Mycobacterium species

Definition:-

Mycobacteria are aerobic, non-spore-forming, non-motile, rod shaped, acid-fast bacilli. Individual species differ in size; the rods of *Mycobacterium bovis* and ***M.*** *avium subsp. avium* are slender and up to 4 mm in length, whereas those of M. avium subsp. paratuberculosis are broad and are usually less than 2 *mm* long(Fig1).

Although mycobacteria are cytochemically Gram positive, the high lipid 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. The mycobacteria include diverse species ranging from environmental saprophytes and opportunistic invaders to obligate pathogens (Fig 2).

Although some pathogenic mycobacteria exhibit a particular host preference, they can occasionally infect other species (Table 1). Mycobacterial diseases in domestic animals are usually chronic and progressive. The closely-related members of the ***M.*** *tuberculosis* complex ***(****M. tuberculosis, M. bovis and M. africanum****)*** cause tuberculosis in humans.

Table 1. **Mycobacteria** which are pathogenic for animals and man.

Species	Host(s)	Significance
***M. tuberculosis* complex: slow growing**		
M. africanum	Humans	Human tuberculosis (mainly West Africa)
'M. canettii'	Humans	Human tuberculosis (mainly East Africa)
M. tuberculosis	Humans, captive primates, dogs, cattle, psittacine birds, canaries	Human tuberculosis (worldwide)
M. bovis	Cattle, deer, badgers, possums, humans, cats, other mammalian species	Bovine tuberculosis
M. microti	Voles, occasionally other mammalian species	Vole tuberculosis. Localized lesions seen in rabbits, calves and guinea pigs
M. caprae	Goats, cattle	Tuberculosis in goats
M. pinnipedii	Seals, sea-lions, occasionally other mammalian species including man	Tuberculosis in pinnipeds
Runyon's groups		
I. Photochromogens: slow-growing (over seven 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	Tuberculosis-like lesions in cervical and intestinal lymph nodes
III. Non-chromogens: (slow growing)		
M. avium complex	Poultry and wild birds	Avian tuberculosis. Generalized form rare in mammals. Lesions in cervical lymph nodes
	Pigs	Intestinal lesions (rare)
	Horses, pigs and others	
M. intracellulare	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 paratuberculosis in cattle)
M. ulcerans	Cats	Nodulo-ulcerative skin lesions
M. xenopi	Cats	Nodulo-ulcerative skin lesions
	Pigs	Tuberculous lesion in lymph nodes of the alimentary tract

