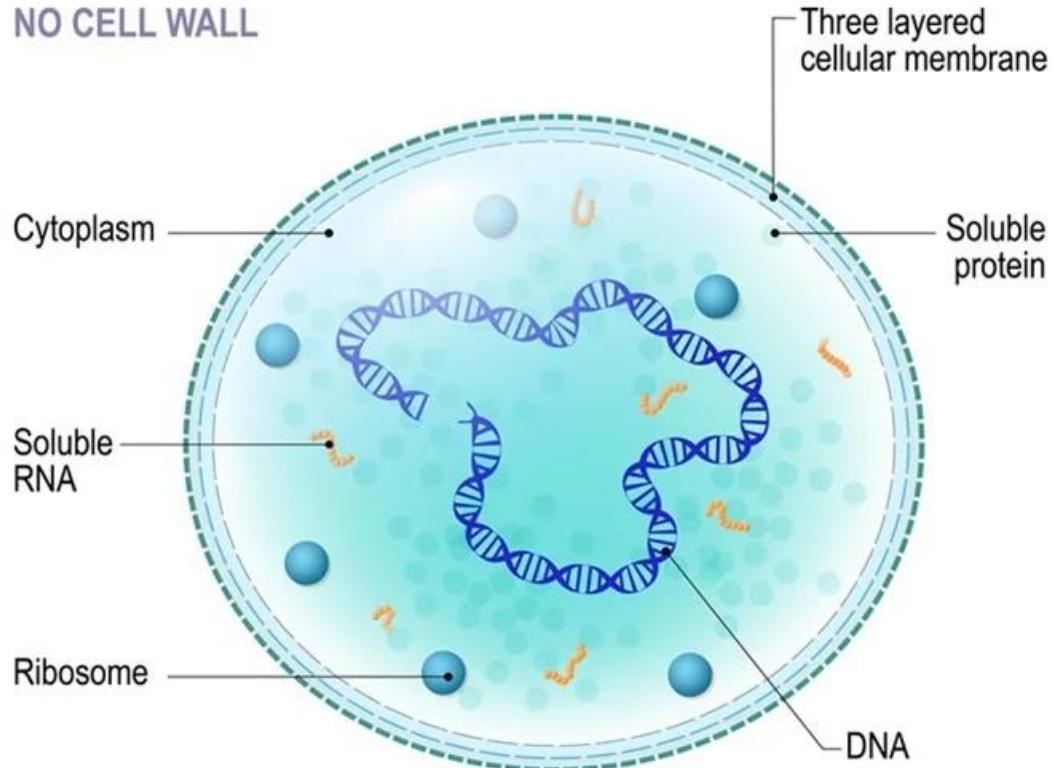


# *Mycoplasma*

NO CELL WALL

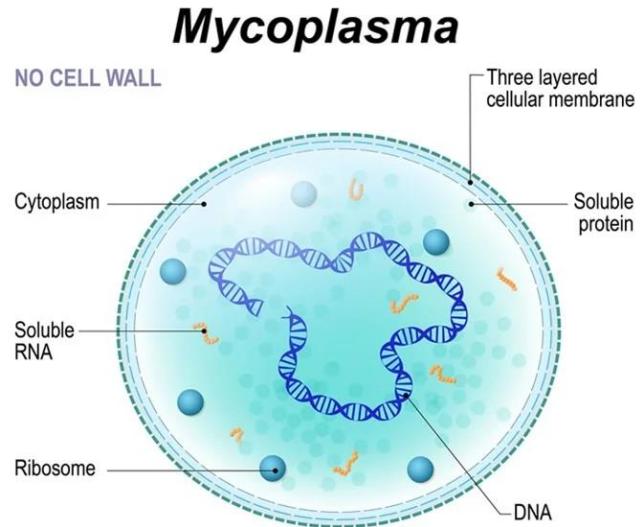


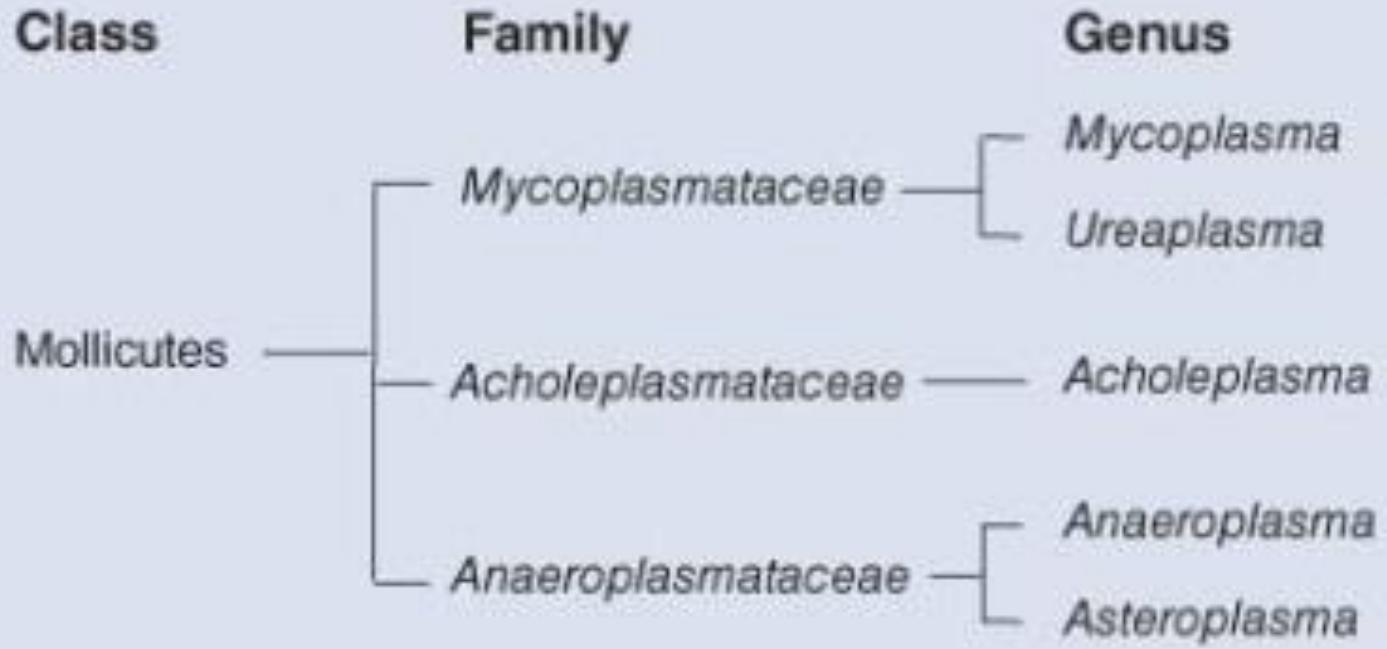
MYCOPLASMA

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# MYCOPLASMA

- Included in Class: Mollicutes
- This class is divided into 3 families
  - Family: Mycoplasmataceae  
G. Mycoplasma  
G. Ureaplasma
  - Family: Acholeplasmataceae  
G: Acholeplasma
  - Family: Anaeroplasmataceae  
G. Anaeroplasma  
G. Asteroplasma
- The first mycoplasma identified in 1890 was *Mycoplasma mycoides* subspecies *mycoides*, the cause of contagious bovine pleuropneumonia
- Mycoplasma also known as PPLO (Pleuro pneumoniae like organisms)
- These are smallest prokaryotic cells capable of self replication and they lack cell walls





