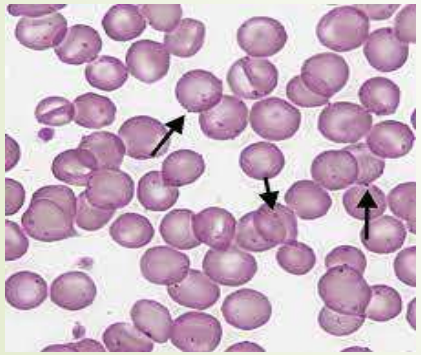
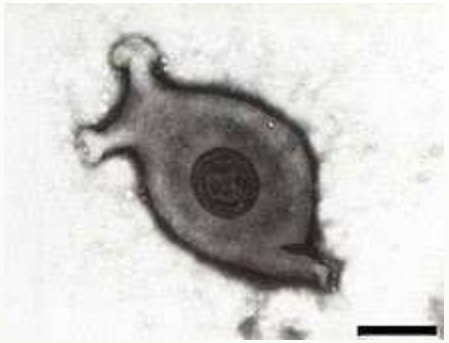
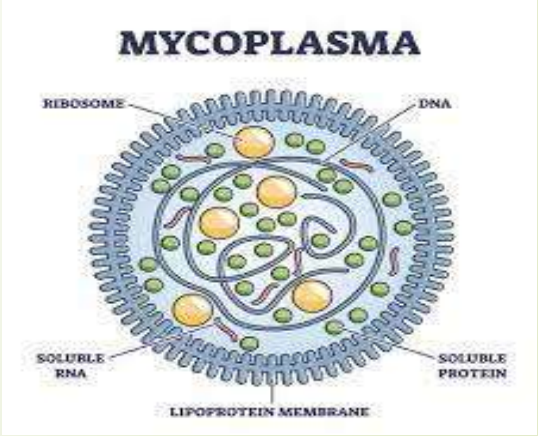
University of Basrah
College of Veterinary Medicine
Department of Microbiology

Mycoplasma







Dr. Tamadher Mohammed

Mycoplasmas are the smallest and simplest self-replicating bacteria. The mycoplasma cell contains the minimum set of organelles essential for growth and replication: a plasma membrane, ribosomes, and a genome consisting of a double-stranded circular DNA molecule .

Unlike all other prokaryotes, the mycoplasmas have no cell walls, and they are consequently placed in a separate class *Mollicutes* (*mollis*, soft; *cutis*, skin).

The trivial term mollicutes is frequently used as a general term to describe any member of the class, replacing in this respect the older term mycoplasmas.

Mycoplasma

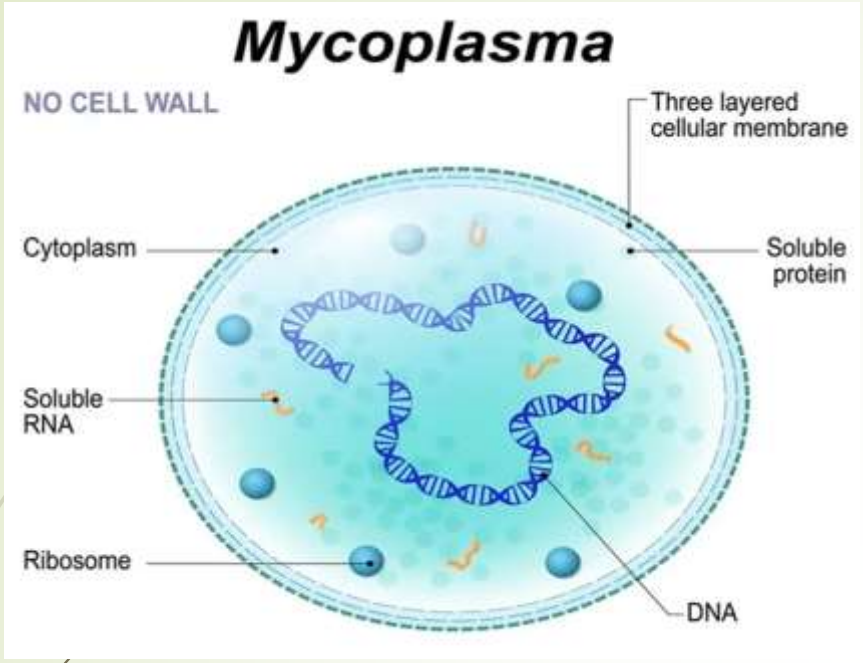
The Mycoplasma are members of the class Mollicutes, order Mycoplasmatales ,can divided 3 families:
Acholeplasmataceae (need not external cholesterol during growth)
Spiroplasmataceae (can form spiral structure).
Mycoplasmataceae (require external cholesterol during growth)
contain two genera Mycoplasma and Ureplasma);
Ureaplasma requires urea to produce an electrochemical gradient; urea is converted to ammonia to produce ATP.
Only *Mycoplasma* and *Ureaplasma* have pathogenic species important in veterinary medicine.

