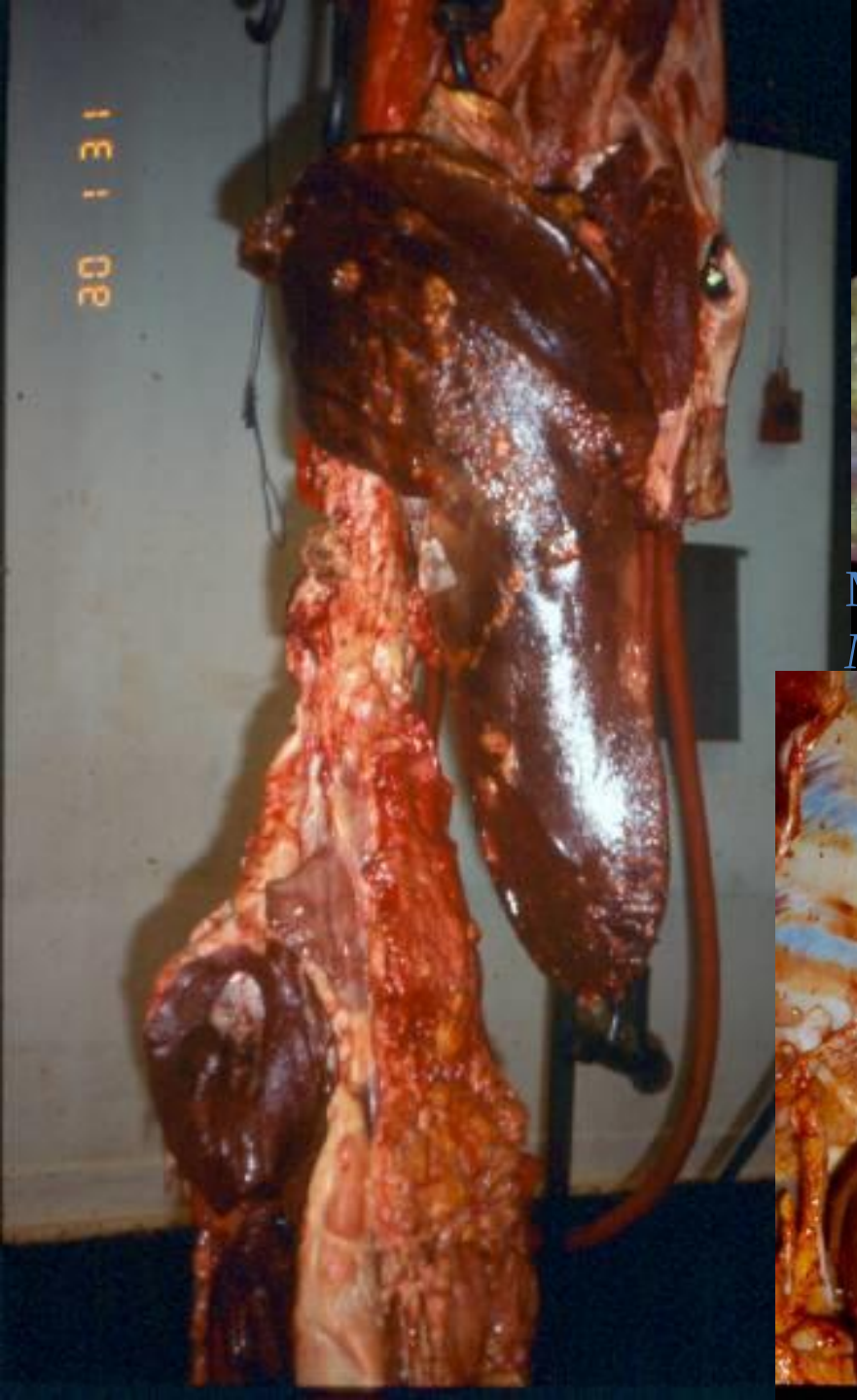

Exposure to Mycobacterium bovis
Bacilli cleared
Innate Response
Bacilli remain viable
Acquired Response
Bacilli controlled
Bacilli not controlled
NO INFECTION (PM NEG.)
A
LATENT INFECTION (PM POS./NEG.)
B
Reactivation
Decreased Immune Status?
DISEASE (PM POS.)
C
Skin test negative
Skin test positive

Epidemiology of bovine TB

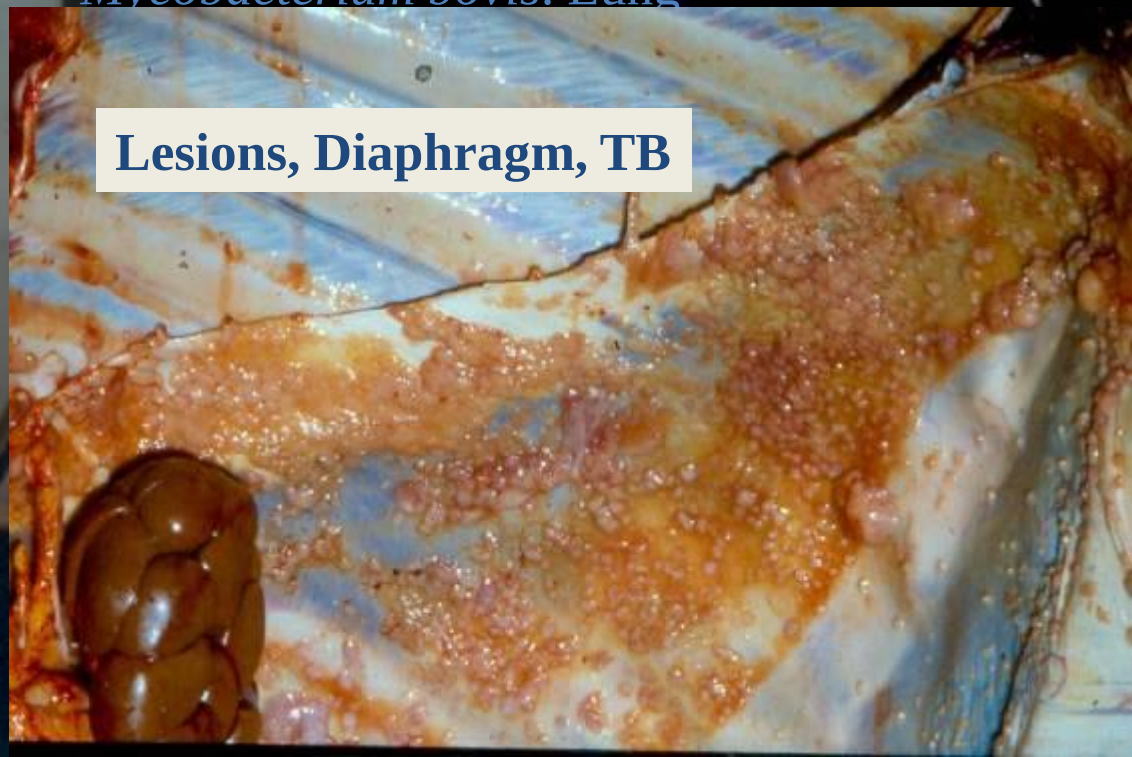
- Cattle transmit infection to cattle via infected respiratory droplets – respiratory route
- Badgers transmit *M. bovis* between themselves by the respiratory route and by biting. Mums transmit to cubs but not by milk
- Cattle may get *M. bovis* from badgers via grazing on pasture contaminated with badger urine, faeces and bronchial pus or badgers urinate and defecate in cattle feeders.
- Aerosol transmission via coughing may be possible or via dried badger saliva in cattle houses
- This may apply to cattle to badger transmission