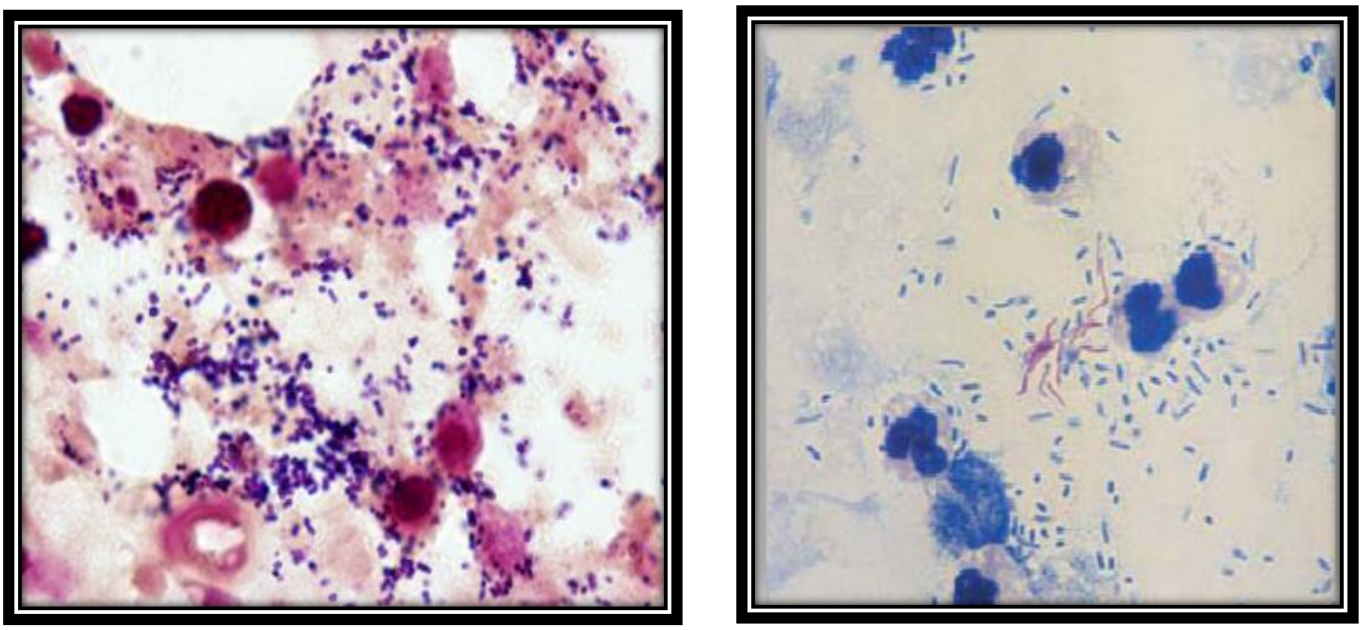

Fig 1. ***Mycobacterium tuberculosis*** **is an acid-fast bacteria**

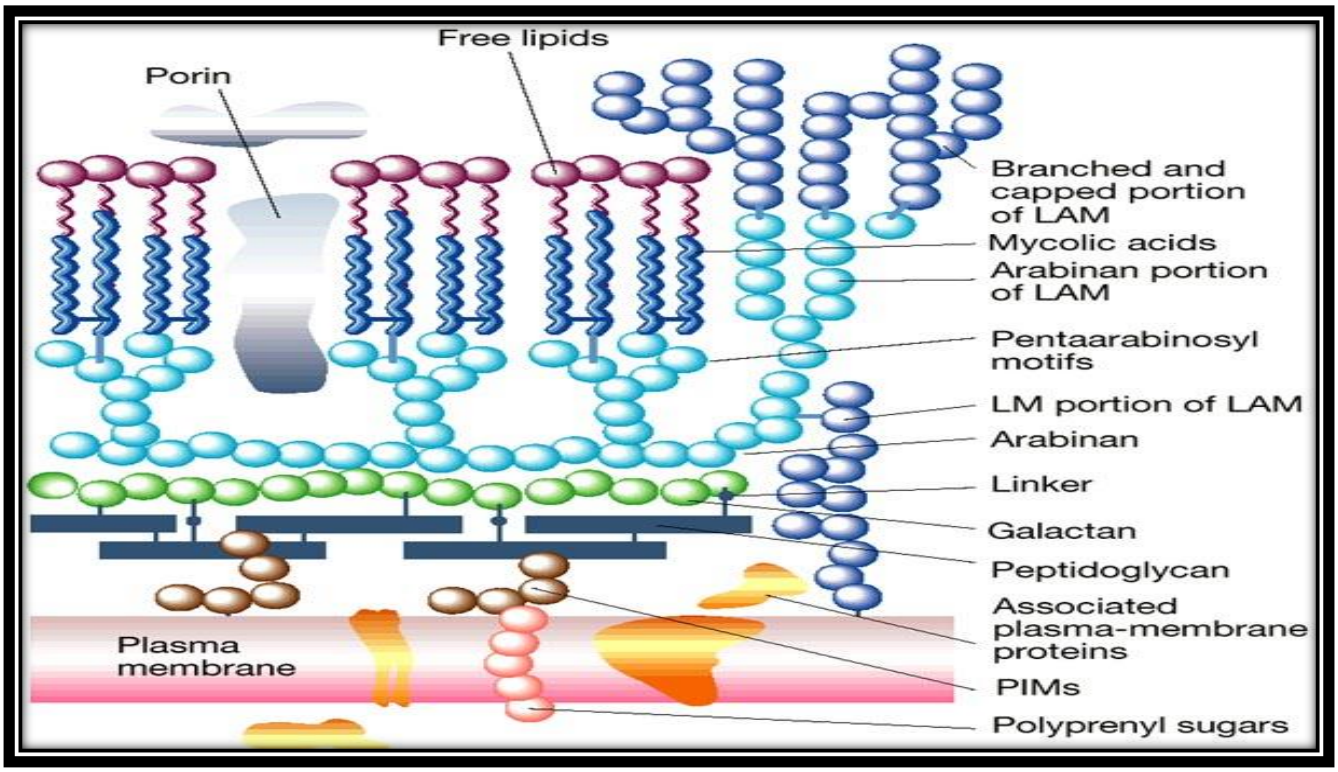

Fig 2. Schematic representation of *M. tuberculosis* cell envelope.