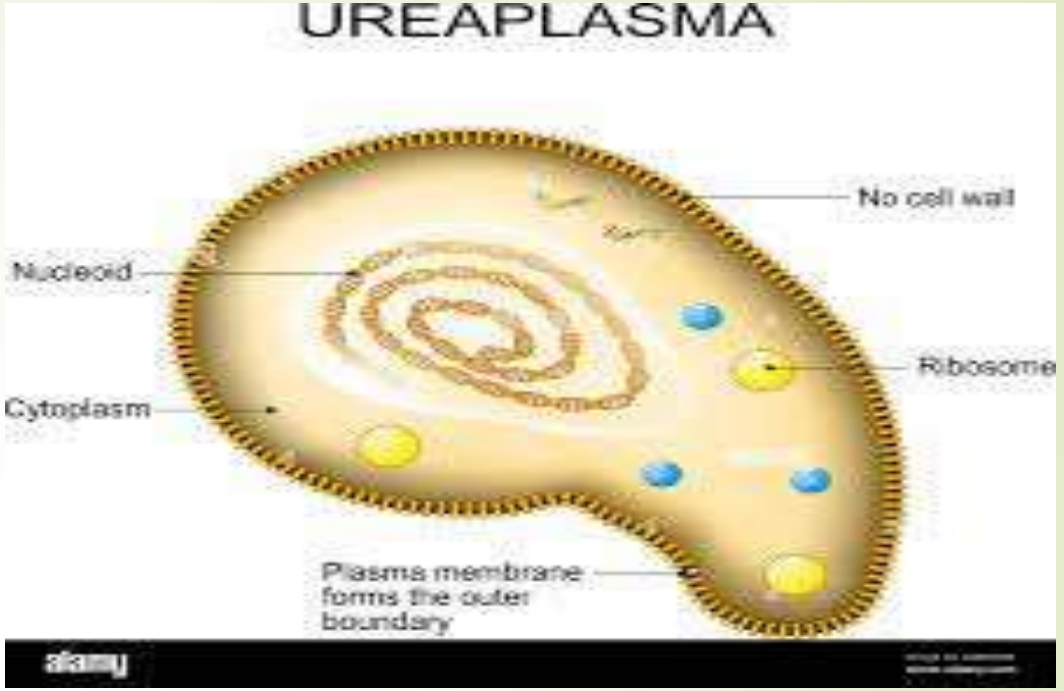
Over 100 species have been included in the genus Mycoplasma ,they are parasites or commensals of humans, animals, and plants.

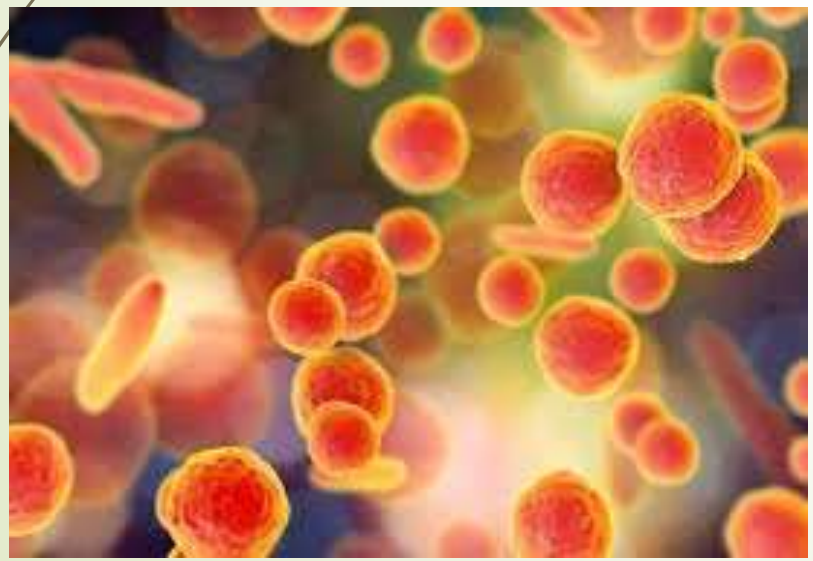
Most pathogenic mycoplasmas and ureaplasmas infect the mucosa of the respiratory and/or urogenital tract

