Mycobacterium species

Pro. Dr. Rasha Munther
Department of Microbiology
Vet. Med. College
University of Basra

The Taxonomy

Scientific classification

Domain:Bacteria

Phylum:Actinobacteria

Order: Corynebacteriales

Family: Mycobacteriaceae

Genus: Mycobacterium

General characteristics

- Acid-fast (ZN-positive) rods
- Cell walls rich in complex lipids and waxes containing mycolic acids
- Complex egg-enriched media required for growth of pathogenic species
- Aerobic, non-motile, non-spore-forming
- Genus includes obligate pathogens, opportunistic pathogens and saprophytes

General characteristics

- 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 (pasteurization)
- Multiply intracellularly and cause chronic, granulomatous infections
- Major diseases include tuberculosis, Johne’s disease and feline leprosy

Usual habitat

- Environmental mycobacteria are found in soil, on vegetation and in water. Obligate pathogens, shed by infected animals, can also survive in the environment for extended periods.
- Safety precautions, including the use of a biohazard cabinet, must be implemented when working with material containing mycobacteria.

Differentiation of pathogenic mycobacteria

- The ZN staining method is used to differentiate mycobacteria from other bacteria.
- Cultural characteristics, Biochemical tests,
- Animal inoculation and chromatographic analyses.
- Molecular techniques are increasingly used for identification of isolates.
- Mycobacteria associated with opportunistic infections can be differentiated on the basis of pigment production, optimal incubation temperature and growth rate

Differentiation of pathogenic mycobacteria

- Pathogenic mycobacteria grow slowly on solid media (for 3 to 6 weeks.)
- Commercially available liquid culture systems such as BACTEC™ (Becton Dickinson and Company, USA) show improved isolation times for pathogenic mycobacteria, ranging from approximately 10 to 20 days.

Differentiation of pathogenic mycobacteria

Molecular techniques:

- DNA probes, complementary to species-specific sequences of rRNA, are commercially available for the *M. tuberculosis* complex, the *M. avium* complex .
- DNA-based typing methods are used in epidemiological studies

Clinical infections

- **The diseases caused by pathogenic mycobacteria are presented in Table 23.1.**
- Diseases in domestic animals caused by mycobacteria include tuberculosis in avian and mammalian species,
- paratuberculosis in ruminants
- feline leprosy.

Clinical infections

- **The diseases caused by pathogenic mycobacteria are presented in Table 23.1.**
- Two other clinical conditions, skin tuberculosis and bovine farcy, are associated with the presence of acid-fast bacteria in lesions.
- In skin tuberculosis of cattle, nodular lesions are located along the course of lymphatics in the limbs.

Clinical infections

- Granulomatous lesions which develop following opportunistic infections with environmental saprophytic mycobacteria are encountered occasionally in domestic animals.
- These saprophytic mycobacteria are grouped on the basis of pigment production and growth rate
- Members of the *M. avium* complex are grouped with those that produce opportunistic infection because they are occasionally involved in mammalian infections..

Tuberculosis in cattle

- Bovine tuberculosis, caused by M. bovis, occurs worldwide.
- The incidence of human infection with *M. bovis* has been reduced to low levels in countries where tuberculosis eradication programmes have been implemented in cattle.
- Cross-infection with *M. tuberculosis* from infected humans has been recorded in cattle on rare occasions.

Epidemiology

- Although ***M. bovis*** can survive for several months in the environment, transmission is mainly through aerosols generated by infected cattle.
- Dairy cattle in particular are at risk because husbandry methods allow close contact between animals at milking and when housed during winter months.

Epidemiology

- Calves can become infected by ingesting contaminated milk and ingestion is the probable route of transmission to pigs and cats.

Pathogenesis and pathogenicity

- The virulence of *M.* bovis relates to its ability to survive and multiply in host macrophages.
- mycobacteria are engulfed by macrophages and by dendritic cells. Mycobacteria engulfed by dendritic cells travel to the draining lymph nodes.

Pathogenesis and pathogenicity

- 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.

Pathogenesis and pathogenicity

- With the development of cell-mediated immunity some weeks after infection, macrophage recruitment accelerates under the influence of cytokines produced by T lymphocytes sensitized to tuberculoprotein.
- In addition, these macrophages become activated through cytokine stimulation and proliferate.

