

# MYCOPLASMA

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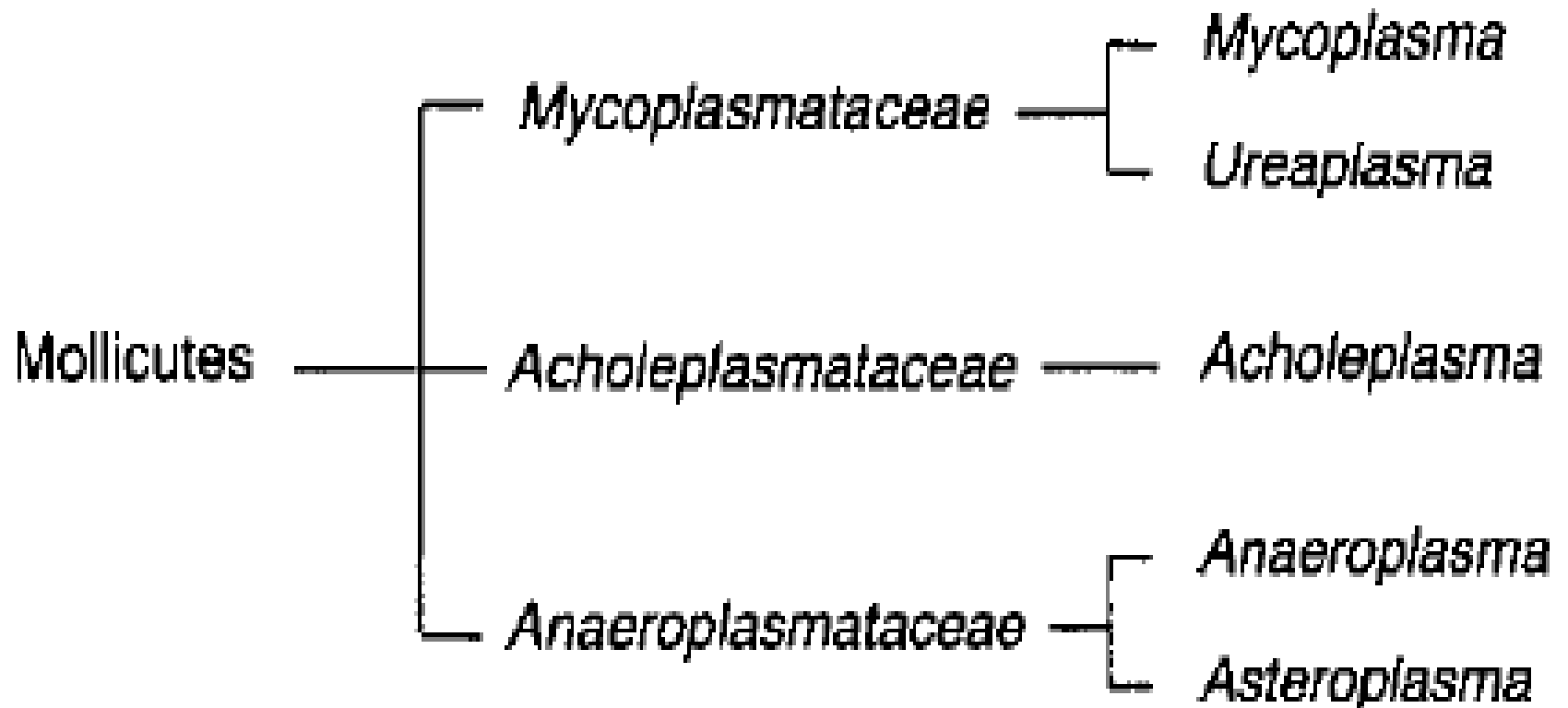
# General Characteristics

- Smallest prokaryotic cells capable of self-replication
- Cannot synthesize Peptidoglycan (*resistant to penicillin*)
- Do not possess rigid cell walls but *have flexible, triple-layered outer membranes*
- Lack rigid cell wall and thus definite shape (*Pleomorphic organisms*)
- *Being pleomorphic, they can pass through bacterial filters of 0.22 μm size.*
- *Require Sterol for growth (except-Acholeplasma)*
- Earlier term ***Pleuropneumonia-like organisms (PPLO)*** was in use for mycoplasmal organisms.

**Class**

**Family**

**Genus**



## Media for Mycoplasma

- Smallest prokaryotes that can be grown on artificial media.
- Fastidious in growth requirement.
- Requires **sterol** and animal protein for growth
- **Penicillin** inhibits growth of Gram positive bacteria
- **Thallium acetate** inhibits Gram negative bacteria and fungi.