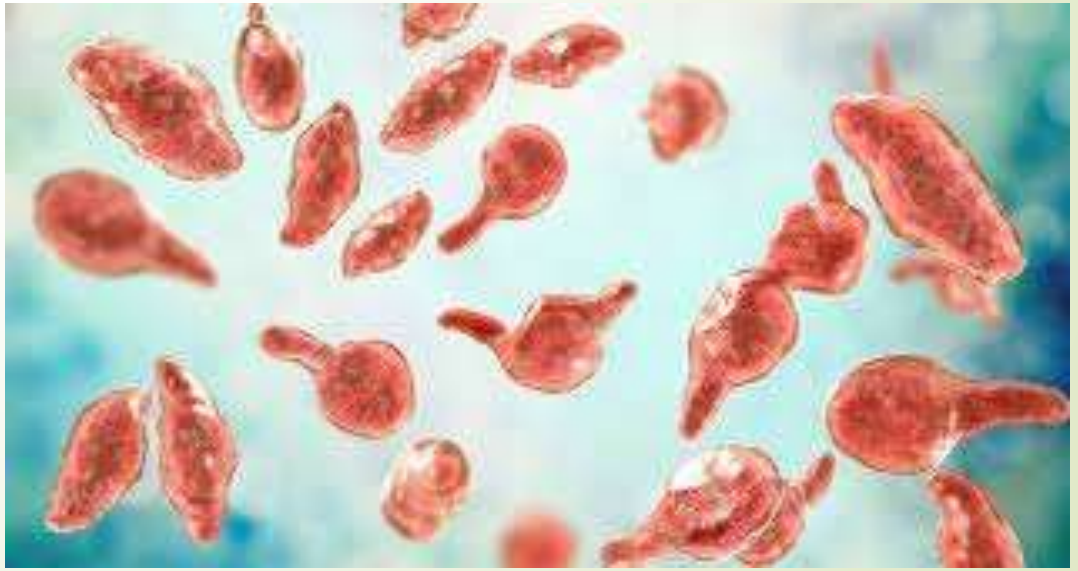
Species have been found in mammals, reptiles, fish, arthropods, insects, and plants .They are also common tissue-culture contaminants.

Infections with mollicutes are parasitic in nature. They are most often subclinical to mild but sometimes fatal diseases can also be observed.


Mycoplasma
NO CELL WALL
Three layered cellular membrane
Cytoplasm
Soluble protein
Soluble RNA
Ribosome
DNA


UREAPLASMA
No cell wall
Nucleoid
Ribosome
Cytoplasm
Plasma membrane forms the outer boundary





Most pathogenic mycoplasmas and ureaplasmas infect the mucosa of the respiratory and/or urogenital tract,but are also found in other body sites such as the conjunctiva, synovia surfaces, and mammary glands. The hemoplasmas infect red blood cells and most commonly cause hemolytic anemia.

