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Lecture title: Mycobacterium spp.

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Summary: Mycobacterium spp.

Key points

1. Classification (According to Bergey's Manual)

2. General characteristics

3. Usual habitat

4. Differentiation of pathogenic mycobacteria

5. Bovine tuberculosis

6. Paratuberculosis (Johne's disease)

1. Acid-fast (Ziehl-Neelsen -positive) rods

2. Cell walls rich in complex lipids and waxes containing mycolic acids

3. Complex egg- media required for growth of pathogenic species

4. Aerobic, non-motile, non-spore-forming

5. Genus includes obligate pathogens, opportunistic pathogens and saprophytes

6. Pathogenic species grow slowly, colonies visible afterseveral weeks

7. Some mycobacteria produce carotenoid pigments

8. Resistant to chemical disinfectants but susceptible to heat treatment (pasteurization)

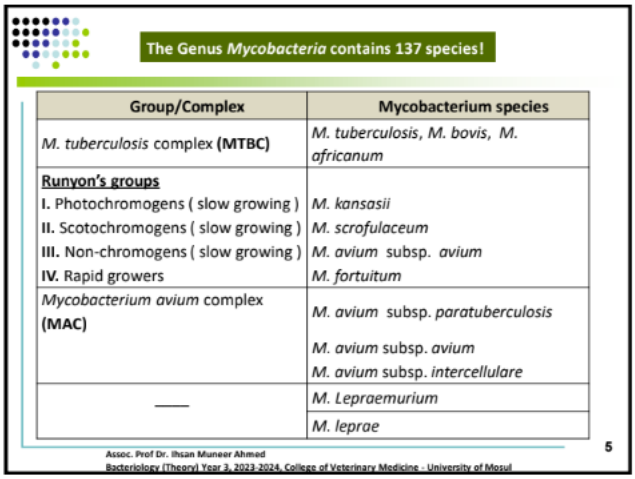
9. Multiply intracellularly and cause chronic, granulomatous infections

10.Major diseases include tuberculosis and Johne's disease.

1. Classification (According to Bergey's Manual)

Family: Mycobacteriaceae

Genus: Mycobacterium



The Genus *Mycobacteria* contains 137 species!

Group/Complex	Mycobacterium species
M. tuberculosis complex **(MTBC)**	*M. tuberculosis, M. bovis, M. africanum*
Runyon's groups	
I. Photochromogens (slow growing)	*M. kansasii*
II. Scotochromogens (slow growing)	*M. scrofulaceum*
III. Non-chromogens (slow growing)	*M. avium* subsp. *avium*
IV. Rapid growers	*M. fortuitum*
Mycobacterium avium complex **(MAC)**	*M. avium* subsp. *paratuberculosis* *M. avium* subsp. *avium* *M. avium* subsp. *intercellulare*
——	*M. Lepraemurium*
	M. leprae

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2. General characteristics

1. Mycobacteria are aerobic, non-spore-forming, non-motile, rod shaped, acid-fast bacilli.

2. Individual species differ in size; the rods of Mycobacterium tuberculosis, M. bovis and M. avium subsp. avium are slender and up to 4 µm in length, whereas those of M. avium subsp. paratuberculosis are broad and are usually less than 2 µm long.

3. 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.

4. Stained by Ziehl-Neelsen (ZN), the cell wall lipids of the mycobacteria bind carbol fuchsin which is not removed by the acid–alcohol

decolorizer used in this staining method. Bacilli that stain red by this method are called acid-fast or ZN-positive.

5. The mycobacteria include diverse species ranging from environmental saprophytes and opportunistic invaders to obligate pathogens. Mycobacterial diseases in domestic animals are usually chronic and progressive (Table 1).

Table 1 Mycobacteria which are pathogenic for animals and humans.