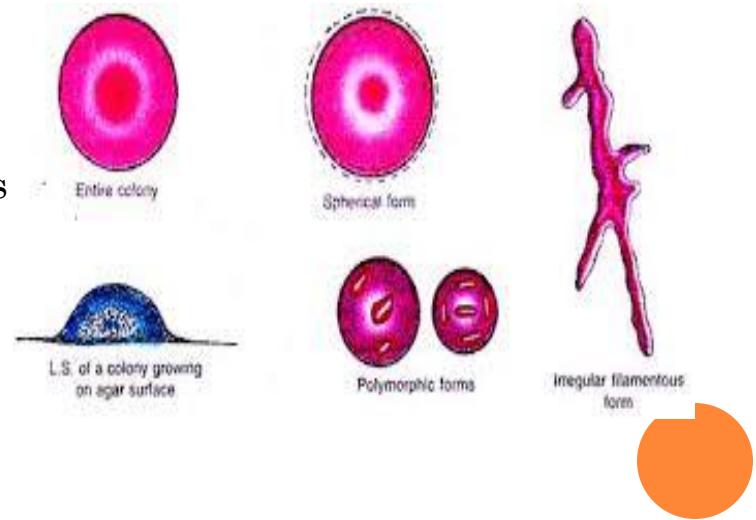
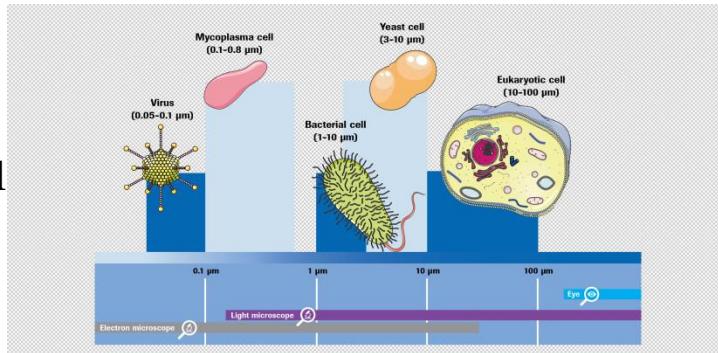
# GROWTH REQUIREMENT

- *Mycoplasma* species and *Ureaplasma* species require enriched media containing animal protein, a sterol component and a source of DNA or adenine dinucleotide.
- Commercially available mycoplasma agar or broth media (often heart infusions) are supplemented with 20% horse serum and yeast extract providing amino acids and vitamins
- In addition, penicillin is used to inhibit Gram-positive bacteria, and thallous acetate is incorporated to inhibit Gram-negative bacteria and fungi
- Media are buffered at pH 7.3 to 7.8 for *Mycoplasma* species and at pH 6.0 to 6.5 for *Ureaplasma* species.
- For culturing ureaplasmas, urea is added to the medium and thallous acetate, which is toxic for these organisms, is omitted

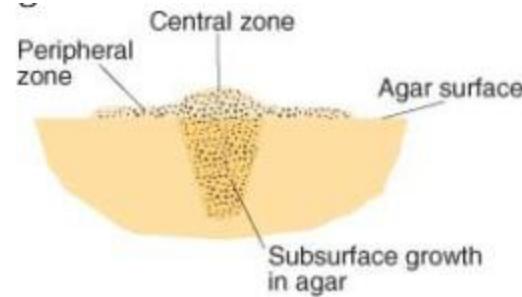
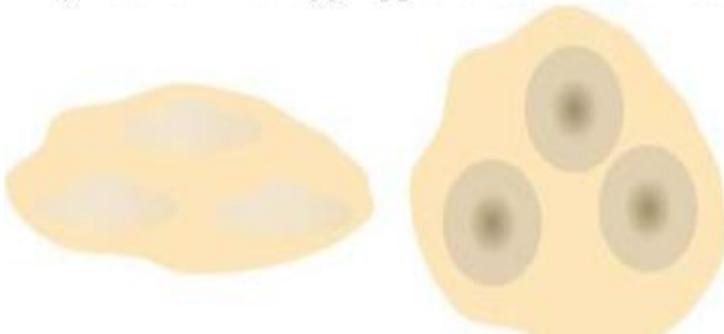


# MORPHOLOGY AND STAINING

- Morphology varies according to the species and environmental conditions and stage of growth because they lack cell wall
- The organisms are pleomorphic spherical, coccoid, coccobacillary, ring, dumb bell, long branching filamentous forms
- The organisms possess a flexible triple layered outer membrane.
- This flexibility allows them to pass through the bacterial membrane filters of pore sizes from  $0.22\text{ }\mu\text{m}$  to  $0.45\text{ }\mu\text{m}$
- Mycoplasmas are susceptible to desiccation, heat, detergents and disinfectants.
- However, they are resistant to antibiotics such as penicillin which interfere with the synthesis of bacterial cell walls
- Organisms divide by binary fission
- Long filamentous form breaks into round forms
- Not stained by Gram staining