### Media for mycoplasma contains:

- Heart infusion broth
- 20% Horse serum
- 1% Peptone
- 1% Albumin
- Penicillin 1000 IU/ml
- Thallium acetate  
1: 2000 to 1: 8000 conc.
- Yeast extract

# Culturing Mycoplasma

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- Can be grown in liquid or solid media
- Facultative anaerobe – **require 5-10% CO<sub>2</sub>**
- Optimal temperature for growth 35-37°C
- Generally slow grower (*generation interval up to 9hrs*)
- For culturing ureaplasmas, ***urea*** is added to the medium and ***thallium acetate is removed***, which is toxic for these organisms

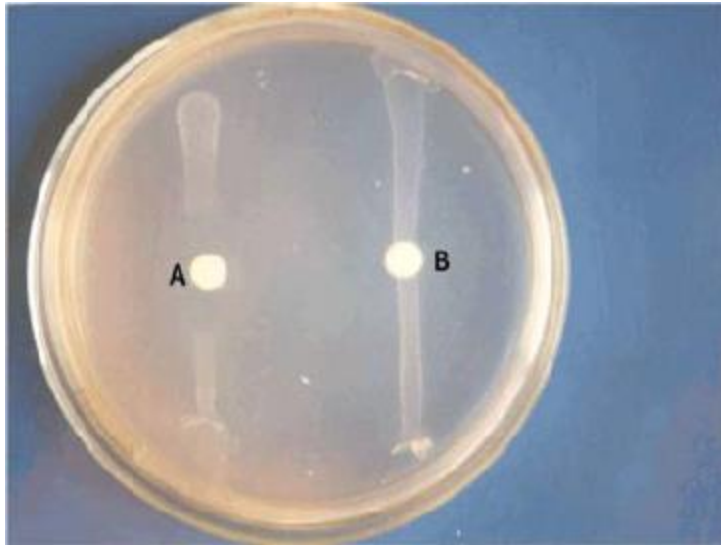
# Mycoplasmal colonies

- *Mycoplasma* colonies are small and can only be observed under a light microscope at low magnification
- **Dienes stain** is used for staining mycoplasmal microcolonies
- ***Nipple Shaped colony*** or Umbonate micro-colonies – ***Fried egg appearance***
- Because of tiny colonies of Ureaplasma- they were also called ***T-mycoplasma***



Genus	Colony size
Mycoplasma	0.1-0.6 mm
Ureaplasma	0.02-0.06 mm
Acholeplasma	Upto 1.5 mm

# Requirement for Sterol and Urea hydrolysis



- Mycoplasmas requires Sterol except members of genus *Acholeplasma*.
- The requirement of sterol is ascertained performing "***Digitonin***" sensitivity.
- Digitonin impregnated discs placed on solid media inhibits growth of isolates that require sterol.
- Members of genus *Ureaplasma* produce ***Urease*** and thus capable of hydrolysis "urea".

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Isolate	Effect of digitonin	Requirement for cholesterol	Urease production	Colony size
<i>Mycoplasma</i> species	Growth inhibition	+	-	0.1 – 0.6 mm
<i>Ureaplasma</i> species	Growth inhibition	+	+	0.02 – 0.08 mm
<i>Acholeplasma</i> species	No growth inhibition	-	-	up to 1.5 mm

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# Mycoplasma and L form of bacteria

*Mycoplasma* and L type bacteria are similar:

- they both lack a cell wall and the cell is pleomorphic
- they can both pass through an antimicrobial filter

The main differences between the two are:

## Mycoplasma

*Mycoplasma* are independent microbes

*Mycoplasma* growth requires cholesterol (10–20% serum in the medium)

*Mycoplasma* do not fade easily after Dienes staining

## L –form of Bacteria

L-type bacteria are variants of normal bacterial cells that have a cell wall

(most L-type cells will revert to their original form)

L-type bacteria does not sterol

L-type bacteria fade easily after Dienes staining

# Habitat

- Mycoplasmas are found on mucosal surfaces of the **respiratory, intestinal and genital tracts**
- Members of *Anaeroplasma* are strict anaerobes – remain in rumen of cattle and sheep
- Members of Spiroplasma causes disease in plants.
- Factors such as extremes of age, stress and intercurrent infection may predispose disease condition.
- In addition, mycoplasmas may exacerbate disease initiated by other pathogens, particularly in the respiratory tract.
- ***Mycoplasma infections cause respiratory diseases of major economic importance in farm animals especially in ruminants, pigs and poultry***