Pathogenesis and pathogenicity

- The gradual accumulation of macrophages in the lesion and the formation of a granulomatous response lead to the development of a tubercle, the typical host response in the delayed-type hypersensitivity to mycobacterial infections

Diagnostic procedures

- The tuberculin test, based on a delayed-type hypersensitivity to mycobacterial tuberculoprotein, is the standard antemortem test in cattle. In cattle is usually detectable 30-50 days after infection.
- Tuberculin, prepared from mycobacteria and called purified protein derivative (PPD), is injected intradermally to detect sensitization

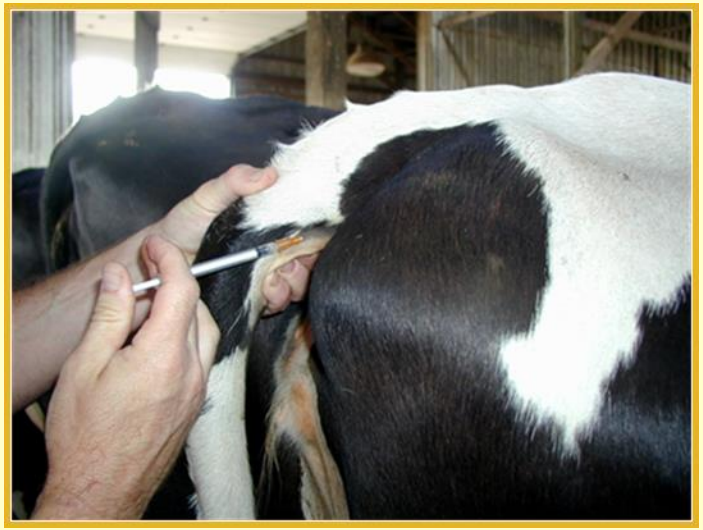
Diagnostic procedures

- Two main methods of tuberculin testing are employed:

1. In the single intradermal (caudal fold) test, 0.1 ml of bovine PPD is injected intradermally into the caudal fold of the tail. The injection site is examined 72 hours later and a positive reaction is characterized by a hard or oedematous swelling.