Usual habitat

Lipid-rich walls render mycobactcria hydrophobic and resistant to adverse environmental influences. 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.

Differentiation of pathogenic 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. In addition, mycobacteria associated with opportunistic infections can be differentiated on the basis of pigment production, optimal incubation temperature and growth rate (Table 2).

1-Safety precautions, including the use of a biohazard cabinet, must **be** implemented when working with material containing mycobacteria.

2-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.

3-*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 to43°C.

4-Cultural features:

a-Pathogenic species of mycobacteria can be distinguished by their colonial appearance on egg-based media.

b-The influence of glycerol and sodium pyruvate on grow*t*h rate is used to differentiate pathogenic species.

c-Supplementation of media with mycobactin is required for *M. avium subsp, paratuberculosis***.** Mycobactin is extracted from laboratory-maintained, rare, non-mycobactin-dependent isolates *of M. avium subsp. paratuberculosis.*

5-Biochemical differentiation, based on specific test methods, aids in the identification of *M. tuberculosis, M. bovis and M*. avium. Some mycobacterial isolates cannot be assigned to a given species using biochemical differentiation as their biochemical profiles are difficult to interpret .

6-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***.**

7-Chromatographic analyses of the lipid composition of some mycobacterial species are used in specialized laboratories.

8-Pigment production and photoreactivity for opportunistic mycobacteria:

a-Non-chromogens produce colonies devoid of orange,carotenoid pigments.

b-Photochromogens, when cultured in the dark, produce non-pigmented colonies which become pigmented after a period of exposure to light.

c- Scotochromogens produce pigment when cultured in the dark or in light.

9-Molecular techniques:

a-DNA probes,complementary to species-specific sequences of rRNA**,**are commercially available for the *M. tuberculosis complex***,** the *M. avium complex* and *M. kansasii.*

b-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

c-DNA restriction endonuclease analyses (DNA finger printing) are used in epiderniologica1

Table 2. Clinical significance, growth characteristics and biochemical differentiation of pathogenic mycobacteria.

	M. tuberculosis	***M. bovis***	***M. avium* complex**	***M. avium* subsp. *paratuberculosis***
1-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
2-Cultural characteristics and requirements				
1-Growth rate	Slow **(3-8**weeks)	Slow (3-8 weeks	Slow (2-6 weeks	Very slow (upto16weeks)
2-Optimalincubation Temperature	37^0c	37^0c	37-43°c	37°c
3-Atmospheric requirements	Aerobic	Aerobic	Aerobic	Aerobic
4- 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
5- Essential growth supplement	None	None	None	mycobactin
6- Effect of added glycerol	Enhanced growth(eugonic)	Growth inhibited (dysgenic	Enhanced growth eugonic)	
7-Effect of added sodium pyruvate	No **effect**	Enhanced growth	No effect	
3- Biochemical differentiation				
- Niacin accumulation	+	-	-	
- Pyrazinamidase production	+	-	+	
- Nitrate reduction	+	-	-	
-Susceptibility to **TCH** (1 0 pglmlla	Resistant	Susceptible	Resistant	

Clinical infections:-

The diseases caused by pathogenic mycobacteria are presented in Table 1. The major pathogenic *Mycobacterium* species which affect domestic animals exhibit a considerable degree of host specificity although they can produce sporadic disease in a number of other hosts.

Diseases in domestic animals caused by mycobacteria include tuberculosis in avian and mammalian species, paratuberculosis in ruminants and feline leprosy. 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. Unspecified acid-fast bacilli have been demonstrated in these lesions. *Mycobacterium senegalense* and *M. farcinogenes* have been isolated from the lesions of bovine farcy. Their aetiological role in this condition, however, is uncertain (fig 3).