Example of *M. bovis* prevalence in wildlife

Wildlife species	Percentage of TB breakdown farms reporting presence of wildlife	*M. bovis* infection prevalence (n)
Badgers	80%	4% (n=21,731)
Deer	Fallow 12% Muntjac 9% Red1% Roe2% Sika 1%	1% (n=1817)
Ferrets/Polecats	6%	4% (n=26)
Foxes	83%	1% (n=954)
Rabbits	80%	0% (n=144)
Rats	76%	1% (n=412)
Stoats / Weasels	35%	0% (n=66)

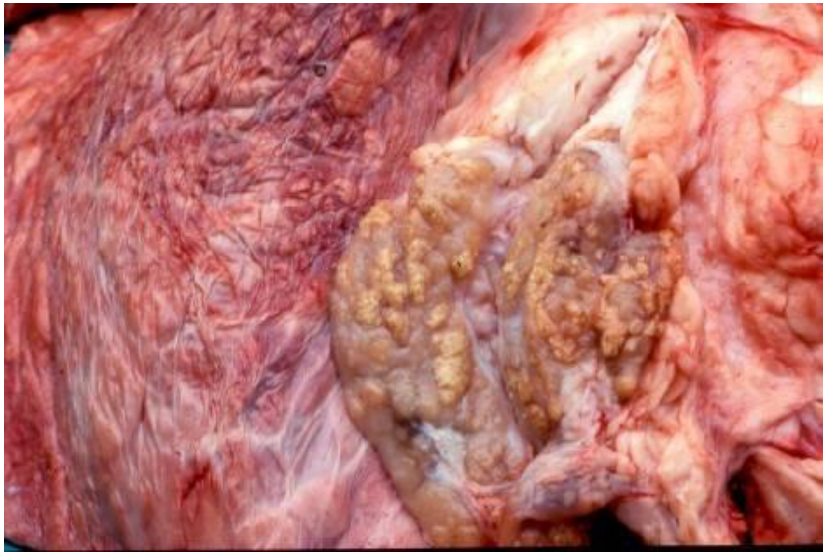


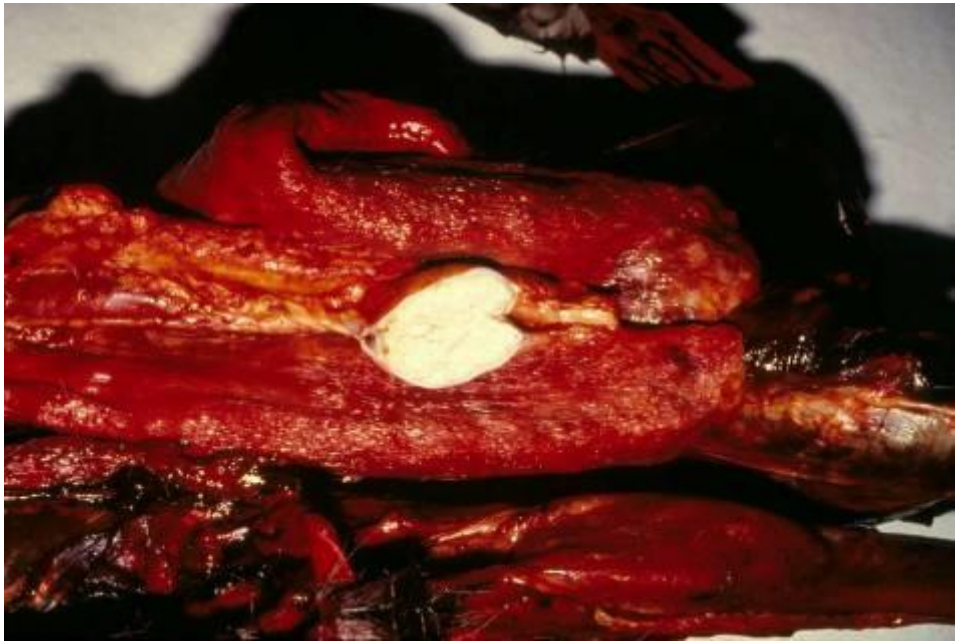
Multifocal to coalescing caseous granulomas. *Mycobacterium bovis*. Lung

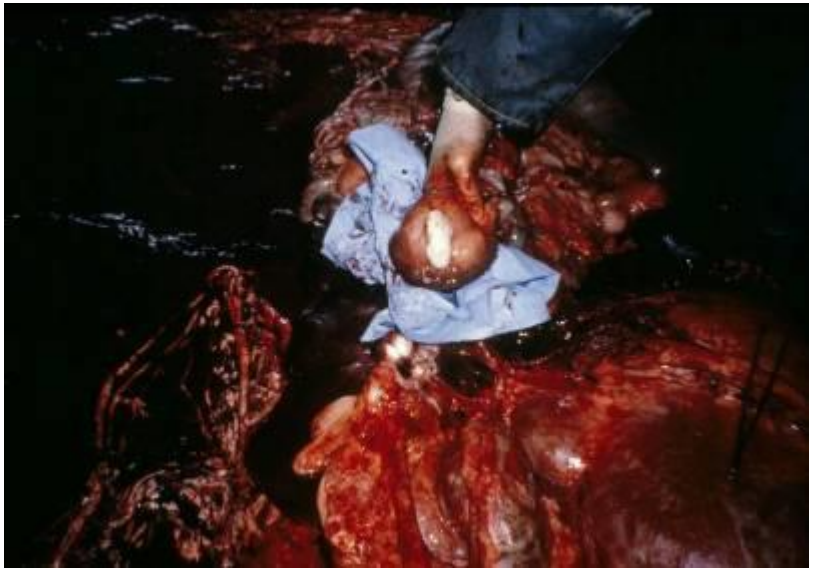


Lesions, Diaphragm, TB

LYMPH NODE, TB







Infected lymph node in a red deer

MILK FROM TUBERCULOUS MASTITIS

Before pasteurisation *M. bovis* infection in man was common (pre-1930's)