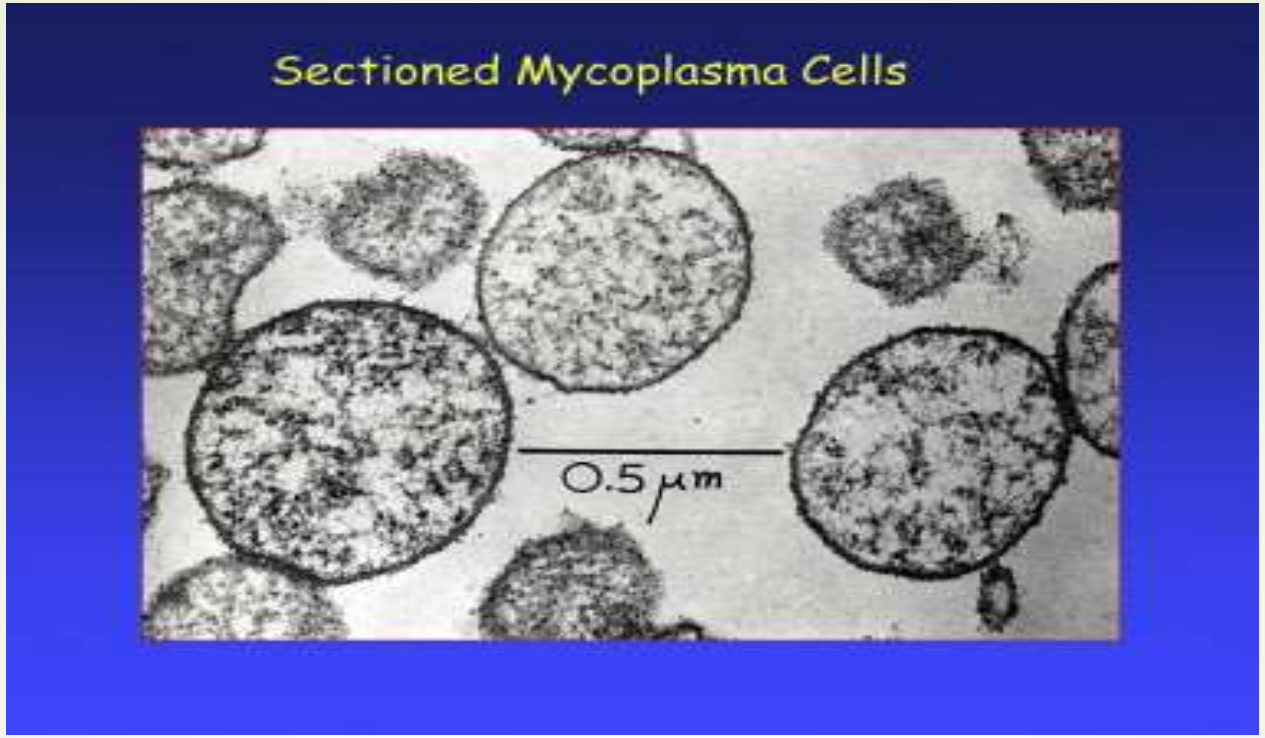
Morphology and Staining

The cell morphology of the mollicutes is extremely pleomorphic. Cell shapes include spherical, ring-shaped, pear shaped, spiral-shaped, and filamentous forms. Cells sometime appear as chains of beads.

The diameter of the spherical form ranges from 0.1 μm to 0.3 μm. Due to the size and shape of these organisms , they are able to pass through 0.22 μm and 0.45 μm membrane filters commonly used to filter sterilize liquid media; therefore, it is difficult to rid tissue culture cell lines of these organisms.

The mollicutes lack the genetic capacity to produce a cell wall.
Cell wall is absent and plasma membrane forms the outer boundary of the cell . Due to the absence of cell walls these organisms can change their shape and leads to pleomorphism.
Lack of nucleus and other membrane-bound organelles.

Instead, they are bound by a single trilaminar membrane composed of proteins, glycoproteins, lipoproteins , phospholipids, and sterols.
Cholesterol in the membrane provides osmotic stability.
The mollicutes have a small genome (ranging from 540 to 1380 kb) relative to other bacteria and are the smallest self-replicating organisms
Genetic material is a single DNA duplex and is naked.



