University of Mosul
Lecture No.:
College of Veterinary Medicine
Date:
Unit of Scientific Affairs
Website:

Lecture title: *Mycoplasma*

Lecturer Affiliation: Department of Microbiology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq. **sabaraheem@uomsul.edu.iq**

Summary: Mycoplasma

General Characteristics of Mycoplasmas:

1. The mycoplasmas are microorganisms in the class Mollicutes. Nine genera in this class, five of them contain species of veterinary interest (Fig. 1)

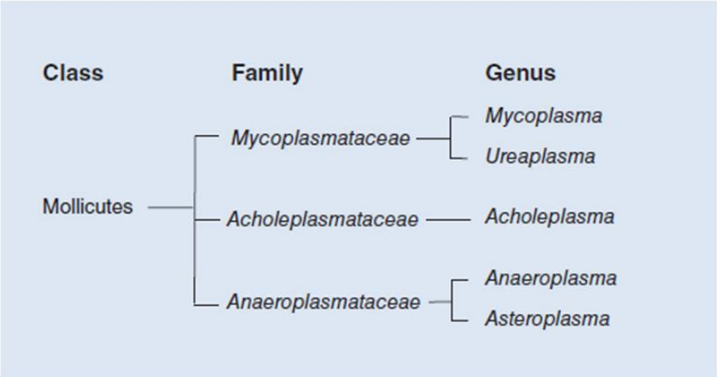

Figure 1 Families and genera of veterinary interest in the class Mollicutes, members of which may be isolated from clinical specimens. Mycoplasma and Ureaplasma are the only genera of pathogenic significance in domestic animals and humans.

2. The genus Mycoplasma, contain more than 100 species, include most of the animal pathogens.

3. Mycoplasmas, the smallest free-living prokaryotic cells capable of self-replication.

4. Pleomorphic organisms ranging from spherical (0.3 to 0.9 µm in diameter) to filamentous (up to 1.0 µm long).

5. They do not possess rigid cell walls because they cannot synthesize peptidoglycan or its precursors, but have flexible, triple-layered outer membranes.

6. Their flexibility allows them to pass through bacterial membrane filters of pore sizes from 0.22 µm to 0.45 µm.

7. Mycoplasmas are susceptible to desiccation, heat, detergents and disinfectants.

8. They are resistant to antibiotics such as penicillin.

9. They require enriched media for growth.

10. They characteristically form fried-egg-shaped microcolonies that grow into agar media. The dense central zone is due to extension of the microcolony into the agar (Fig. 2).

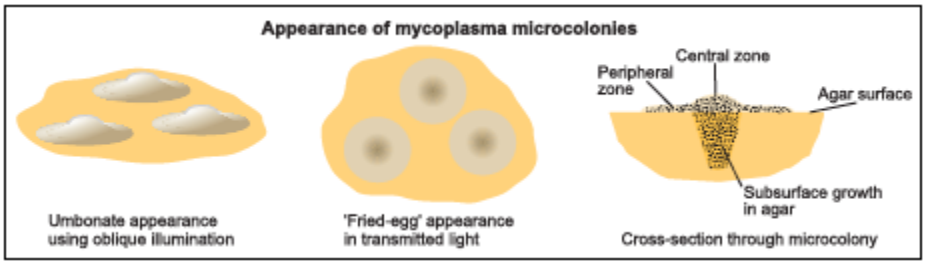

Figure. 2 Appearance of Mycoplasma microcolonies

11. Mycoplasmas have relatively small genomes (approximately 800 genes), they have lost the genes required for many metabolic processes.

12. They are dependent on the host cell for essential nutrients that they cannot produce and are fastidious in their growth requirements when cultured in vitro.

13. Most mycoplasmas are facultative anaerobes and some grow optimally in an atmosphere of 5 to 10% CO2.

14. Non-pathogenic anaerobic mycoplasmas are found in the rumens of sheep and cattle.

15. The genera Mycoplasma and Ureaplasma contain animal pathogens.

16. Most of them are host-specific.

17. Organisms in genus Mycoplasma are known as the haemotropic mycoplasmas or 'haemoplasmas' they parasitize red blood cells.

Usual Habitat:

1. The mycoplasmas (Mollicutes) occur worldwide as free-living saprophytes or as parasites of animals.

2. Mycoplasmas are found on mucosal surfaces of the conjunctiva, nasal cavity, oropharynx and intestinal and genital tracts of animals and humans.

3. Some species have tropisms for particular anatomical sites while others are found in many locations. The haemotropic mycoplasmas are found on the surface of red blood cells.

4. In general, they are host-specific although some species have a broad host range. 5. Many mycoplasmas are non-pathogenic and constitute part of the normal flora of their host.

6. Mycoplasmas survive for several days in the environment.

Pathogenesis:

1.The parasitic mycoplasmas tend to adhere firmly to the host's mucous membranes and some species have been shown to affix to cells by specific attachment structures.