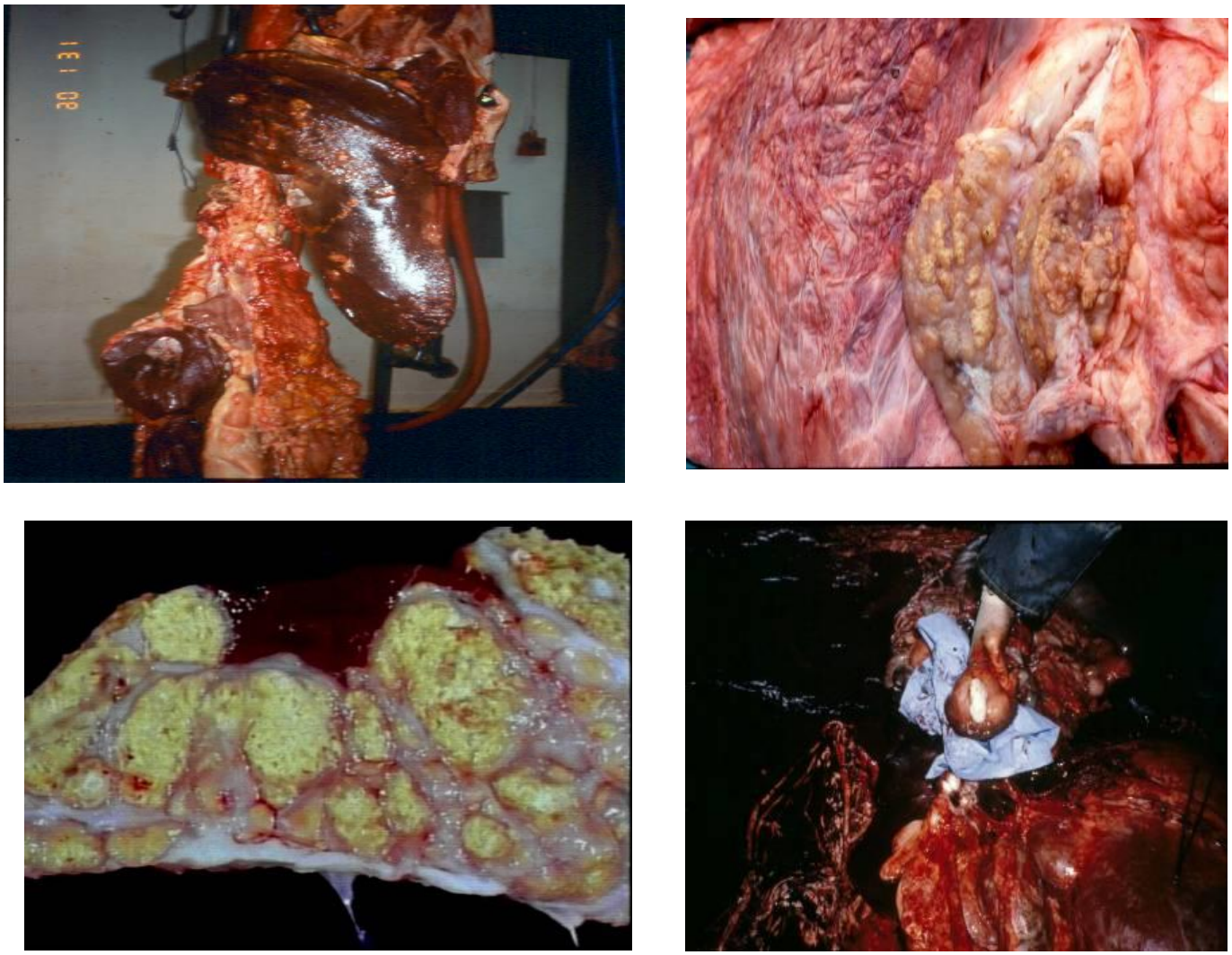

Fig 3. Lesion of tuberculosis in the lungs and liver.

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 (Table 2). Members of the ***M.*** avium complex are grouped with those which produce opportunistic infection because they are occasionally involved in mammalian infections.