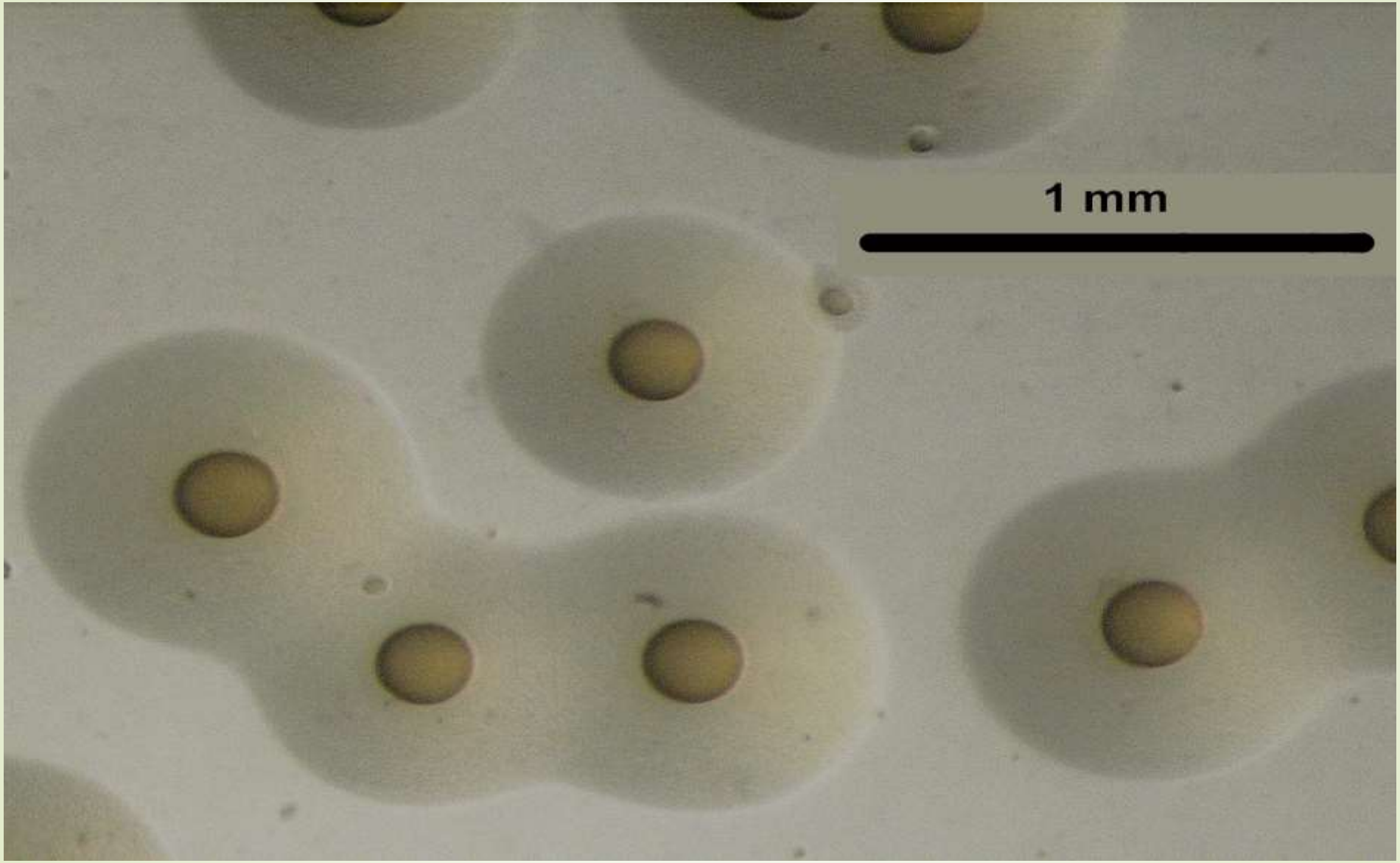
Electron micrograph of thin-sectioned mycoplasma cells. Cells are bounded by a single membrane showing in section the characteristic trilaminar shape. The cytoplasm contains thin threads representing sectioned chromosome and dark granules representing ribosomes.

Growth Characteristics

Generally slow growing and often require 3–10 days (or more) of incubation before colonies are apparent on agar. *In vitro* growth is generally best at 33–38 ◦C in an atmosphere of increased CO2.
media supporting growth of the various mycoplasmas and ureaplasmas are often highly enriched and complex. Most are based on beef heart infusion ,peptone , yeast extract, and serum along with various other supplements. Optimal pH for growth ranges from 6.0 to 8.0 for mollicutes depending on the normal host environment in which they are found.

colonies are small and difficult to visualize with the unaided eye. Colony sizes vary from 0.01 to 1.0 mm. When observed with a dissecting microscope ,many species exhibit a “fried egg” morphology .

“fried egg” morphology



Mollicutes can survive outside the host for substantial lengths of time in moist, cool environments. They are very susceptible to heat and most detergents (tween) and disinfectants (quaternary ammonium, iodine, and phenol based compounds).