Tuberculin skin test

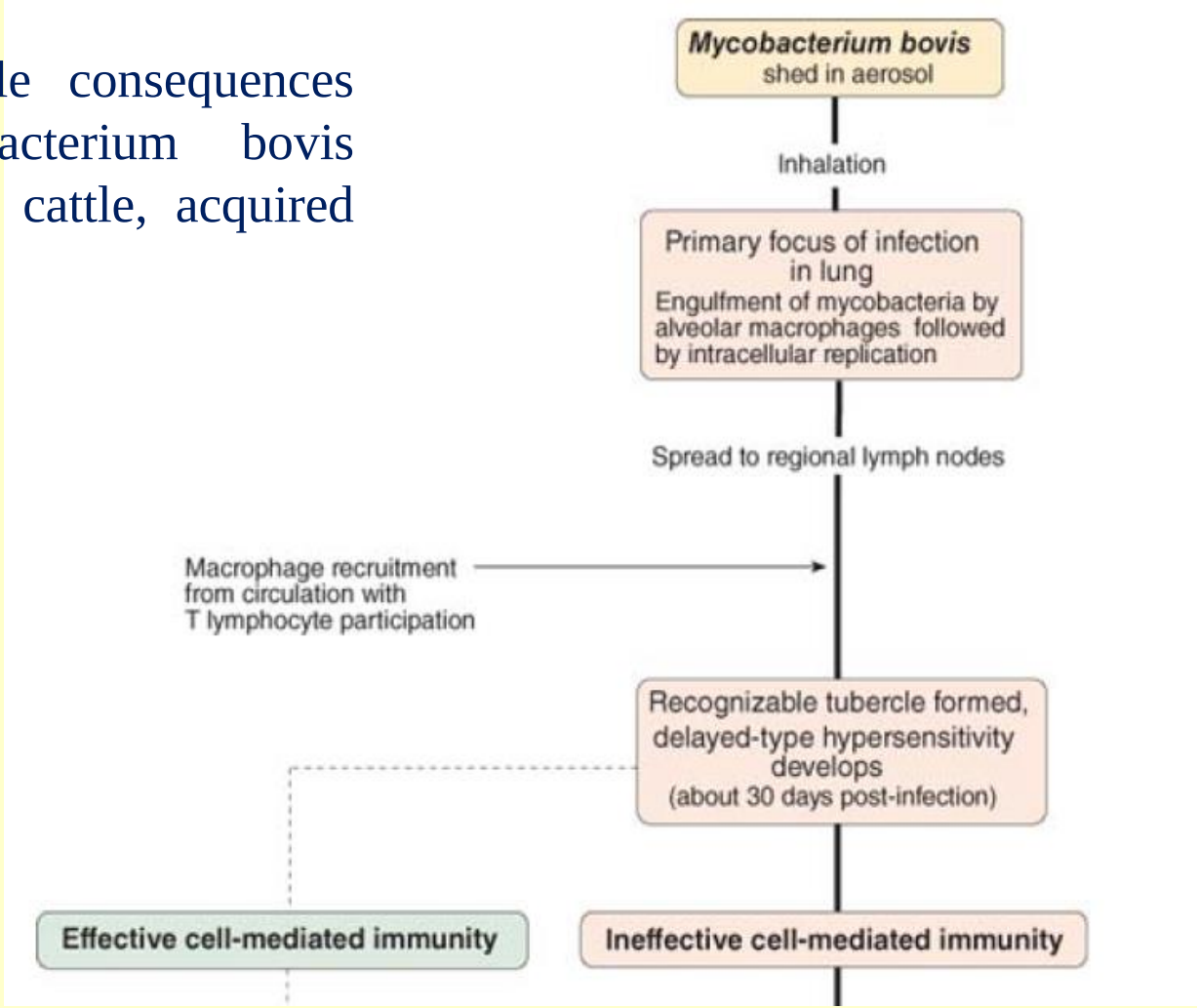
- Diagnosis: Live Cattle
- Caudal fold test : test for detection tuberculosis in cattle
- Preliminary screening of cattle