Now *M. bovis* rare in humans

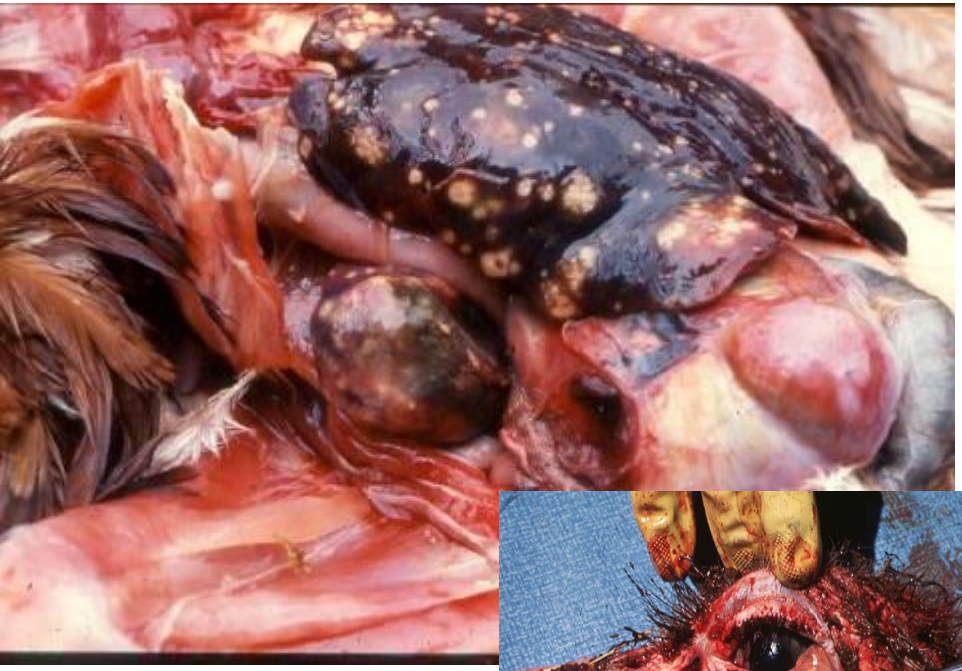
Causes <1% of all human TB cases in developed countries

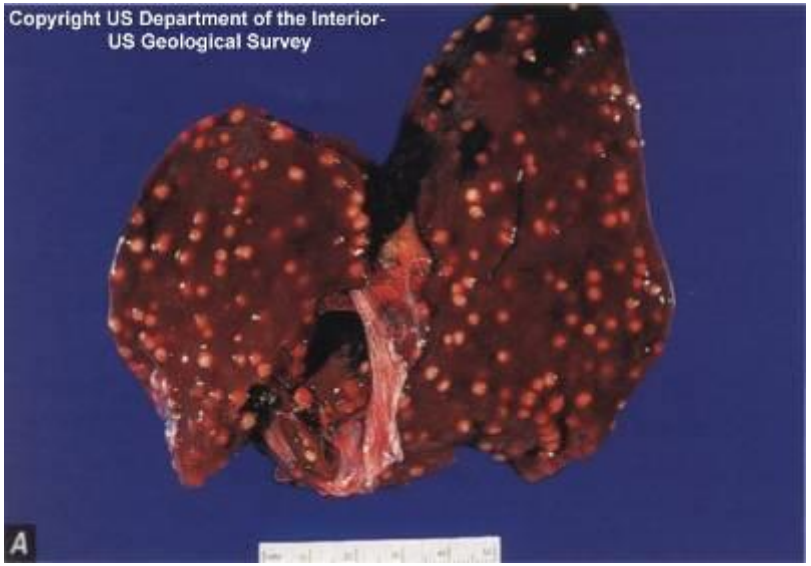
Elderly (inc. reactivated infections)
Immunosuppressed (e.g. HIV, cancer)
Foreign travellers

Mycobacterium avium

- *M. avium* subspecies *avium* and the taxonomically closely related *M. intracellulare* (both organisms referred to as the *M. avium complex*)
- Widest host range among Mycobacteria
- *M. avium* serovars 1, 2 and 3 isolated from tuberculous lesions in avian species (avian TB – progressive disease)
- Other *M. avium* serovars produce minimal disease (microscopic foci in liver and spleen) in chickens
- Non human primates, cattle and pigs infection by *M. avium ss avium* is confined to lymph node infection (Mycobacteriosis in pigs)
- *M. avium-intracellulare* causes disseminated disease in HIV/AIDS patients