While there are several clinically and economically important species of mollicutes, primary pathogens such as *M. mycoides* ssp. *mycoides*, small colony (SC) variants in cattle are the exception rather than the rule

Pathogenesis.

Attachment to host cells is the first step in establishing infection and is facilitated by the anionic surface layer on most mycoplasmas . Host receptors for attachment are surface proteins, especially glycoconjugates that allow colonization of mucosal surfaces.

M. mycoides ssp. *mycoides* SC variant is considered the most virulent of the bovine mycoplasmas. It causes a respiratory disease, contagious bovine pleuropneumonia (CBPP), in cattle that ranges from a persistent, subclinical infection to an acute, sometimes fatal disease.
Clinical signs include respiratory distress, coughing, nasal discharge, and reluctance to move. In severe cases,

Antibiotics are used to treat *Mycoplasma* and *Ureaplasma* infections. The lack of a cell wall renders mollicutes resistant to the action of antimicrobial agents that target the cell wall or its synthesis, such as the glycopeptides and *β*-lactams, but are sensitive to compounds that interfere with protein and nucleic acid synthesis.
Mycoplasmas show sensitivity to the following antibiotics: tetracyclines, macrolides,lincosamides ,ketolides , aminoglycosides, cephalosporins, pleuromutilins, and fluoroquinolones.

Mycoplasma	Hosts	Primary site of isolation (other)	*Disease*
M. capricolum subsp. *capripneumoniae*	Goat (sheep)	Lungs	CCPP
M. mycoides subsp. *capri*	Goat (sheep)	Respiratory tract (joints)	Plp, A, C
M. capricolum subsp. capricolum	Goat, sheep	Joints/resp. tract (udder)	Plp, M, A
M. ovipneumoniae	Sheep, goat	Respiratory tract	P
M. conjunctivae	Sheep, goat	Eyes	KC
M. agalactiae	Sheep, goat	Udder (joints, eyes)	M, A, Ke, P, S
M. putrefaciens	Goat (sheep)	Udder (joints)	M, A
M. arginini	Ubiquitous	Respiratory tract	None

Source: Nicholas (2002), Nicholas et al. (2008; 2009), CCPP – Contagious caprine pleuropneumonia, Plp – Pleuropneumonia, P – Pneumonia, M – Mastitis, A – Arthritis, C – Conjunctivitis, Kc – Keratoconjunctivitis

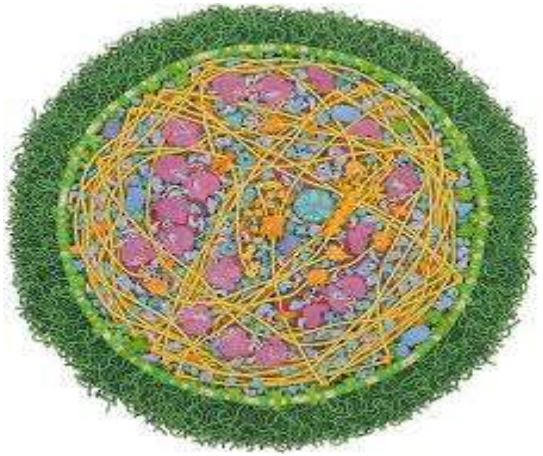
Because of the absence of cell walls in mycoplasma:

1-They do not stain with the Gram stain although they are Gram negative bacteria (Giemsa and Dienes stains are used)

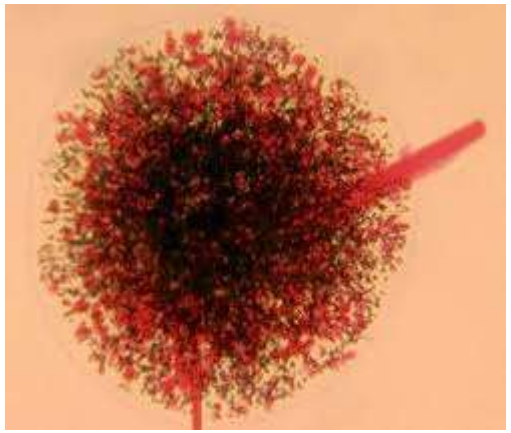
2-They are more pleomorphic with no fixed shape or size. The absence of a rigid cell wall and small size makes Mycoplasma able to cross filters that are otherwise permeable only to viruses.