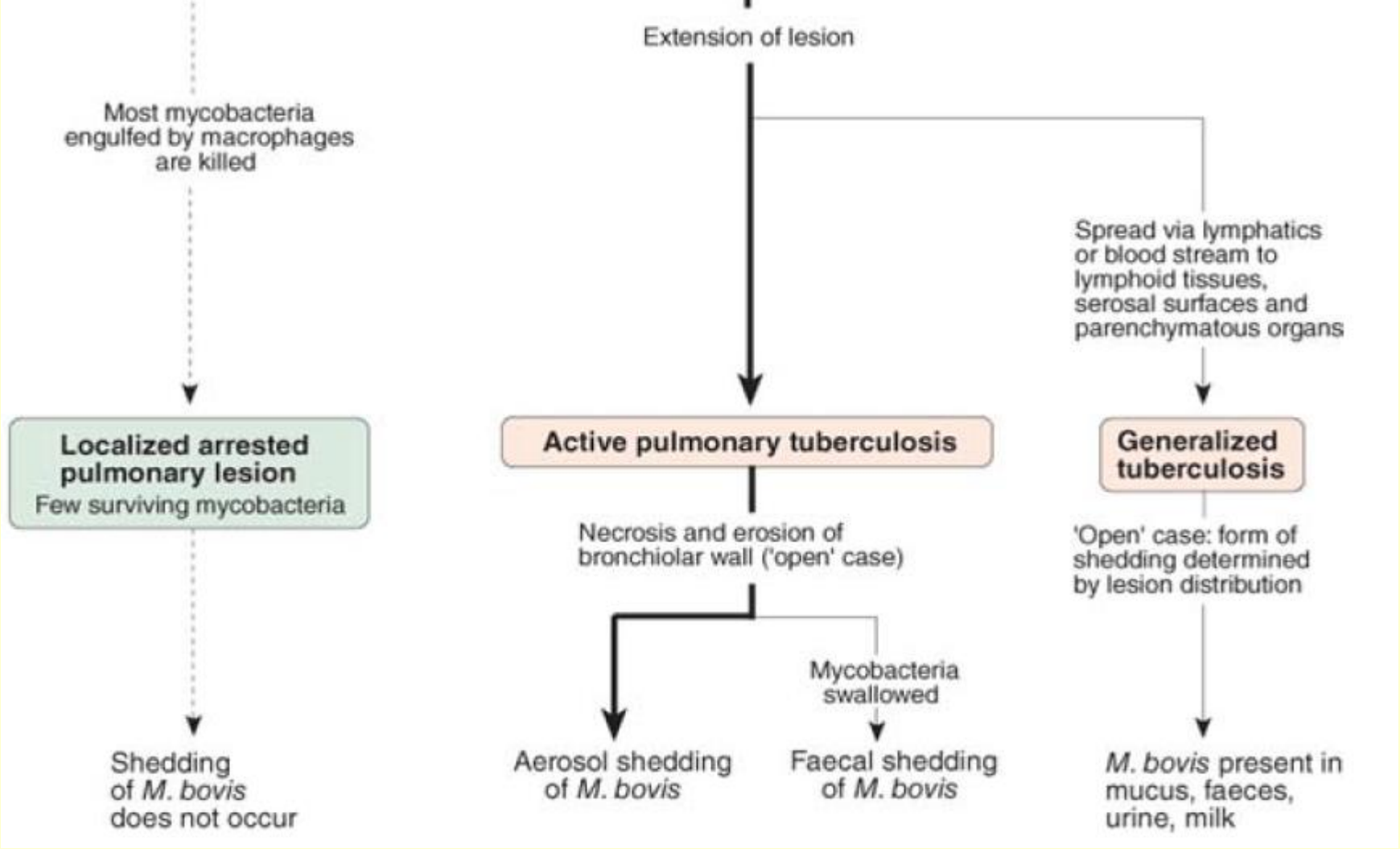


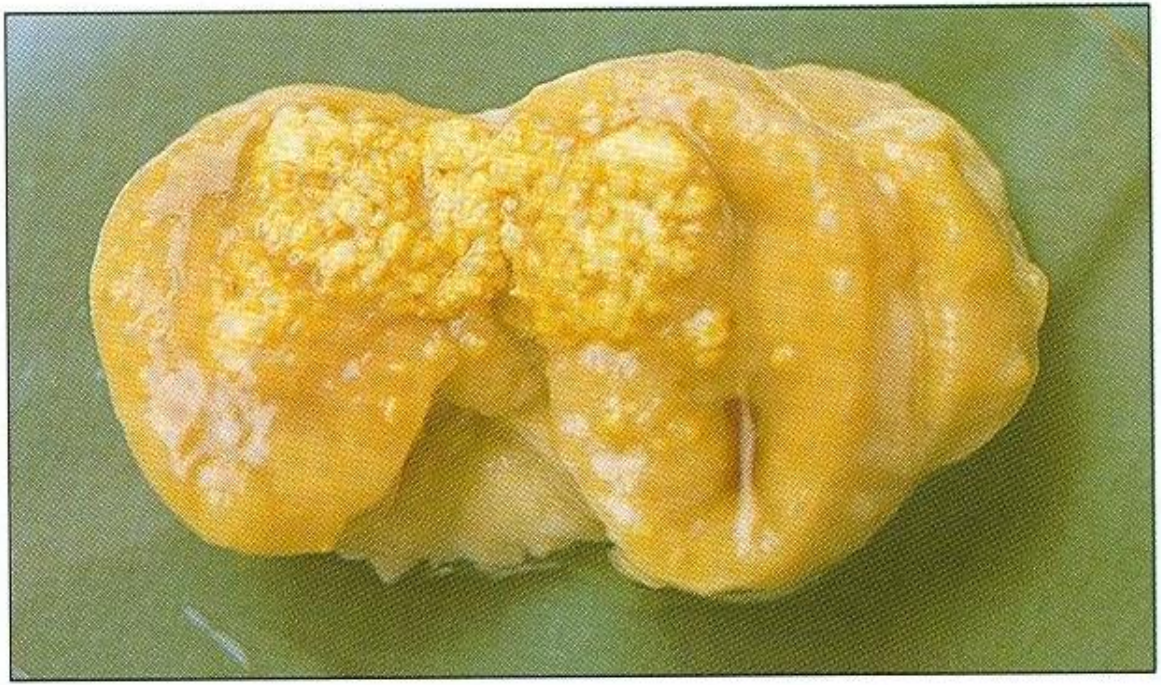
Diagnostic procedures

2. the comparative intradermal test, 0.1 ml of avian PPD and 0.1 ml of bovine PPD are injected intradermally into separate clipped sites on the side of the neck about 12 cm apart. Skin thickness at the injection sites is measured with calipers before injection