<i>Mycoplasma</i> species	Hosts	Disease conditions
<i>M. mycoides</i> subsp. <i>mycoides</i> (small colony type)	Cattle	Contagious bovine pleuropneumonia
<i>M. bovis</i>	Cattle	Mastitis, pneumonia, arthritis
<i>M. agalactiae</i>	Sheep, goats	Contagious agalactia
<i>M. capricolum</i> subsp. <i>capripneumoniae</i> (F38)	Goats	Contagious caprine pleuropneumonia
<i>M. capricolum</i> subsp. <i>capricolum</i>	Sheep, goats	Septicaemia, mastitis, polyarthritis, pneumonia
<i>M. mycoides</i> subsp. <i>mycoides</i> (large colony type)	Goats, sheep	Pleuropneumonia, mastitis, septicaemia, polyarthritis
<i>M. mycoides</i> subsp. <i>capri</i>	Goats	Septicaemia, pleuropneumonia, arthritis, mastitis

<i>M. hyopneumoniae</i>	Pigs	Enzootic pneumonia
<i>M. hyorhinis</i>	Pigs (3-10 weeks of age)	Polyserositis
<i>M. hyosynoviae</i>	Pigs (10-30 weeks of age)	Polyarthritis
<i>M. gallisepticum</i>	Chickens Turkeys	Chronic respiratory disease Infectious sinusitis
<i>M. synoviae</i>	Poultry	Infectious synovitis
<i>M. meleagridis</i>	Turkeys	Airsacculitis, bone deformities, reduced hatchability and growth rate

# Contagious Bovine Pleuro-pneumonia (CBPP)

- *A severe contagious disease of cattle caused by **M. mycoides subspecies mycoides (small colony type)***
- Transmission is by aerosols- requires close contact
- In severe outbreaks the mortality rate may be high
- Death can occur 1 to 3 weeks after the onset of clinical signs.

# CBPP- Symptoms

- Acute form - sudden ***onset of high fever, anorexia, depression, drop in milk yield, accelerated respiration and coughing.***
- Animals adopt a **characteristic stance** with the head and neck extended and elbows abducted.
- ***Expiratory grunting and mucopurulent nasal discharge*** may be present.
- Arthritis, synovitis and endocarditis may be present in affected calves.



# CBPP- PM findings

- ***Pneumonic lungs have a marbled appearance.***
- Grey and red consolidated lobules
- ***Interlobular septa*** are distended and oedematous.
- ***Serofibrinous exudate in the pleural cavity.***
- In chronic cases, ***fibrous encapsulation of necrotic foci*** is commonly found.
- These necrotic foci contain viable mycoplasmas



# CBPP- Diagnosis and Control

## Diagnosis

- Clinical signs and characteristic PM findings helps in presumptive diagnosis.
- PCR, FAT can be used on pleural fluid to confirm the presence of the pathogen.
- Broncho-alveolar lavage, pleural fluid, lung tissue or the broncho-pulmonary lymph nodes

## Serological tests:

- -Rapid field serum agglutination test
- -Passive haemagglutination screening test
- -Complement fixation test –
- Dot-blot technique for confirmation

## Treatment and control

- Treatment is generally unsatisfactory



Pneumonia,  
Mastitis,  
Polyarthrits

- *Mycoplasma bovis* causes severe pneumonia in calves
- It can exacerbate respiratory disease caused by *Pasteurella* and *Mannheimia* species
- Most important mycoplasmal species causing mastitis.
- Mycoplasmal mastitis is common in large dairy herds.
- Milking machines, milkers' hands and cloths which are then important sources of infection Causes severe mastitis, systemic involvement is uncommon.
- subclinical carriers are important sources of infection
- Dramatic loss of milk production and the serous or purulent mastitic exudates
- Infection often results in agalactia