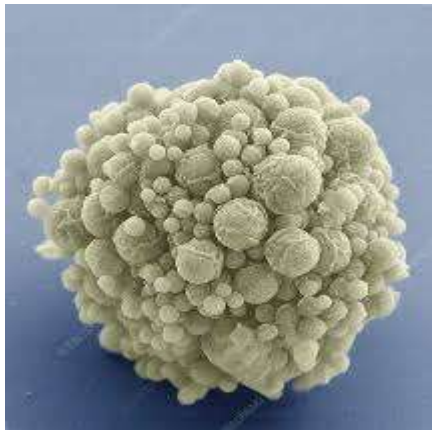
3-They are resistant to antibiotics that interfere with the synthesis of cell wall (Penicillins , Cephalosporins , Vancomycin) but susceptible to tetracycline, erythromycin

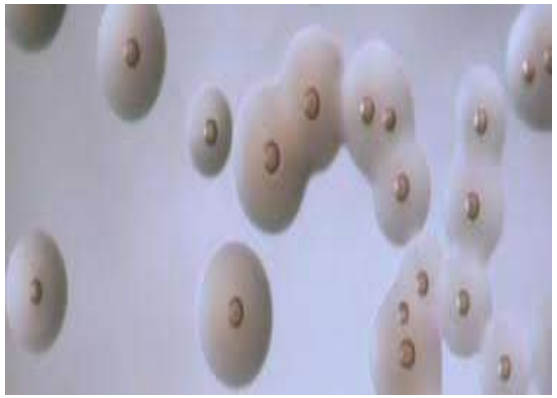
Mycoplasma mycoides



Class:	***Mollicutes***
Order:	**Mycoplasmatales**
Family:	**Mycoplasmataceae**
Genus:	***Mycoplasma***
Species group:	***Mycoplasma mycoides group***
Species:	***M. mycoides***







Mycoplasma mycoides is a bacterial species of the genus Mycoplasma in the class Mollicutes.
This microorganism is a parasite that lives in ruminants.
Mycoplasma mycoides comprises two subspecies, mycoides and capri, which infect cattle and small ruminants such as goats respectively.
Mycoplasma mycoides belongs to the Mycoplasma mycoides cluster, or Mycoplasma mycoides group, a group of closely related infectious mycoplasmas, first named by Weisburg et al.

The cluster contains the genera Mycoplasma mycoides and Mycoplasma capricolum and comprises six species and subspecies:

M. mycoides subsp. mycoides biotype Small Colony (MmmSC)
M. mycoides subsp. mycoides biotype Large Colony (MmmLC)
M. mycoides subsp. capri (Mmc)
M. capricolum subsp. capricolum (Mcc)
M. capricolum subsp. capripneumoniae (Mccp)
Mycoplasma sp. 'bovine group 7' (MBG7)

The first genome of *Mycoplasma mycoides* to be sequenced was that of strain $PG1^T$, the Causative Agent of Contagious Bovine Pleuropneumonia (CBPP).
It has a single circular chromosome contains 985 putative genes.

Very small and pleomorphic.
Non motile.
Facultatively anaerobic
Lacks a cell wall.

Hosts	Disease	Clinical picture
Cattle, water buffalo and yak	Contagious bovine pleuropneumonia (CBPP)	Fever, passivity, decreased milk production, rumination ceases, cough, difficulty in breathing, mucopurulent nasal flow, nasal congestion, curved back and weight loss. If the animal appears to become healthy, well-defined necrotic areas (so-called sequesters) can form in the lungs. These sequesters may contain live bacteria, which can spread to other animals if the general condition of the host animal deteriorates.