CHICKEN TB

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US Geological Survey
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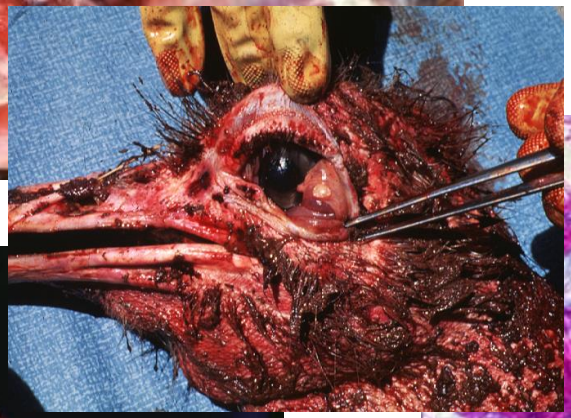
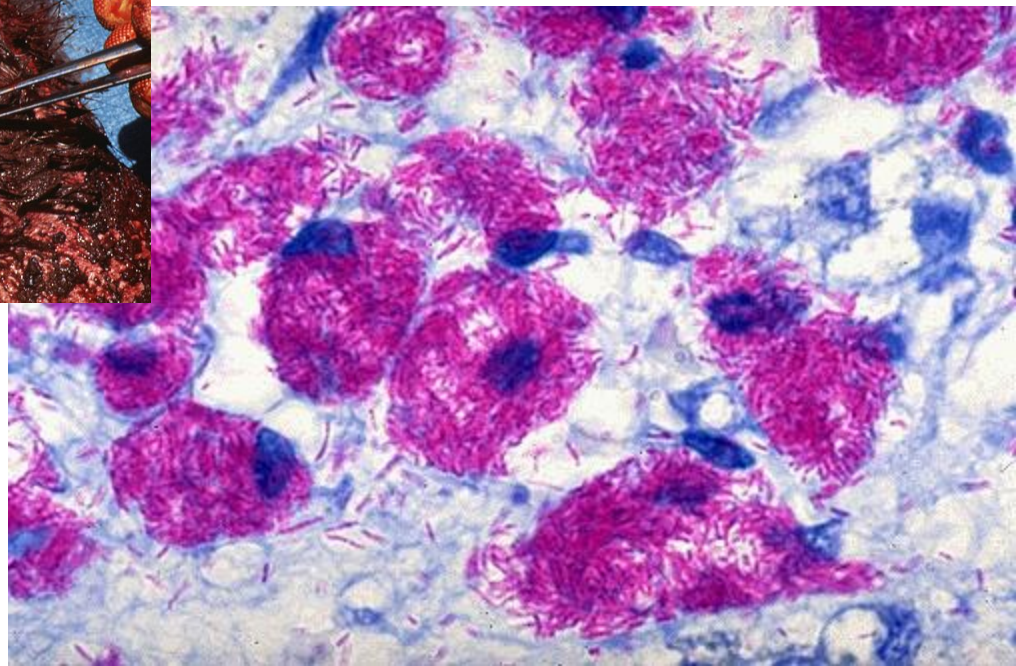
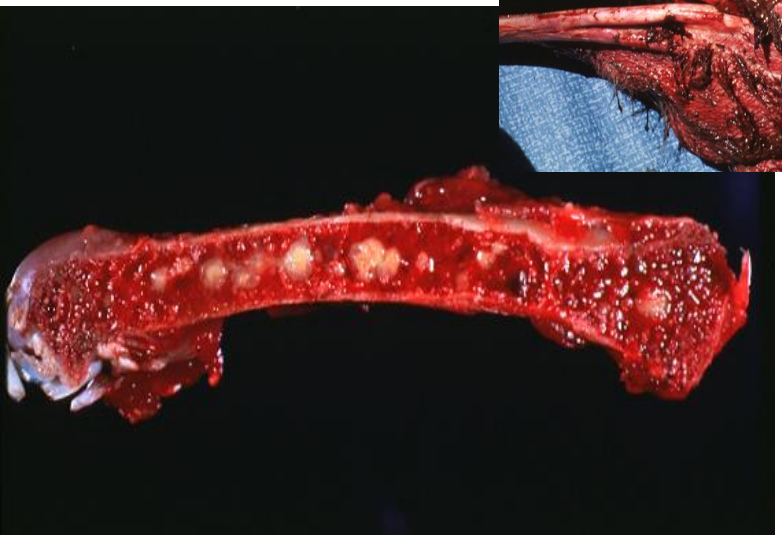






M. avium sub spec*. paratuberculosis*

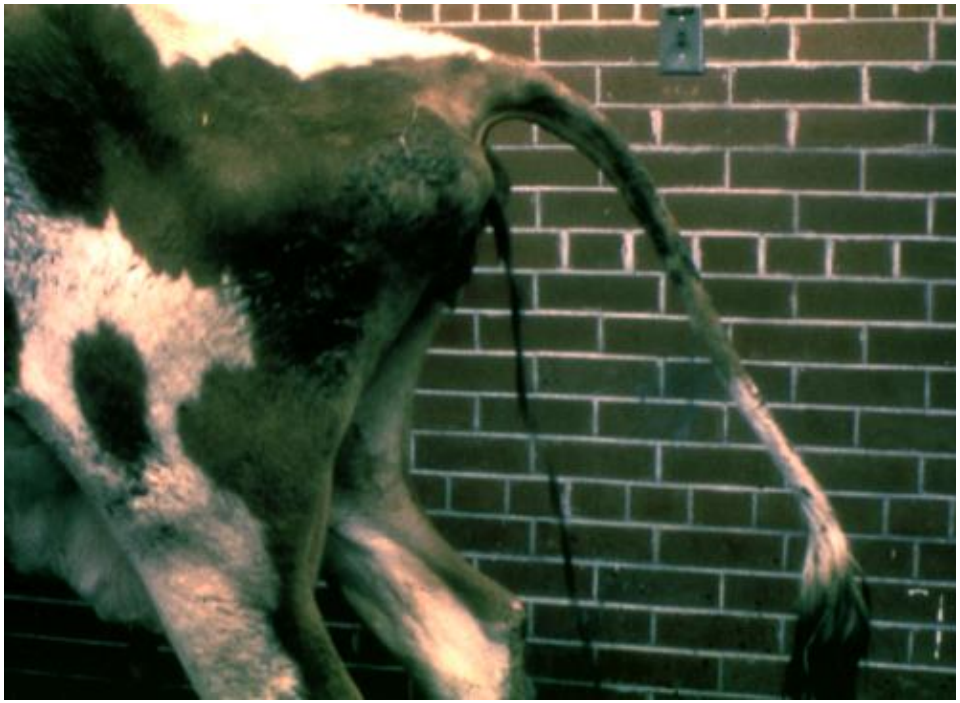
This organism causes a transmissible chronic and progressive enteritis in cattle sheep and goats, but not swine or horses.

First observed by Johne and Frothingham in 1895 – **Johne's disease**.