## Mycoplasmal infections in Goats

<b>Contagious caprine pleuropneumonia</b>	<ul style="list-style-type: none"><li>• Caused by <i>M. capricolum</i> subspecies <i>capripneumoniae</i> (<i>Mycoplasma</i> strain F38)</li><li>• Similar in many respects to CBPP</li><li>• <i>Highly contagious disease</i>; transmission through <b>aerosols</b>.</li><li>• The disease is characterized by <i>pneumonia, fibrinous pleurisy, profuse pleural exudate</i></li><li>• <i>Marbled appearance</i> on the cut surface of affected lungs is seen.</li><li>• Inactivated vaccines give satisfactory protection.</li><li>• Pleuropneumonia in goats can occasionally be caused by<ul style="list-style-type: none"><li>- <i>M. mycoides</i> subspecies <i>capri</i></li><li>- <i>M. mycoides</i> subspecies <i>mycoides</i> (large colony type)</li><li>- <i>M. capricolum</i> subspecies <i>capricolum</i></li></ul></li></ul>
<b>Contagious agalactia of sheep and goats</b>	<ul style="list-style-type: none"><li>• Caused by <i>M. agalactiae</i></li><li>• Characterized by <i>mastitis, arthritis and conjunctivitis</i>.</li><li>• Observed soon after parturition</li><li>• <u>Pregnant animals may abort</u></li><li>• Can be fatal in young animals due to pneumonic complications.</li><li>• The organism is shed in milk</li><li>• Organism may remain localized in the supra-mammary lymph nodes</li></ul>
<b>Mastitis, Pneumonia, Arthritis</b>	<ul style="list-style-type: none"><li>- <i>M. mycoides</i> subspecies <i>capri</i></li><li>- <i>M. mycoides</i> subspecies <i>mycoides</i> (large colony type)</li><li>- <i>M. capricolum</i> subspecies <i>capricolum</i></li></ul>

## Mycoplasmal infections in Pigs

<b>Enzootic pneumonia</b>	<ul style="list-style-type: none"><li>• Caused by <i>M. hyopneumoniae</i>,</li><li>• Affects intensively reared pigs.</li><li>• Extremes of temperature, sudden change in temperature, poor ventilation, overcrowding</li><li>• Pigs of all ages are susceptible</li><li>• Characterized by coughing, poor growth rates and, in some cases, respiratory distress.</li><li>• At post mortem, <i>pulmonary consolidation is confined to the apical and cardiac lobes</i> with clear demarcation from normal lung tissue.</li></ul>
<b>Polyserositis</b>	<ul style="list-style-type: none"><li>• <i>Caused by Mycoplasma hyorhinis</i></li><li>• Chronic progressive polyserositis in pigs up to 10 weeks of age.</li><li>• It is characterized by <i>fever, laboured breathing, lameness and swollen joints.</i></li></ul>
<b>Polyarthritis</b>	<ul style="list-style-type: none"><li>• Caused by <i>M. hyosynoviae</i></li><li>• Affects pigs from 10 to 30 weeks of age.</li><li>• This self-limiting arthritis and synovitis produces transient lameness.</li></ul>

# Poultry infections

- *Mycoplasma gallisepticum* causes **chronic respiratory disease** in chickens and **infectious sinusitis** in turkeys.

## Transmission:

- Through infection of the embryo in the egg
- By aerosols
- **In chickens:** affects upper respiratory tract.
- **In turkeys:** Causes swelling of the paranasal sinuses

## Diagnosis:

- Isolation and identification of the pathogen
- Flock testing using the serum plate agglutination test
- Haemagglutination inhibition and ELISA tests
- Specific-pathogen-free (SPF) flocks
- Eggs used for hatching should be dipped in a tylosin

# Poultry infections

<i>Mycoplasma gallisepticum</i>	<i>Chronic respiratory disease</i> in chickens <i>Infectious sinusitis</i> in turkeys
<i>Mycoplasma meleagridis</i>	<b>Causes airsacculitis in young poults and joint and bone deformities in growers</b>  <i>Transmission is mainly through eggs</i>
<i>Mycoplasma synoviae</i>	<b><i>Infectious synovitis</i></b> <ul style="list-style-type: none"><li>- In chickens and turkeys</li><li>- Transmitted mainly by aerosols.</li><li>- Arthritis and respiratory signs are the main clinical features</li></ul>

# Diagnosis of Mycoplasmal infections

- Suitable samples include mucosal scrapings, tracheal exudates, aspirates, pneumonic tissue, mastitic milk and fluids from joints or body cavities.
- The presence of Mycoplasma in samples can be demonstrated by:
  - Fluorescent antibody techniques
  - Peroxidase-antiperoxidase procedures on paraffin embedded tissues
  - Polymerase chain reaction techniques

# Diagnosis of Mycoplasmal infections

- Isolation can be attempted in mycoplasma medium under 10% CO<sub>2</sub> at 37<sup>0</sup> C for upto 14 days.
- Identification criteria for isolates:
  - 'Fried-egg' microcolonies
  - Microcolony size
  - Cholesterol requirement for growth (digitonin sensitivity test)
  - Biochemical profile including urease production
  - Fluorescent antibody technique on microcolonies
  - Growth inhibition test with specific antisera

# Serological tests

- Complement fixation tests
- Tests based on ELISA
- Rapid plate agglutination tests
- Haemagglutination-inhibition tests



**THANKS**