Table 3. classification of mycobacteria of environmental origin which infrequently produce opportunistic infections.

classification of pathogenic mycobacteria	Example
1-Photochromogens	*M,*. kansasii ***M. marinum***
2-Scotochromogens	***M.*** *scrofulaceum*
3-Non-chromogens	***M.*** **avium** complex ***M.*** *genavense*
4-Rapid growers	***M. chelonae*** group *M.*. ***forfuitum*** group ***M. phlei*** ***M. smegmutis***

Tuberculosis in cattle

Bovine tuberculosis, caused by *M. **bovis,*** occurs worldwide. Because of the zoonotic implications of the disease and production losses due to its chronic progressive nature, eradication programs have been introduced in many countries. When eradication programs are successfu1, infections in cattle caused by members of the ***M. avium*** complex and by other saprophytic mycobacteria are occasionally encountered. 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. In addition, pasteurization of milk has eliminated **exposure of** humans **to** infection **from** dairy products. Cross-infection with M. *tuberculosis* from infected humans has been recorded on rare occasions in cattle.

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. Calves can become infected by ingesting contaminated milk and ingestion is the probable route of transmission to pigs and cats. Wildlife reservoirs of ***M.*** *bovis* are major sources of infection for grazing cattle in some countries. They include the badger in Europe, the brush-tailed possum in New Zealand and the Cape buffalo and other ruminants in Africa. Deer, both wild and farmed, are particularly susceptible and may act as reservoirs of infection for cattle.

Pathogenesis and pathogenicity

1-The virulence of ***M.*** *bovis* relates to its ability to **survive and multiply in host macrophages**.

2-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.

3-Migration of macrophage containing viable mycobacteria can **disseminate infection**

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

5-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).

Clinical signs

1-Clinical signs are **evident only in advanced disease** and cattle with extensive lesions can appear **to be in good health**.

2-Loss of condition may become evident as the disease progresses.

3-In advanced pulmonary tuberculosis, animals may eventually develop a cough and intermittent pyrexia.

4-Involvement of mammary tissue **may** result in marked induration of affected quarters, often accompanied by supramammary lymph node enlargement.

5-Tuberculous mastitis is of facilitates spread of infection to calves and cats, and is of major public health importance.

6−In the early stages of the disease, lesions may be difficult to detect at postmortem examination. These small lesions are composed of aggregates of macrophages, termed epithelioid cells. Multinucleate Langhans' giant cells, formed from the fusion of macrophages, may also be present.

7−In older lesions, fibroplasia produces early capsule formation and there is central caseous necrosis, detectable grossly as yellowish cheesy material (Fig 4).

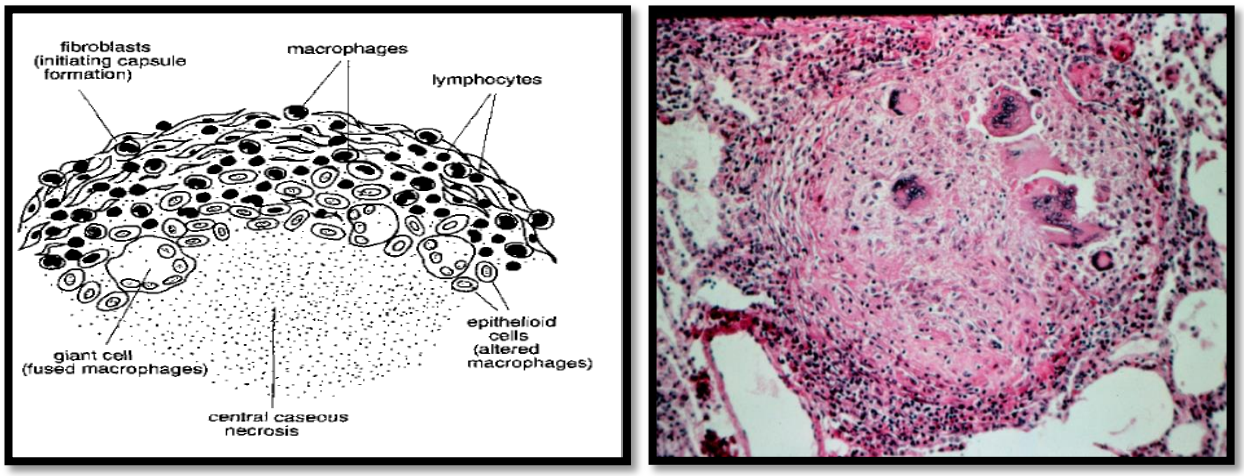

Fig 4. The characteristic histological appearance of a typical tubercle

Diagnostic procedures

A-The tuberculin test, based on a delayed-type hypersensitivity to mycobacterial tuberculoprotein, is the standard antemortem test in cattle. The test can be adapted for use in pigs and farmed deer. Reactivity 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

Two main methods of tuberculin testing are employed:

a- 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.

b-In 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 of tuberculins and after 72 hours. An increase in skin thickness at the injection site of bovine PPD which exceeds that at the avian PPD injection site by 4 mm or more is interpreted as evidence of infection and the animal is termed a reactor.