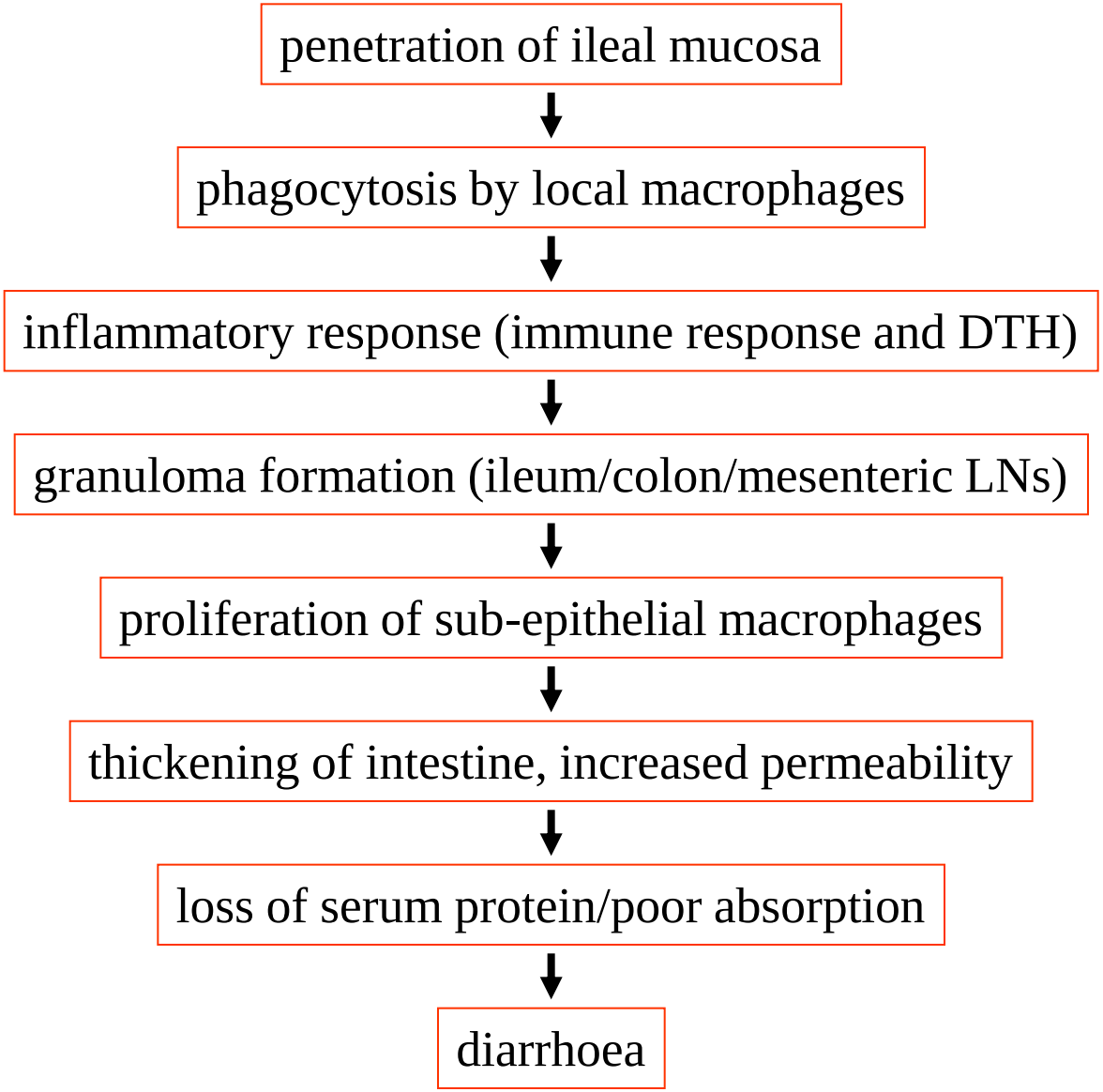
Infection usually occurs within the first month but may take 6 months to 5 years to become apparent. Clinical course (1-4 months) starts with general signs of illness (weight loss, int. diarrhoea), followed by severe diarrhoea, emaciation and death.

Impaired intestinal function due to chronic inflammation. Evidence of diffuse granulomatous changes. Accumulation of lymphocytes and epitheloid cells in the lamina propria and submucosa.

Johne's disease: *Mycobacterium paratuberculosis*



Pathogenesis of paratuberculosis (Johne's disease)



Gross pathology of Johne's disease: *Mycobacterium paratuberculosis*

thickened and corrugated infected ileum

normal ileum

Mycobacterium paratuberculosis
Johne's disease

- **Caused by bacterium *Mycobacterium avium* subsp. *Paratuberculosis***
- **Shed in manure of adult cattle**
- **Bacteria can survive 1 year in environment**

Transmission

- **Entry into herd:**
  - infected adult cattle enter
  - adult carrier sheds bacteria in feces
  - susceptible calf ingests bacteria
  - infected calf sheds bacteria at adult age

Calves are infected early in life (birth to 1 year of age)
Calves are most susceptible at less than 6 months of age
Incubation period is 2+ years

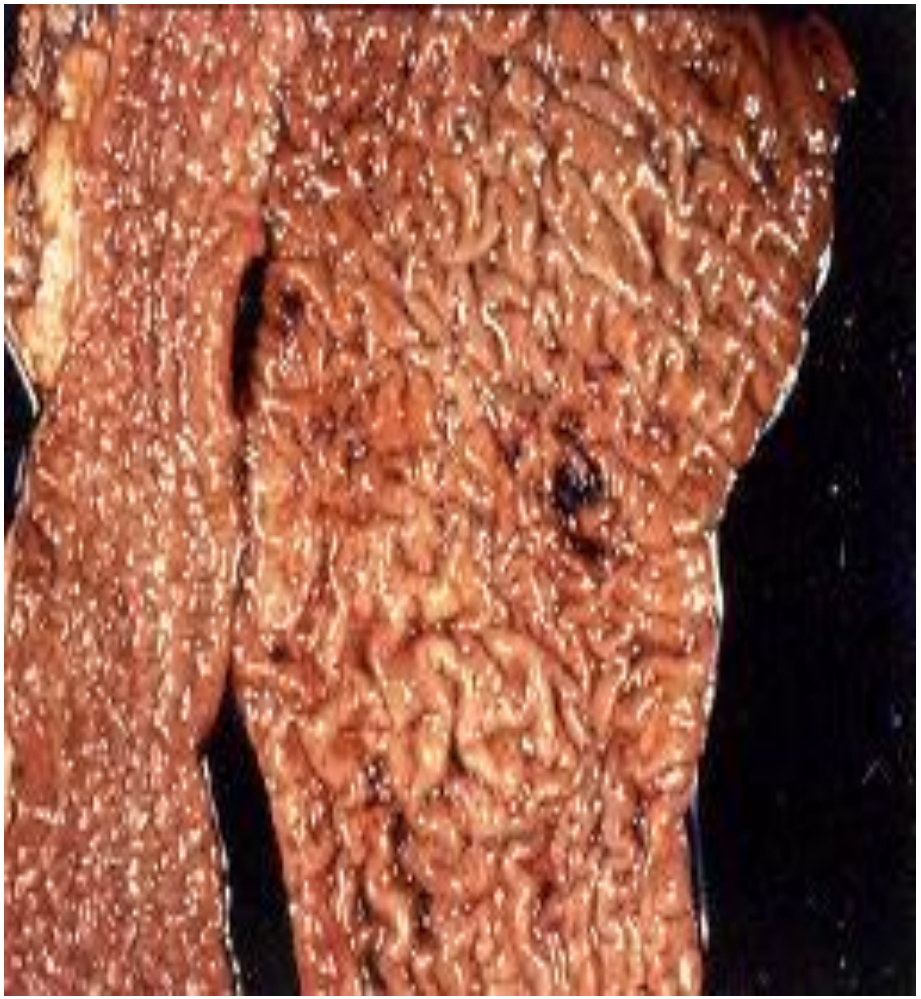
- **Transmission to calves within herd:**
  - Ingest contaminated milk
  - In utero transplacental transfer
  - Lick manure-contaminated teats
  - Lick manure-contaminated haircoat
  - Eat manure-contaminated food
  - Drink manure-contaminated water