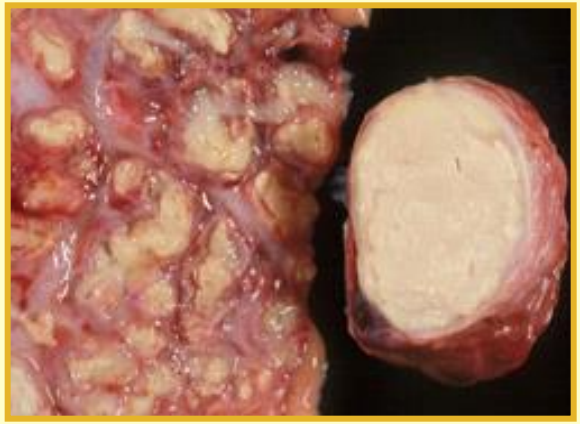
The possible consequences of Mycobacterium bovis infection in cattle, acquired via aerosols.

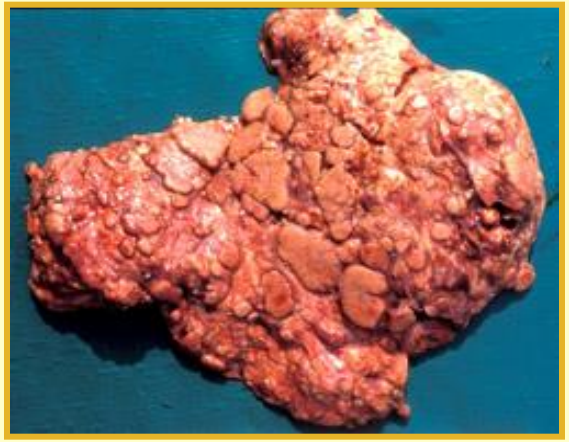



Extension of lesion
Most mycobacteria engulfed by macrophages are killed
Spread via lymphatics or blood stream to lymphoid tissues, serosal surfaces and parenchymatous organs
Localized arrested pulmonary lesion
Few surviving mycobacteria
Active pulmonary tuberculosis
Generalized tuberculosis
Necrosis and erosion of bronchiolar wall ('open' case)
'Open' case: form of shedding determined by lesion distribution
Mycobacteria swallowed
Shedding of M. bovis does not occur
Aerosol shedding of M. bovis
Faecal shedding of M. bovis
M. bovis present in mucus, faeces, urine, milk



182 A tuberculous lesion in a bovine lymph node (*Mycobacterium bovis* infection).





Tuberculosis in poultry and other avian species

Mycobacterium avium

Mycobacterium avium

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 feces of birds with advanced lesions, can survive for long periods in soil.

Mycobacterium avium

Diagnosis is based on the post-mortem findings and on the demonstration of large numbers of ZN-positive bacilli in smears from lesions. Ante-mortem diagnosis of avian tuberculosis in free-range poultry is based on tuberculin testing, using avian PPD injected into the skin of a wattle

Pathogenesis

- *M.avium* are shed in faces
- Transmission is usually by the fecal-oral route and in birds the lesions are characteristically found in the liver.
- Lesions can also be present in the intestines spleen and bone marrow.
- *M.avium* may sensitize cattle to the tuberculin test

Paratuberculosis (Johne's disease)

Mycobacterium paratyperculosis

Paratuberculosis (Johne's disease)

- Paratuberculosis is a chronic, contagious, invariably fatal enteritis which can affect domestic and wild ruminants.
- The a etiological agent, *M. avium* subsp. *paratuberculosis*, is an acid-fast organism formerly referred to as *Mycobacterium johnei*.