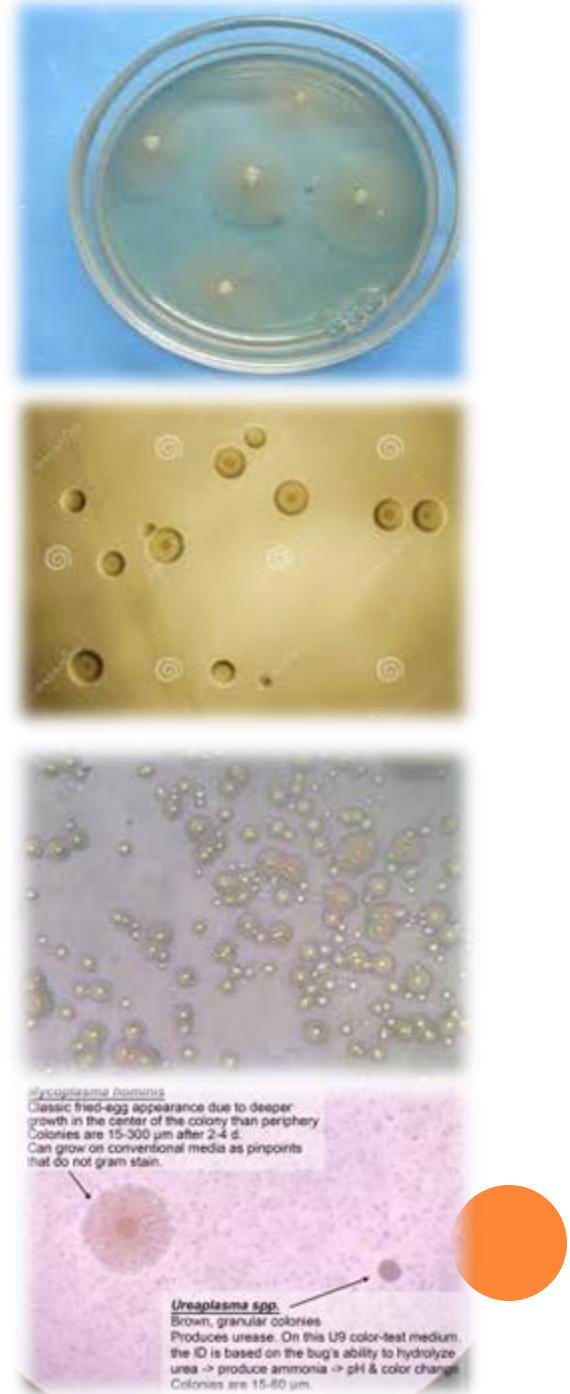


**Figure 38.2** The appearance of mycoplasma microcolonies in oblique illumination (A) and in transmitted light (B). When illuminated obliquely, the microcolonies have an umbonate appearance. They have a ‘fried-egg’ appearance in transmitted light.



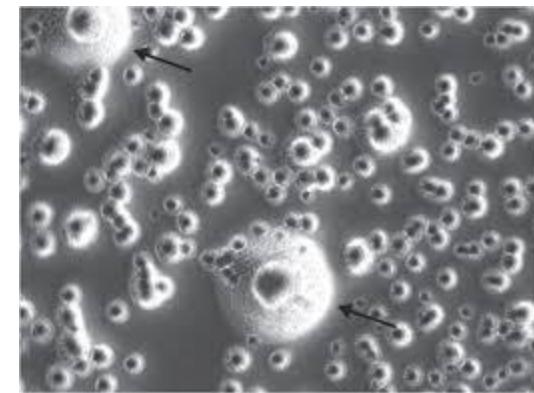
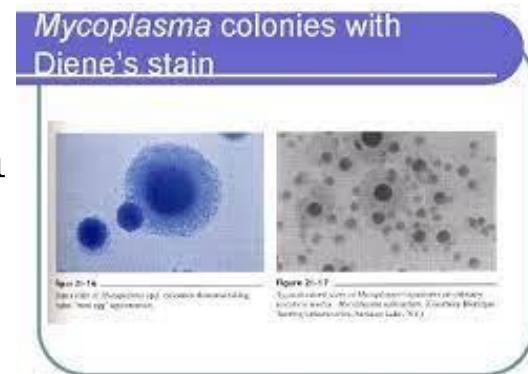
# COLONY MORPHOLOGY