PATHOGENESIS

- Bacteria invade mucosa of ileum of small intestine
- Intestinal wall thickens
- Absorption of water and nutrients altered
- Chronic emaciation
- Mal-digestive enteritis

Debiliated Condition of Animal Suffering from M. paratuberculosis

Bottle jaw appearance



Corrugation of Intestine

DIAGNOSIS

Sample collection

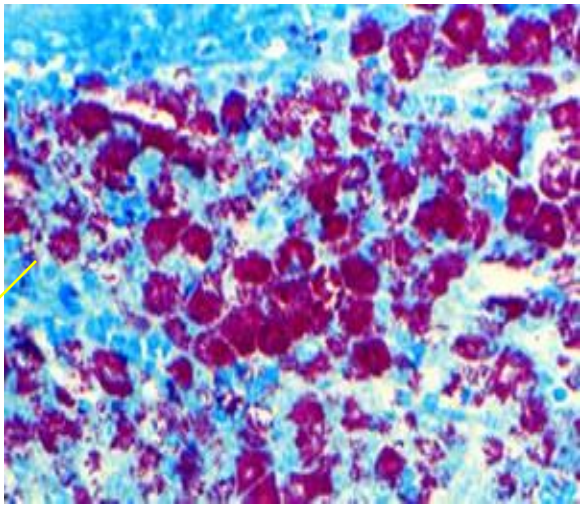
From cattle, samples collected from the iliocecal area(intestine/lymph nodes) are best but mucosal scrapping from the rectum in the live animals are easier to obtain.
In sheep and goats, examination of the iliocecal lymph nodes is the most rewarding.

Direct examination :

Impression smears of lymph nodes or smears of rectal or intestinal scrapping

↓ Stained by ZN - Procedure

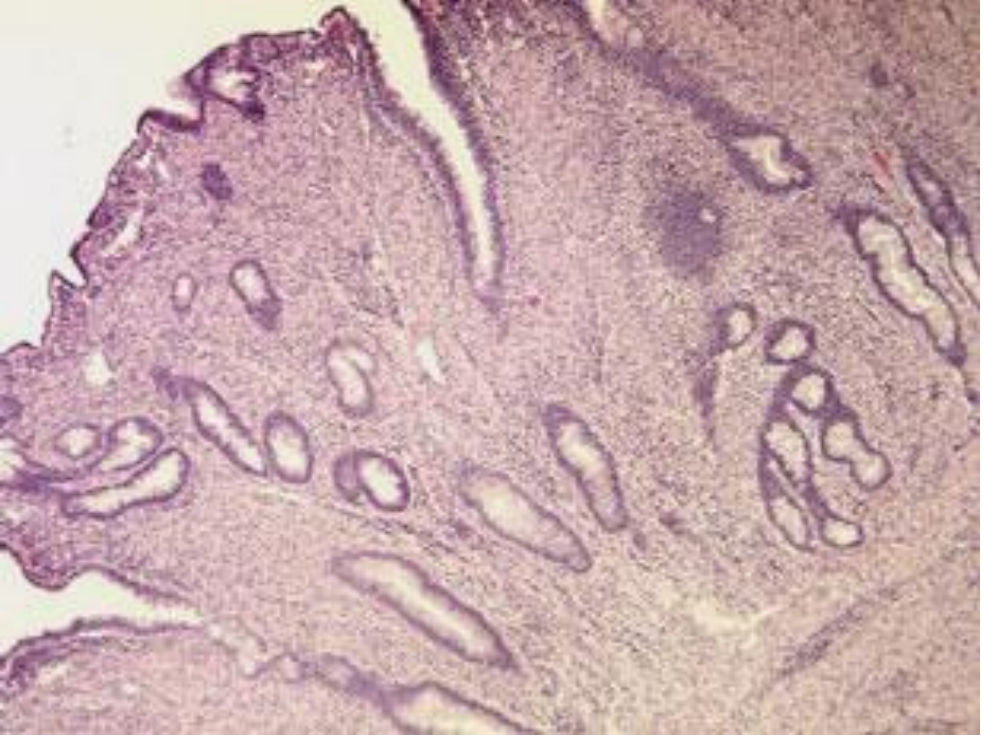
M.paratuberculosis (acid-fast) they are short, slender rods, occurring in bunches other acid-fast staining structures in samples(saprophytic mycobacteria, bacterial endospores) will be solitary and quite large.



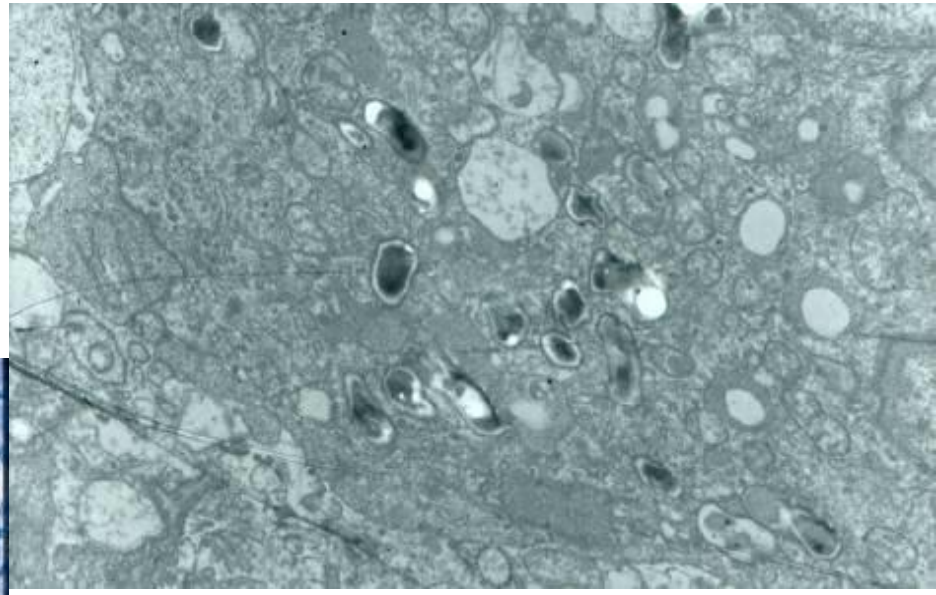
Zeil Neelsen staining of magenta-coloured colonies of MAP in gut of a cow