Mycobacterium paratyperculosis

Uncertainty exists regarding the association between infection with *M. avium* subsp. *paratuberculosis* and Crohn's disease, a chronic enteritis in humans

Pathogenesis and pathogenicity

- *Mycobacterium avium* subsp. *paratuberculosis* is an intra-cellular pathogen and cell-mediated reactions are mainly responsible for the enteric lesions.
- Ingested mycobacteria are taken up by **microfold (**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.

Pathogenesis and pathogenicity

- 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.*

Pathogenesis and pathogenicity

- As the disease progresses, an immune-mediated granulomatous reaction develops, with marked lymphocyte and macrophage accumulation in the lamina propria and submucosa.
- Two types of lesion are recognized, multibacillary (lepromatous) and
- paucibacillary (tuberculoid), which appear to be correlated with host immune response.

Diagnosis

- 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.

Cont.

- After decontamination of the specimen with 0.3% benzalkonium chloride and concentration by centrifugation, slants of Herrold’s egg-yolk medium with 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.

Cont.

- More rapid isolation techniques, which are based on growth in liquid media and detection of growth using radiometric methods (e.g. BACTEC), are available.
- PCR techniques for the detection of insertion sequence IS900 which is specific for M. avium subsp. paratuberculosis.

Cont.

- Strict safety precaution must be enforced when working with samples suspected of containing *M. bovis*, *M. tuberculosis*.
- As the mycobacteria are very resistant to disinfectants.

Media for the Mycobacterium

- The egg- based Lowenstein-Jensen and Stonebrinks media are most commonly used in veterinary bacteriology.

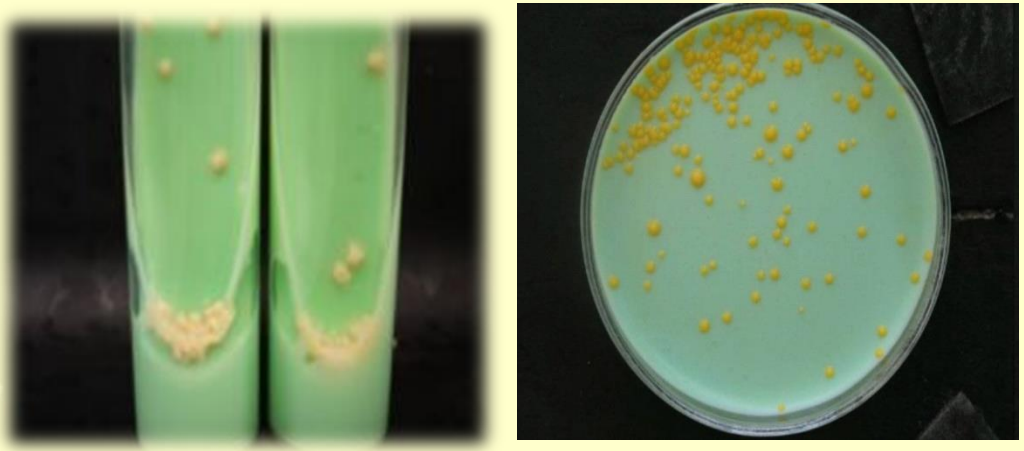


Figure 1: colonies of *M. tuberculosis* on Lowenstein-Jensen medium dry, heaped -up yellow to buff-colored

Pigment production and response to light

- The Mycobacteria that produce yellowish-orange carotenoid pigments are called chromogenic; the term photochromogenic is applied to those mycobacteria that produce pigment only if exposed to light.
- Pigments formation is produced by young well developed colonies on Lowenstein-Jensen medium