B-Blood-based tests which have been developed for use in conjunction with the tuberculin test include:

1- Gamma interferon assay
2- ELISA for detecting circulating antibodies
3-Lymphocyte transformation and related assays

C-Staining of samples

1-Specimens suitable for laboratory examination include: lymph nodes, tissue lesions, aspirates and milk.

2- Specimens are stain by Z.N. and bacteria appear resistant to decolorization red in color and background blue in color (fig 5).

D- Isolation of *M. bovis* requires:

1-Decontamination of specimens to eliminate fast growing contaminating bacteria. Ground-up specimens are treated for up to 30 minutes with 2-4% sodium hydroxide or 5% oxalic acid, followed by neutralization of the alkali or acid.

2-Centrifugation is used to concentrate the mycobacteria and the supernatant fluid is discarded.

3-Slants **of Lowenstein-Jensen medium**, without glycerol and containing 0.4% sodium pyruvate, are inoculated with the centrifuged deposit and incubated aerobically at 37°C for up to 8 weeks (fig 6).

The Identification criteria for isolates:

a- Growth rate and colonial appearance

b-Positive ZN-staining of bacilli in smears from colonies

c- Biochemical profile (Table1.13)

d-Analytical and molecular techniques

Commercially-available, rapid, automated systems can be used for isolating pathogenic mycobacteria of the M. ***tuberculosis*** complex.