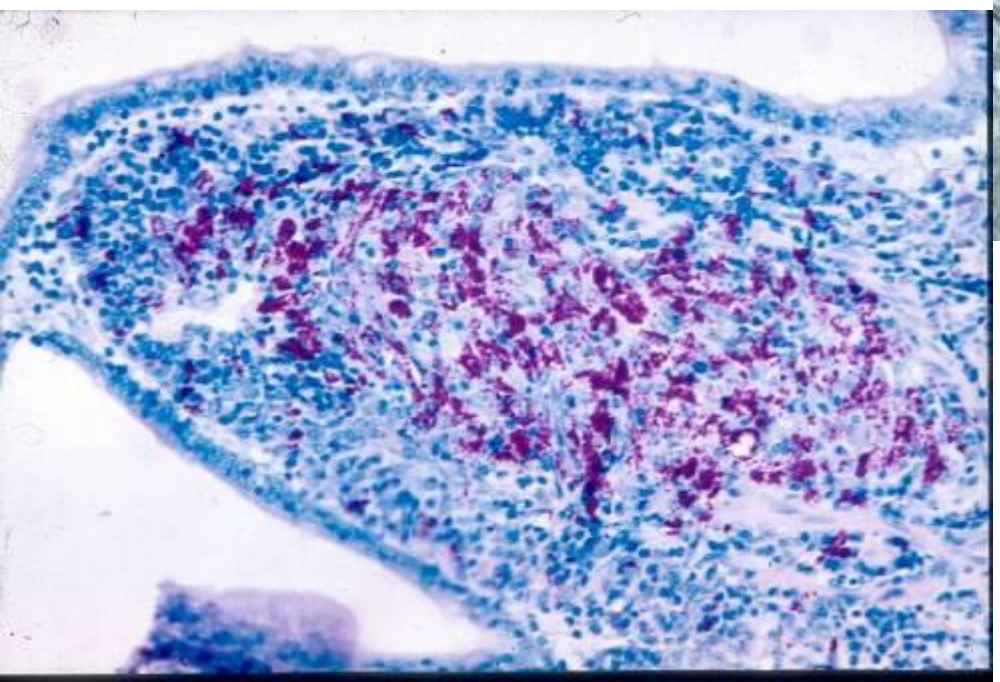
TREAT MENT

- **Use a combination of drugs to help prevent the emergence of resistant strains**
  - **Isoniazid**
  - **Rifampin**
- **Para-aminosalicylic acid**
  - **Ethambutol**
  - **Streptomycin**
  - **Antibiotics must be given over an extended period of time – at least 9 months**



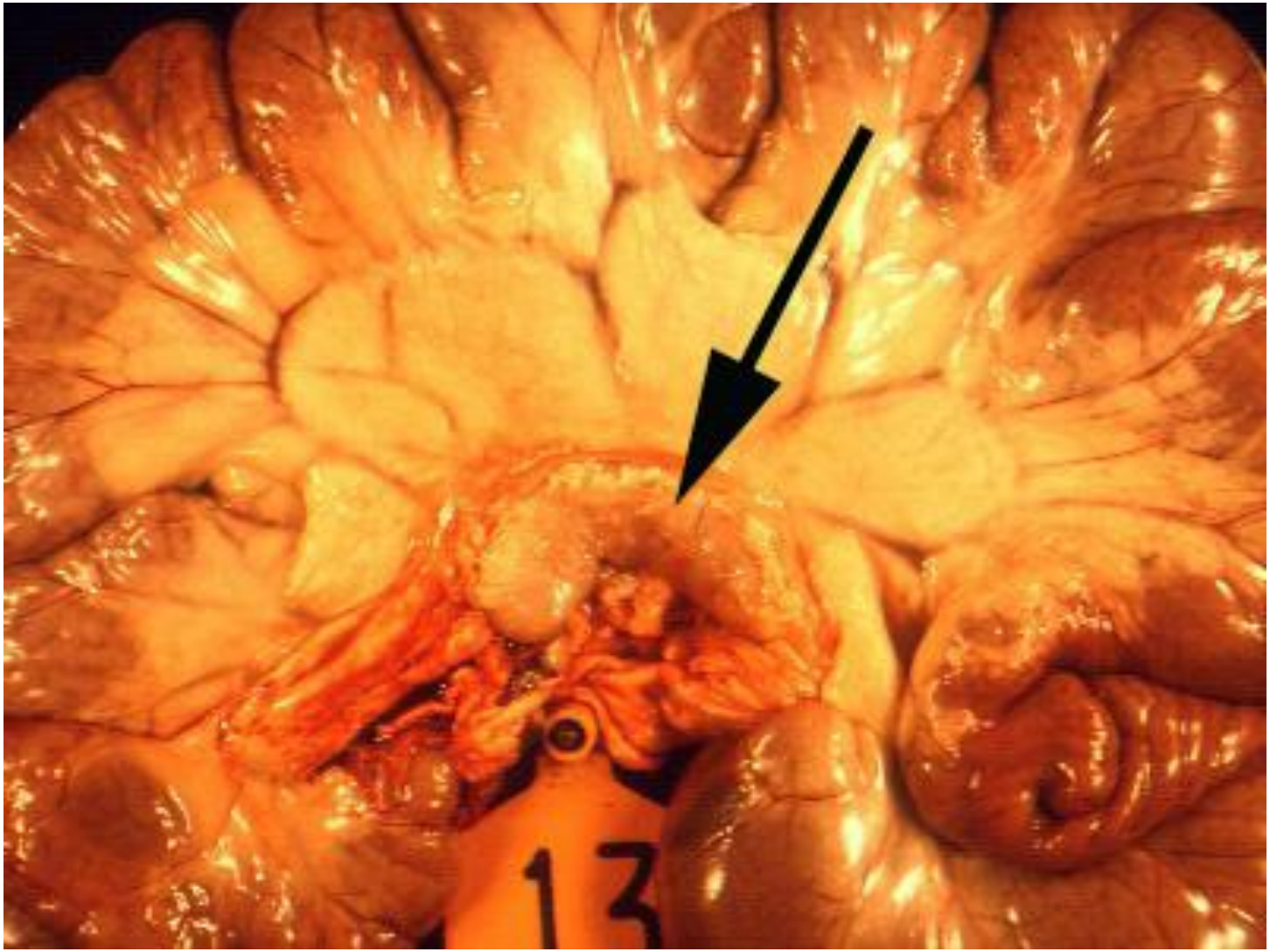
Thickened granulomatous ileum as a consequence of
M. paratuberculosis infection