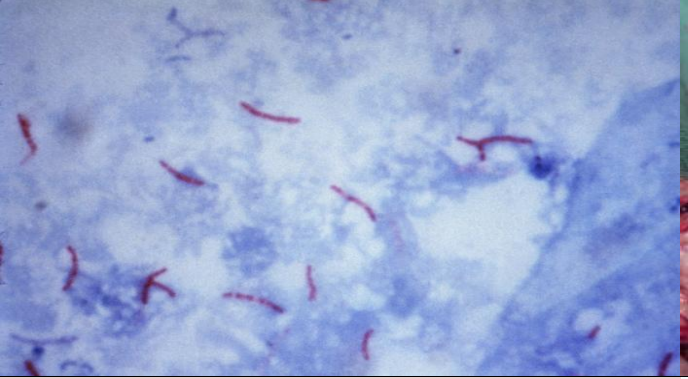


Figure 1*: M. tuberculosis* **bacteria using acid-fast Ziehl-Neelsen stain**

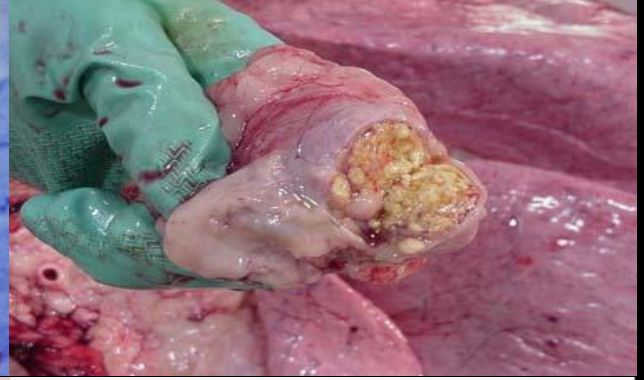


Figure 1: Lesions of bovine tuberculosis caseous lesion lymph nodes of the respiratory system.

Figure 1: colonies of *M. tuberculosis* on Lowenstein-Jensen medium dry, heaped -up yellow to buff-colored

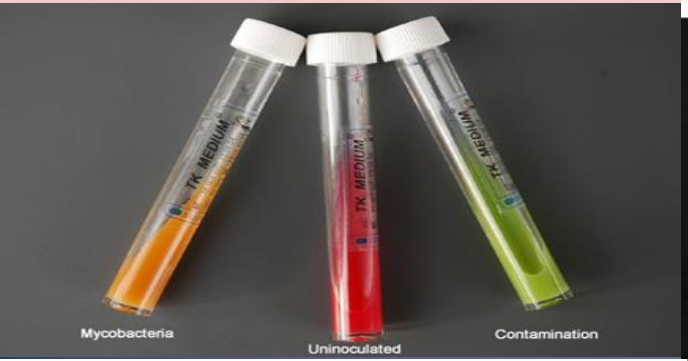


Figure : *TK MEDIA*® indicates growth of mycobacteria by changing its color allowing differentiation from contamination prior to microscopic examination. The metabolic activity of bacteria changes the color of the medium for a positive identification long before bacteria colonies appear.

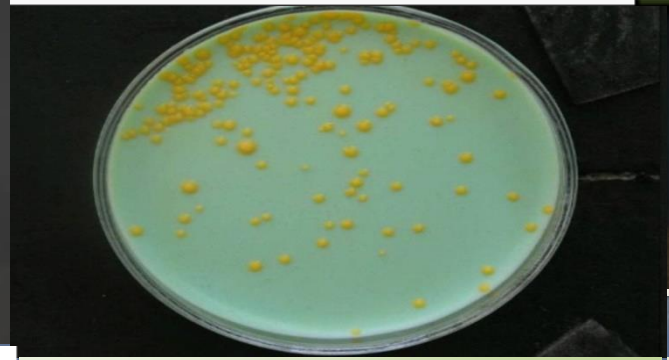


Figure 1: *M. paratuberculosis* growth in Lowenstein-Jensen medium

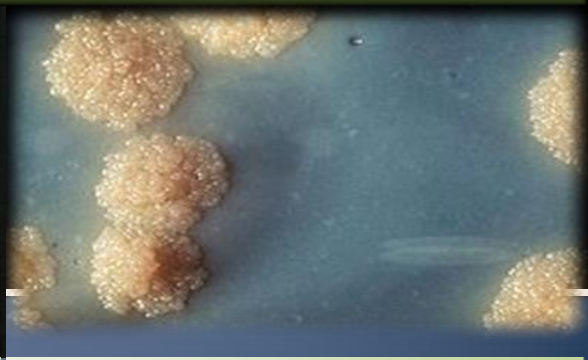


Figure 1: *M. tuberculosis* bacterial colonieson Lowenstein-Jensen medium granular waxy pattern of growth

References and Textbooks

Veterinary Microbiology and Microbial Disease, 2nd Edition.P. J. Quinn, B. K. Markey, F. C. Leonard, P. Hartigan, S. Fanning, E. S. Fitzpatrick