- Most mycoplasmas are facultative anaerobes and some grow optimally in an atmosphere of 5 to 10% CO<sub>2</sub>.
- When examined under low power magnification colonies have 0.1-0.6 mm diameter and have a typical fried egg or bull's eye appearance.
- Some species produce colonies upto 1.5 mm diameter which can be seen without magnification
- Colonies of ureaplasma are usually 0.02-0.06 mm and they lack typical peripheral zone
- Because of tiny appearance of colony Ureaplasma are known as tiny mycoplasma or T- mycoplasma
- 



# DIENE'S STAINING

- Best staining method for demonstration of colonies of organism by Diene's staining method
- In this method a piece of agar containing the colony is placed on the coverslip with surface growth in contact with stain
- The centre will be stained dark blue and periphery light blue
- This staining will be retained for long time and this method is used for differentiation of Mycoplasma from L – form of bacteria
- In L form the stain will be destained within 15 minutes



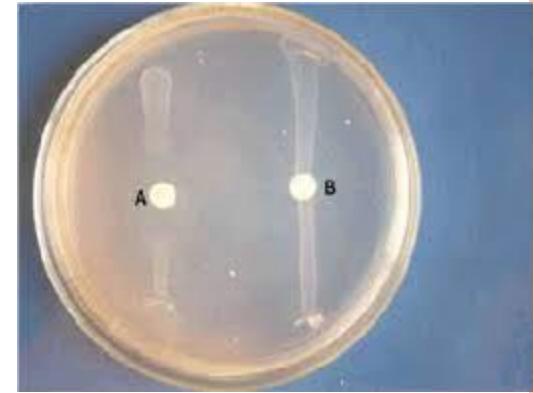
## OTHER METHODS OF CULTIVATION

- Embryonated eggs through **yolk sac route**. The avian strain cause death of embryo within 2-4 days with extensive cutaneous haemorrhage and generalised oedema
- There are various cell culture employed for isolation of organism : **Hela cells and chicken heart fibroblast**



# DIGITONIN SENSITIVITY TEST

- *Mycoplasma* species and *Ureaplasma* species require sterols for growth and this is reflected in their sensitivity to inhibition by digitonin
- As *Acholeplasma* species are sterol-independent, they are resistant to inhibition by digitonin.
- In the digitonin sensitivity test, a filter paper disc impregnated with digitonin is placed on medium inoculated with the isolate
- A zone of growth inhibition exceeding 5 mm around the disc indicates sensitivity to digitonin



# DIFFERENTIATION BETWEEN VARIOUS GENERA

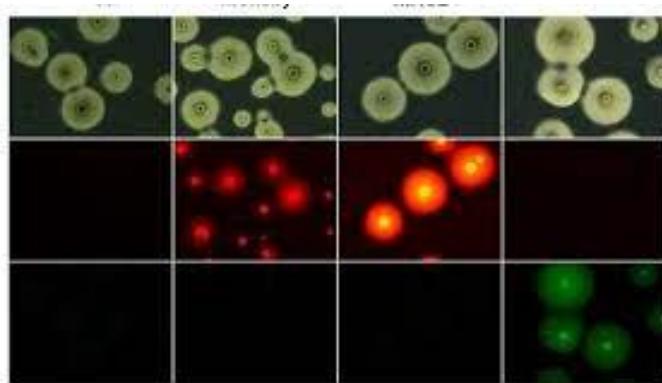
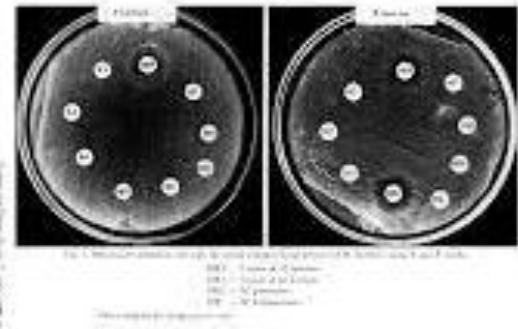
organism	Effect of digitonin	Colony size	Reqt of cholesterol	Urease production
Mycoplasma	Growth inhibition	0.1-0.6mm	Requir cholesterol	-ve
Ureaplasma	Growth inhibition	0.02-0.06 mm	Require cholesterol	+ve
Achleplasma	No Growth inhibition	Upto 1.5 mm	Don't require cholesterol	-ve