Acid fast staining of
***M. paratuberculosis* in ileal tissue**

Enlarged mesenteric lymph node as a consequence of *M. paratuberculosis* infection



Differential Characteristics of Commonly Isolated Mycobacterium spp.

Organism	Niacin	Nitrate Reductase	Heat-Stable Catalase	Tween-80 Hydrolysis	Iron Uptake	Arylsulfatase	Urease
M. tuberculosis	+	+	−	−		−	+
M. kansasii	−	+	+	+		−	+
M. avium complex	−	−	+/−	−		−	−
M. fortuitum	−	+	+	V	+	+	+
M. chelonae	V	−	V	V	−	+	+

Iron uptake

- **Iron uptake test utilized to identify rapidly growing mycobacteria capable of converting ferric ammonium citrate to an iron oxide**
- **LJ slant inoculated with the organism incubated until visible growth develops, aqueous ferric ammonium citrate added, and the slant incubated for up to 21 days at 37^{o}C**
- **Development of reddish brown color in the colonies indicates production of iron oxide and is a positive result**

New methods

- Morphologic properies
- Analysis of cell wall lipids
- Nuclic acid probes
- Nucleic acid sequencing
- Rapid radiometric mycobacterial detection system
- Gas liquid chromatography
- immunological techniques- use monoclonal antibodis

Laboratory Diagnosis of Tuberculosis

Culture of acid-fast bacilli

- **Egg based medium (Lowenstein-Jensen)**

- **Agar and broth based medium (Middlebrook)**

Media for the growth of Mycobacterium:

- Lowenstein Jensen (glycerol)
- Stonebrink (M. bovis)
- Egg yolk Citrate
- Potato Agar
- Petragnani
- Dubos Broth

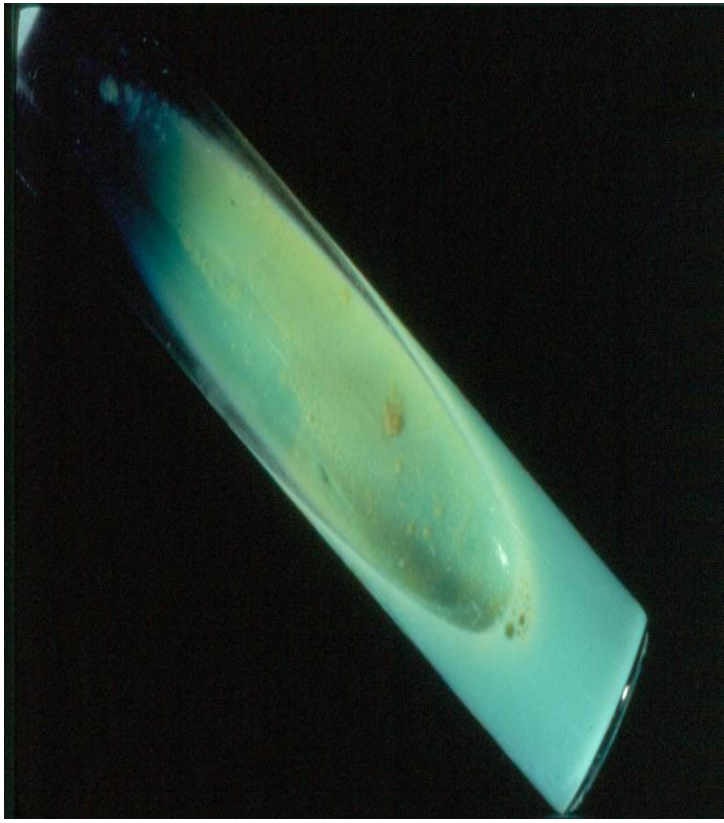
colonies appear after 4 – 6 weeks

Lowenstein-Jensen Egg Base Medium

- Coagulated whole eggs
- Potato flour
- Glycerol
- Defined salts
- Malachite Green (0.025 g/100 mL)

  (Petragnani 0.052 g/100 mL)

  (ATS 0.020 g/100 mL)



Middlebrook Agar Base 7H10 Medium

- **Defined salts**
- **Vitamins and Cofactors**
- **Oleic acid**
- **Albumin**
- **Catalase**
- **Glycerol**
- **Dextrose**
- **Malachite Green (0.0025g/100 mL)**

Middlebrook Agar Base 7H11 Medium

- **Same composition as Middlebrook 7H10 except 0.1% casein hydrolysate added for enhanced recovery of fastidious isoniazid-resistant *Mycobacterium tuberculosis***
- **Selective 7H11 contains carbenicillin, amphotericin B, polymixin B, and trimethroprim to inhibit oropharyngeal commensals**

Cultivation

- 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 arc incubated aerobically at 37°C for up t.o 16 **weeks** and examined weekly for evidence of growth**.**
- Medium containing mycobactin supports growth
- Colonies less than 1 mm in diameter, usually colourless and hemispherical appear in 5-16 weeks.
- Isolates from sheep may be pigmented

The Comparative intradermal test

1. The tuberculin test is carried out at 1,2,3, or 4 year intervals depending on the frequency of TB in the area. National average 2.7% dairy farms.

2. Animal identified and two sites prepared on the side of the neck, approx. 13 cm apart. Hair clipped 2 cm radius, and the skin fold measured.

3. Inject PPD, usually the *M. avium* preparation in the upper site.

4. Re-measure fold after 72 hrs. Reaction to *M. bovis* PPD is 5 mm greater than to the *M. avium* then defined a reactor. If 1-4 mm then retested within 40-60 days.

5. Rest of the herd analysed using 'severe interpretation' which is 3 mm.

Figure 2
Schematic representation of the spectrum of pathological and immune responses in cattle infected with *Mycobacterium bovis*