2. Mycoplasmas can adhere to neutrophils and macrophages and can also impair phagocytic functions.

3. The organisms are extracellular and produce haemolysins, proteases, nucleases and other toxic factors that can lead to the death of host cells or to a chronic infection.

4. Production of H2O2 can induce toxic damage to host cells.

5. One species Mycoplasma neurolyticum produces a neurotoxin.

6. The respiratory tract and lungs are frequent sites of infection. Some species tend to infect joints and serous cavities. Mycoplasmas are capable of destroying the cilia of cells in the respiratory tract, thus predisposing to secondary bacterial invasion.

7. Latency can occur and various stresses predispose to mycoplasmal diseases.

8. The infections are frequently chronic or low grade.

9. Infections are either endogenous or exogenous.

10.Transmission is usually venereal, vertical or by aerosols and many important avian mycoplasmas are egg-transmitted.

Pathogenic Mycoplasma and Ureaplasma species in animals

Hosts	Pathogen	Clinical conditions
Cattle	*M. mycoides subsp. mycoides (small colony type)*	Contagious bovine pleuropneumonia (CBPP) (Africa, Middle East, China)
	M. bovis	Mastitis, arthritis, pneumonia, genital infections, abortion
	Mycoplasma alkalescens	Mastitis
	M. bovigenitalium	Seminal vesiculitis, vaginitis, mastitis
	M. bovirhinis	Mastitis
	M. bovoculi	Role in keratoconjunctivitis
	M. californicum	Mastitis
	M. canadense	Mastitis
	M. dispar	Pneumonia in calves

	M. leachii	Mastitis, polyarthritis, pneumonia
	Ureaplasma diversum	Vulvitis, infertility, abortion
	M. wenyonii	Mild anaemia
Sheep, goats	*M. agalactiae*	Contagious agalactia (USA, Mediterranean, Europe, Asia)
	M. conjunctivae	Keratoconjunctivitis
	M. capricolum	Polyarthritis, mastitis, pneumonia
	Acholeplasma oculi	Keratoconjunctivitis
Sheep	*M. ovipneumoniae*	Pneumonia
	M. ovis	Haemolytic anaemia, varying in severity
Goats	*M. mycoides subsp. mycoides (large colony type)*	Septicaemia, polyarthritis, pneumonia, mastitis, conjunctivitis (North America)
	M. mycoides subsp. capri	Contagious caprine pleuropneumonia (CCPP) (Africa, Mediterranean)
	Mycoplasma strain F-38	Contagious caprine pleuropneumonia (CCPP) (Africa)
	M. putrefaciens	Mastitis, arthritis
Horses	*M. felis*	Pleuritis
	M. equigenitalium	Implicated in abortion
Cats	*M. felis*	Conjunctivitis
	M. gateae	Arthritis, tendosynovitis
Dogs	*M. cynos*	Implicated in the kennel cough complex
	M. haemocanis	Mild or subclinical anaemia; more severe signs in splenectomized animals
Pigs	*M. suis*	Mild anaemia, poor growth rates
Poultry	*M. gallisepticum*	Chickens: chronic respiratory disease (CRD). Turkeys: infectious sinusitis. Infections in game birds and imported Amazon parrots
	M. synoviae	Chickens and turkeys: infectious synovitis
	M. meleagridis	Turkeys: Mycoplasma meleagridis disease (MM disease), an air sacculitis and bursitis in young birds
	M. iowae	Turkey poults: air sacculitis, stunting and leg deformities. Mortality of turkey embryos can occur
	M. anatis	Ducks: sinusitis

Laboratory Diagnosis:

A. Specimens for laboratory examination:

1. Must collect early in the c

ourse of a disease.

2. Should be kept refrigerated and delivered to a laboratory within 48 hours because Mycoplasmas are fragile.

3. Suitable samples include mucosal scrapings, tracheal exudates, aspirates, pneumonic tissue, mastitic milk and fluids from joints or body cavities.

4. Swabs from lesions or suspect material should be placed in mycoplasmal transport media for transfer to the laboratory.

5. A simple transport medium for mastitic milk samples is the sample itself with 5 mg/ml ampicillin.

B. Direct microscopy:

Fragility, pleomorphism and weak staining by various methods make direct examination of stained smears of little value for the diagnosis of mycoplasmal diseases. Some workers have developed a fluorescent antibody technique to identify M. dispar and ureaplasmas in bronchial epithelium of calves with pneumonia.

C. Isolation:

 Culture media:

The basic medium is a good quality beef infusion with supplements.

❖ Supplements:

1. 20% horse serum (source of cholesterol) for Mycoplasma and Ureaplasma species.