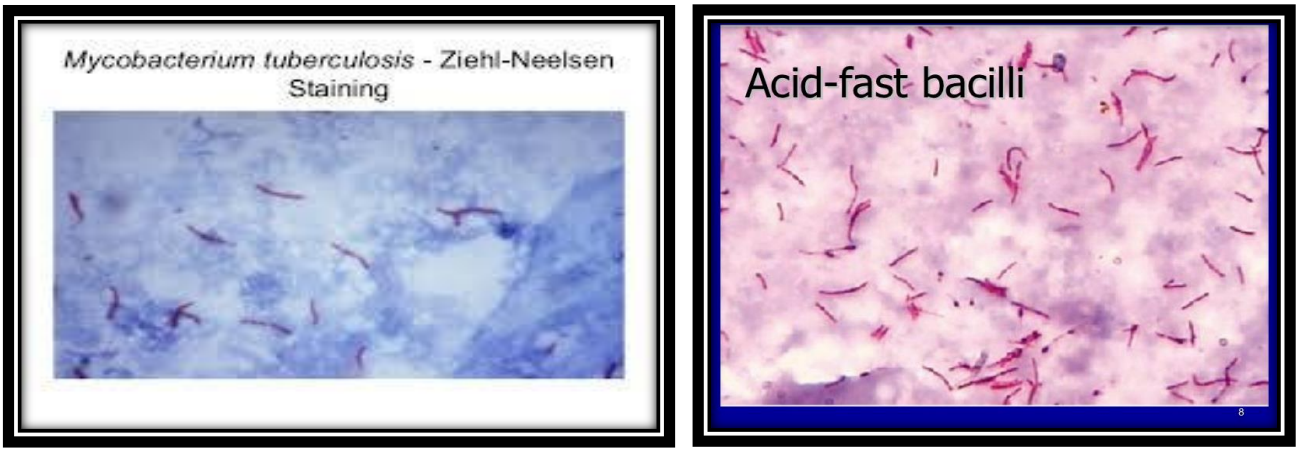

Fig 5. TB ZN (Ziehl-Neelsen) stain

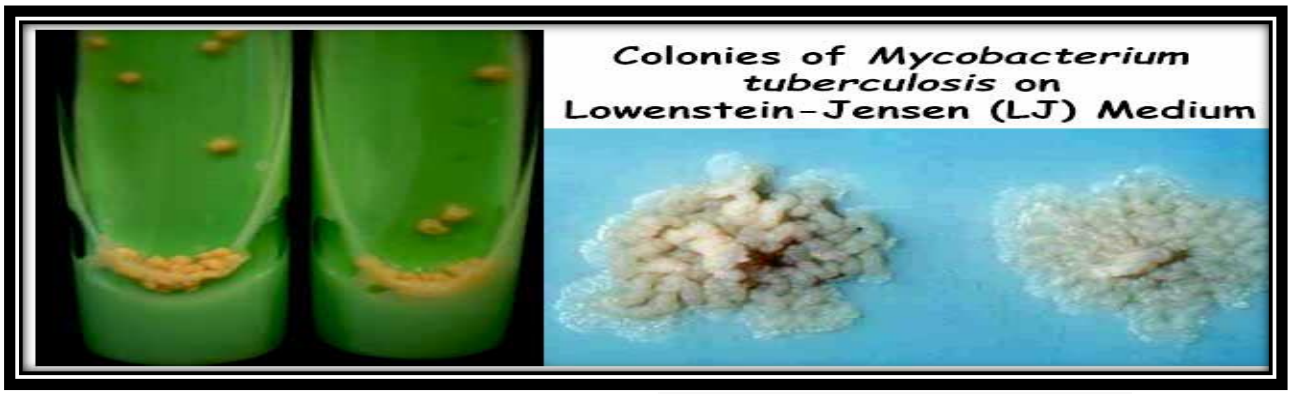

Fig 6. *Mycobacterium tuberculosis* on Löwenstein-Jensen medium

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

Non-specific clinical signs including dullness, emaciation and lameness develop in affected birds only when the disease is at an advanced stage. At postmortem examination, granulomatous lesions are characteristically present in the liver, spleen, bone marrow and intestines. Diagnosis is based on the postmortem findings and on the demonstration of large numbers of ZN-positive bacilli in smears from lesions. Antemortem diagnosis of avian tuberculosis in free-range poultry is based on tuberculin testing, using avian PPD injected into the skin of a wattle. *M. tuberculosis* occasionally infects parrots and canaries and M. genavense has been isolated from pet birds (fig 6).

Members of the *M. avium* complex cause opportunistic infections in immunocompromized humans. Rare cases of generalized disease in cats, dogs and horses caused by members of the complex have been reported. Pigs infected through the ingestion of uncooked swill contaminated with *M. avium* often develop small tubercles in the retropharyngeal, submaxillary and cervical lymph nodes.

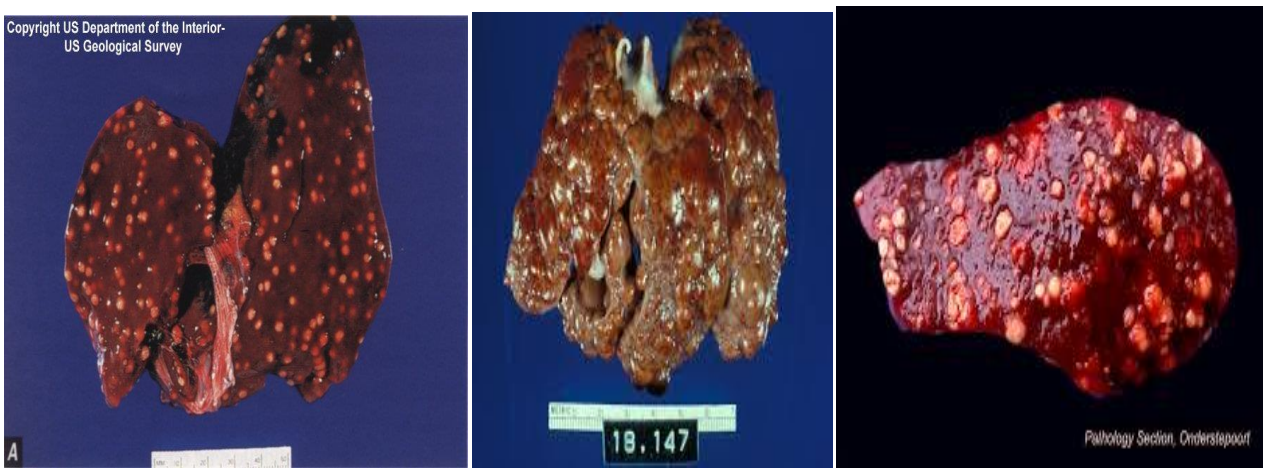

Fig 5. Firm nodules within the liver associated with avian tuberculosis.