Mycobacterium species	Host	Clinical conditions
M. tuberculosis	Humans	Human tuberculosis
M. bovis	Humans + Cattle	Bovine tuberculosis
M. kansasii	Cattle, Deer, Pigs	Tuberculosis like disease
M. scrofulaceum	Cattle, Buffalos	Tuberculosis lesion in cervical and intestinal lymph nodes
M. avium subsp. avium	Poultry and wild birds , Humans	Avian tuberculosis
M. fortuitum	Cattle , Cat	Granulomatous lesions, ulcerative, pyogranulomatous lesion of skin
M. avium subsp. paratuberculosis	Cattle, Sheep, Goats	Paratuberculosis
M. lepraemurium	Cat, Rodent's	Feline and murine Leprosy
M. leprae	Humans	Leprosy

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3. Usual Habitat

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

2. Environmental mycobacteria are found in soil, on vegetation and in water.

4. Differentiation of pathogenic mycobacteria

• The ZN staining method is used to differentiate mycobacteria from other bacteria. Pathogenic mycobacteria can be differentiated on the basis of growth characteristics and biochemical differentiation.

Cultural features

Safety precautions, including the use of a biohazard cabinet when working with material containing mycobacteria.

1. Pathogenic mycobacteria grow slowly on solid media, (3 to 6 weeks). In contrast, the colonies of rapidly growing saprophytes are visible within days.

2. 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 37 to 43 °C.

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

5. Bovine tuberculosis

1- Pathogenesis and pathogenicity

1. The virulence of M. bovis relates to its ability to survive and multiply in host macrophages (Figure 1).

2. Survival within the phagosome of macrophages is promoted by interference with phagosome-lysosome fusion and failure of lysosomal digestion.

3. Migration of macrophages containing viable mycobacteria can disseminate infection.

4. The gradual accumulation of macrophages around the developing lesion and the formation of a central necrotic core result in a tubercle or granuloma.

2- Clinical signs and pathology

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

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

3. Tuberculous mastitis facilitates spread of infection to calves and cats.

4. Consumption of unpasteurized milk is of major public health importance.

3- Diagnostic procedures

1. The tuberculin test, based on a delayed-type hyper-sensitivity to mycobacterial tuberculin, is prepared from mycobacteria and called purified protein derivative (PPD), is injected intradermally to detect sensitization.

2. ELISA for detecting circulating antibodies.

3. Isolation and identification of M. bovis requires:

5. Paratuberculosis (Johne's disease)

1. Paratuberculosis is chronic, contagious enteritis which can affect domestic and wild ruminants.

2. The etiological agent, M. avium subsp. paratuberculosis (Map), is an acid-fast organism formerly referred to as Mycobacterium johnei.

3. First described by Johne and Frothingham 1895.

2- Pathogenesis and pathogenicity

1. Mycobacterium avium subsp. paratuberculosis is an intra-cellular

pathogen and cell-mediated reactions are mainly responsible for the enteric lesions.

2. Ingested mycobacteria are taken up by M cells over Peyer's patches.

3. The organisms cross the intestinal epithelial layer and are engulfed by macrophages in which they survive and replicate.

4. Prevention of phagosome–lysosome fusion appears to be important for intracellular survival of M. avium subsp. paratuberculosis as is the case for M. bovis.

5. As the disease progresses, an immune-mediated granulomatous reaction develops.

6. The resulting enteropathy leads to loss of plasma proteins and malabsorption of nutrients and water.

3- Clinical signs and pathology

1. Clinical signs develop in most ruminant species after a prolonged subclinical phase of infection (more than 2 years of age).

2. The disease is clinically evident only in mature sheep and goats.

3. The main clinical feature in cattle is diarrhea. Progressive weight loss results without loss of appetite.

4. Thickening of the intestinal mucosa is less marked in sheep, and necrosis and caseation may be present in the regional lymph nodes.

4- Diagnosis

1. Paratuberculosis requires differentiation from other chronic wasting diseases in ruminants.

2. Specimens for direct microscopy from live animals include scrapings or pinch biopsies from the rectum. feces may be submitted for culture and

serum for serological tests.

3. Specimens for microscopical examination should be stained by the ZN

technique.