2. Yeast extract as source of DNA and possibly nucleotides.

3. Penicillin to inhibit the growth of Gram-positive bacteria

4. Thallium (Thallus) acetate to inhibit fungi and Gram-negative bacteria.

5. Addition of urea require for growth of Ureaplasmas and a final pH of 6.0.

6. Thallium acetate is omitted from Ureaplasma media as it is harmful to them.

7. For Mycoplasma species final pH of the medium is adjusted to be between 7.2 and 7.8 for.

 Inoculation of culture media:

For routine isolation of mycoplasmas, the specimen should be inoculated into two broths and onto two plates of agar (one suitable for mycoplasmas and the other for ureaplasmas).

The inoculation technique will vary according to the nature of the specimen:

1. Fluid materials such as foetal fluids and exudates can be inoculated directly into broth medium and spread over the surface of the agar medium.

2. Some specimens such as semen, joint fluids and tissues may contain inhibitors for mycoplasmas. Both undiluted specimen and ten-fold dilutions in mycoplasmal broth (up to 10-6) should be cultured.

3. With tissues, such as pieces of lung, a freshly cut surface on a block of the tissue can be moved across the surface of an agar plate for inoculation. Alternatively, the tissue can be homogenized in broth, ten-fold dilutions made and broth cultures inoculated.

Notes on cultured media:

1. Its better if inoculate duplicate agar plates and incubated in a humid atmosphere at 37°C, one aerobically and one under 5% CO2 and 95% N2. A candle-jar may be satisfactory.

2. The plates should be examined after 48hr and 96hr of incubation.

3. The plates are viewed under a stereoscopic microscope (transmitted light) or under the low-power objective of a light microscope, for the mycoplasmal 'fried-egg' microcolonies.

4. The cultures can be regarded as negative if no microcolonies are seen after 14 days of incubation.

5. The tubes of broth, incubated aerobically at 37°C, are checked daily for growth. When a slight turbidity is seen, loopfuls of the broth can be used to inoculate agar media.

D. Identification criteria for isolates:

a. ‘Fried-egg’ microcolonies. With or without stained by Dienes' stain.

b. Differentiation from bacterial L-forms

c. Microcolony size.

d. Identification of the genus and species:

▪ Cholesterol requirement for growth (digitonin sensitivity test).

▪ Biochemical profile including urease production.

▪ Fluorescent antibody technique on microcolonies.

▪ Growth inhibition test with specific antisera.

Biochemical tests of Mollicutes isolated from domestic animals

Isolate	Effect of digitonin	Requirement for cholesterol	Urease production	Colony size
Mycoplasma species	Growth inhibition	+	–	0.1–0.6 mm
Ureaplasma species	Growth inhibition	+	+	0.02–0.06 mm
Acholeplasma species	No growth inhibition	–	–	up to 1.5 mm

Differentiation from bacterial L-forms:

 Bacterial L-forms: Its bacteria that have temporarily failed to form cell walls (L-forms) and can produce microcolonies similar to those of the mycoplasmas. The failure to form cell walls is often due to the exposed to penicillin or other antibacterial agents that affect cell wall formation.

Differentiation between mycoplasmal and bacterial L-form microcolonies can be carried out as follows:

a) Subculturing the suspected bacterial L-form on media without antibacterial substances should cause reversion of the L-form with the formation of normal-sized bacterial colonies. Up to five subcultures may be necessary.

b) Staining microcolonies with the Dienes' stain is useful for the differentiation between mycoplasmal and bacterial L-form microcolonies. Mycoplasmal colonies retain the Dienes' stain indefinitely, whereas bacterial L-form microcolonies tend to decolorize in about 15 minutes.

Identification of Mycoplasma species:

The following methods are examples of the techniques that can be used to identify mycoplasmal species:

1. Fluorescent antibody (FA) staining to identify M. dispar and Ureaplasmas in bronchial epithelium of calves with pneumonia.

2. Enzyme-linked immunoperoxidase used on sections of porcine bronchial epithelium to detect M. hyopneumoniae.

3. Agar gel diffusion tests using known antisera to identify prepared mycoplasmal antigen from broth cultures.

4. ELISA and complement fixation test (CFT) for antigen identification using known antisera.

5. Species-specific DNA probes have been developed for the identification of some species.

6. Biochemical tests such as glucose fermentation, arginine hydrolysis, phosphatase activity and reduction of tetrazolium.

7. PCR Techniques.

Treatment:

Among the drugs used for treatment are tylosin, tetracyclines, tiamulin and fluoroquinolones.

Specific-Pathogen-Free (SPF) Programmes:

Flocks of poultry (chickens and turkeys) free from the major avian mycoplasmas and pig herds free from M. hyopneumoniae have been established in some countries with specific pathogen-free programmes. There are usually two phases in these programmes:

a. Detection of infections and culling or isolation of affected pigs or birds.

b. Followed by serological monitoring of the flocks or herds to demonstrate continued freedom from infection.