BIOCHEMICAL TESTS: USED FOR DIFFERENTIATION OF MYCOPLASMA SPECIES AFFECTING SHEEP AND GOATS

Test	<i>M. agalactiae</i>	<i>M. capricolum</i> <i>subsp.</i> <i>capricolum</i>	<i>Mycoplasma</i> <i>mycoides</i> <i>subsp.</i> <i>mycoides</i>
Glucose fermentation	-ve	+ve	+ve
Arginine hydrolysis	-ve	+ve	-ve
Phosphatase activity	+ve	+ve	-ve
Casein digestion	-ve	+ve	+ve

# SPECIES IDENTIFICATION

- Certain immunological tests utilising specific antiserum are required for species identification
- **Growth inhibition test**
- Filter paper disc specific antisera are placed on agar surface and cultured with mycoplasma
- If there is inhibition of growth then +ve for that species
- **Metabolic inhibition test**
- In the presence of specific antiserum, there metabolic activity will get inhibited
- **Fluorescent antibody staining for individual colonies**



<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>	Goats	Contagious caprine pleuropneumonia
<i>M. capricolum</i> subsp. <i>capricolum</i>	Sheep, goats	Septicaemia, mastitis, polyarthritis, pneumonia
<i>M. mycoides</i> subsp. <i>capri</i> includes strains previously classified as <i>M. mycoides</i> subsp. <i>mycoides</i> (large colony type)	Goats, sheep	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>	Chickens, turkeys	Infectious synovitis
<i>M. meleagridis</i>	Turkeys	Airsacculitis, bone deformities, reduced hatchability and growth rate
<i>M. haemofelis</i>	Cats	Feline infectious anaemia

# DISEASES BY MYCOPLASMA: CATTLE

- **Mycoplasma mycoides subspecies mycoides (small colony type)** is considered as most important in bovine mycolasma
- This organism causes **contagious bovine pleuropneumoniae or Brahmaputra valley disease.**
- It can be subclinical acute or fatal, characterised by rise in temperature and respiratory disturbances, cough and nasal discharge
- Animal will be reluctant to move
- In severe cases animal stand with neck extended and mouth open to facilitate breath
- Subclinically affected animal is the source of spreading and maintaining disease in a herd

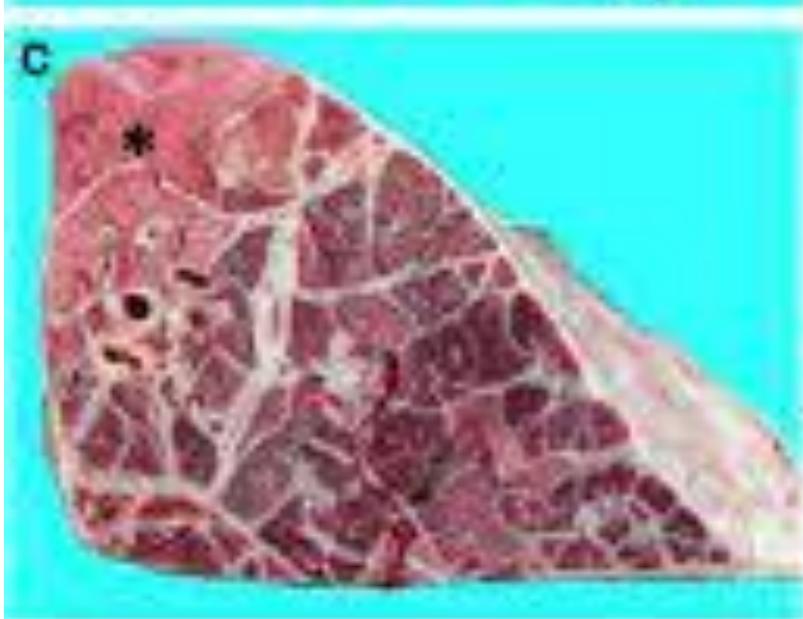


## CLINICAL SIGNS AND PATHOLOGY

- Clinical signs in the acute form of CBPP include 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.
- Death can occur 1 to 3 weeks after the onset of clinical signs.
- Arthritis, synovitis and endocarditis may be present in affected calves
- 