Feline leprosy:-

It is generally considered that feline leprosy, a cutaneous disease of worldwide distribution, is caused by *M. lepruemurium***,** the aetiological agent of rat leprosy. Sporadic transmission of the organism to cats probably occurs through bites from infected rodents, the wildlife reservoirs. Nodular lesions, involving subcutaneous tissues, may be solitary or multiple and are usually confined to the head region or the limbs. The nodules, which are fleshy and freely movable, tend to ulcerate. Large numbers of ZN positive bacilli are present in smears from the lesions. Histopathological examination demonstrates many infiltrating macrophages which contain densely –packed mycobacteria.

Mycobacterium lepruemurium, a slow-growing fastidious organism, requires a specially formulated culture medium for growth. It does not appear to be infectious for other species of domestic animals or for humans. Diagnosis is based on the histopathological features of the lesions and negative cultural results for *M. bovis* and opportunistic mycobacteria, which can also cause granulomatous dermatitis in cats. Surgical excision of lesions is the preferred treatment (fig 6).

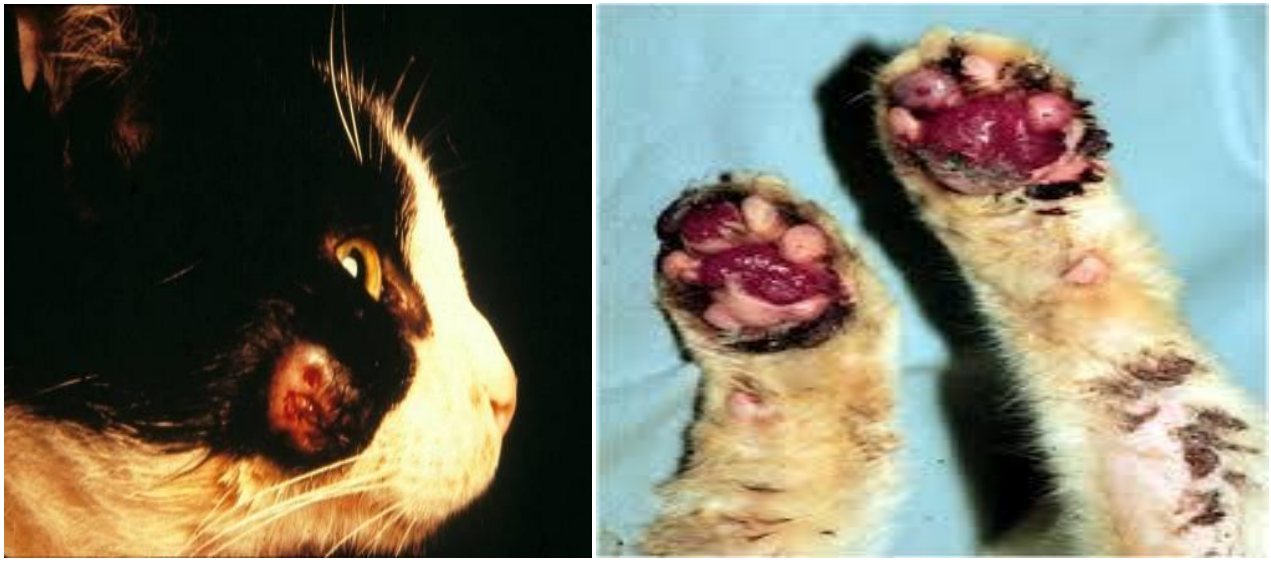

Fig 6. Cutaneous leprosy in cat, showing numerous periocular lesions

Paratuberculosis (Johne's disease):-

Paratuberculosis is 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.

Uncertainty exists regarding an 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 intracellular pathogen and cell-mediated reactions are mainly responsible for the enteric lesions. Ingested mycobacteria, engulfed by macrophages in which they survive and replicate, are found initially in Peyer's patches. **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.