- At post-mortem, the pneumonic lungs have a marbled appearance.
- Grey and red consolidated lobules alternate irregularly with pink emphysematous lobules and the interlobular septa are distended and oedematous.
- There may be abundant serofibrinous exudate in the pleural cavity
- In chronic cases,fibrous encapsulation of necrotic foci is commonly found
- These necrotic foci contain viable mycoplasmas, and breakdown of the capsules in chronically affected animals is a major factor in the persistence and spread of CBPP in endemic areas.



# MYCOPLASMA BOVIS

- Most important cause of Mycoplasma mastitis in cattle
- There will be decrease in milk production and milk will become thick, intermixed with watery secretion and may progress to a purulent exudate
- *M.bovis* and *M.dispar* upper respiratory infection in calf along with other respiratory pathogen
- *M. bovigenitalium* : causes urogenital infection in cattle



## DISEASES CAUSED BY MYCOPLASMA : GOAT

- *Mycoplasma mycoides* subsp. *mycoides* causes mastitis, arthritis, pneumoniae in goat and septicaemia in kids
- *Mycoplasma mycoides* subsp. *capri* causes pleuropneumoniae in goat
- *Mycoplasma capricolum* subsp. *capripneumoniae* causes **contagious caprine pleuropneumoniae (CCPP)**
- The disease is characterized by pneumonia, fibrinous pleurisy, profuse pleural exudate and a marbled appearance on the cut surface of affected lungs. Although similar in many respects to contagious bovine pleuropneumonia (CBPP), well developed necrotic areas in the lungs in chronic CCPP are rare
- *Mycoplasma agalactiae* causes contagious agalactiae in sheep and goat



## DISEASES BY MYCOPLASMA : PIG

- *Mycoplasma hyorhinis* causes atropic rhinitis along with *Pasteurella multocida* and *Bordetella bronchiseptica*
- *Mycoplasma hyopneumoniae* causes enzootic pneumoniae in pig
- *Mycoplasma hyosynoviae* arthritis in pig



- *Mycoplasma ovipneumoniae*: pneumoniae in sheep
- *Mycoplasma conjunctivae*: keratoconjunctivitis
- In birds *Mycoplasma gallisepticum* cause **chronic respiratory disease (CRD)** in chicken and infectious sinusitis in turkey
- *Mycoplasma synoviae*: Infectious synovitis in chicken
- *Mycoplasma meleagridis*: air sacculitis in turkey
- *Mycoplasma pneumoniae* also known as Eaton's agent

## DIAGNOSIS

- Clinical material are mucoid scrappings, tracheal exudate, pneumonic lung, mastitic milk, fluids from joints and other body fluids and swabs from lesions or suspected material to be transported in mycoplasma transport medium
- Diagnosis
- Presence of mycoplasma antigen can be demonstrated immunologically and nucleic acid detection (FAT, IPT and PCR of Nucleic acid)
- Material are inoculated on Mycoplasma medium and incubated aerobically or increased carbon dioxide in humid atmosphere at 37°C for 2 weeks
- Fluid sample can be inoculated into PPLO agar/ broth
- Colonies are identified by fried egg appearance, size, digitonin sensitivity and biochemical test



- FAT on microcolonies and growth and metabolic inhibition using specific antiserum
- Serological test
- CFT, ELISA, Rapid plate agglutintion test for poultry and CCPP
- HI for avian mycoplasmosis



## TREATMENT AND CONTROL

- Most of this drugs are unsatisfactory in chronic condition
- In countries where disease is exotic, slaughter of affected and incontact animals
- In endemic areas control is based on prohibitory movement of suspected animals
- Mandatory quarantine and elimination of carrier animal by serological test can be detected
- Autogenic vaccine from affected tissues also practised
- Attenuated vaccine are there and annual vaccination practised



## AVIAN MYCOPLASMA ( MYCOPLASMA GALLISEPTICUM)

- Antimicrobial medication with tetracycline or tylosin
- Establishment of mycoplasma free flock
- The eggs used for hatching should be dipped in tylosin
- Modified live vaccine available