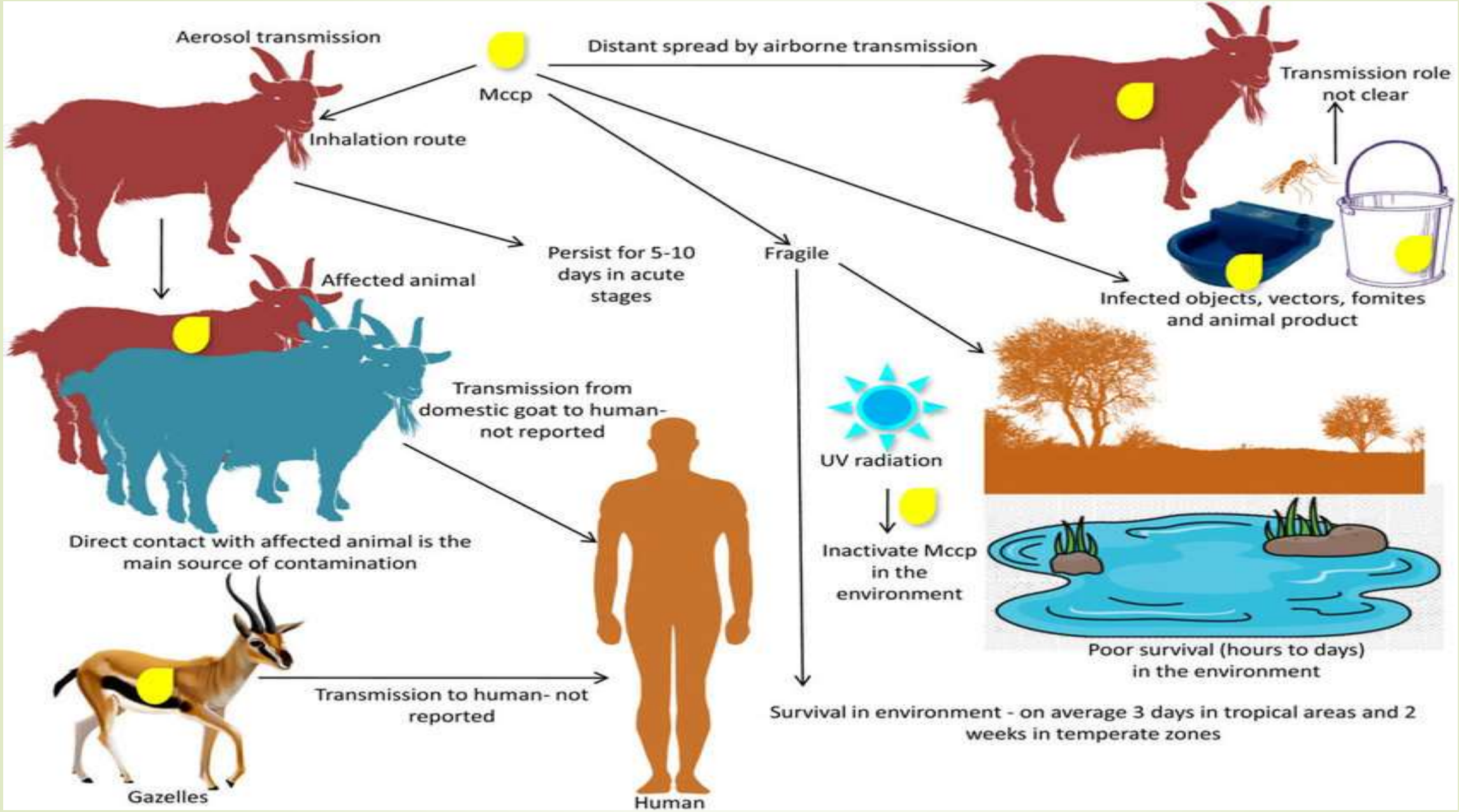
Mycoplasma capricolum subsp. capripneumoniae

Mycoplasma capricolum subspecies *capripneumoniae* is a member of *Mycoplasma mycoides* cluster ,which comprises five pathogens of ruminants including the mycoplasmas causing contagious bovine pleuropneumonia, contagious caprine pleuropneumonia (CCPP) and contagious agalactia which have been listed by the World Organisation for Animal Health because of their damaging socio-economic impacts .

CCPP is characterized by respiratory distress, coughing, nasal discharge and severe fibrinous pleuropneumonia.

It is a significant disease of goats in Africa, the Middle East and parts of Asia, and can cause mortality of up to 80% in immunologically naïve herds

The members of the *M. mycoides* cluster show close genetic and immunological similarity which has led to difficulties for differential diagnosis



Transmission of Mycoplasma capricolum subsp. capripneumoniae.(Mccp)

Pathogenesis of Mycoplasma capricolum subsp. capripneumoniae.

(1)Inhalation is the commonest route of infection through aerosol transmission

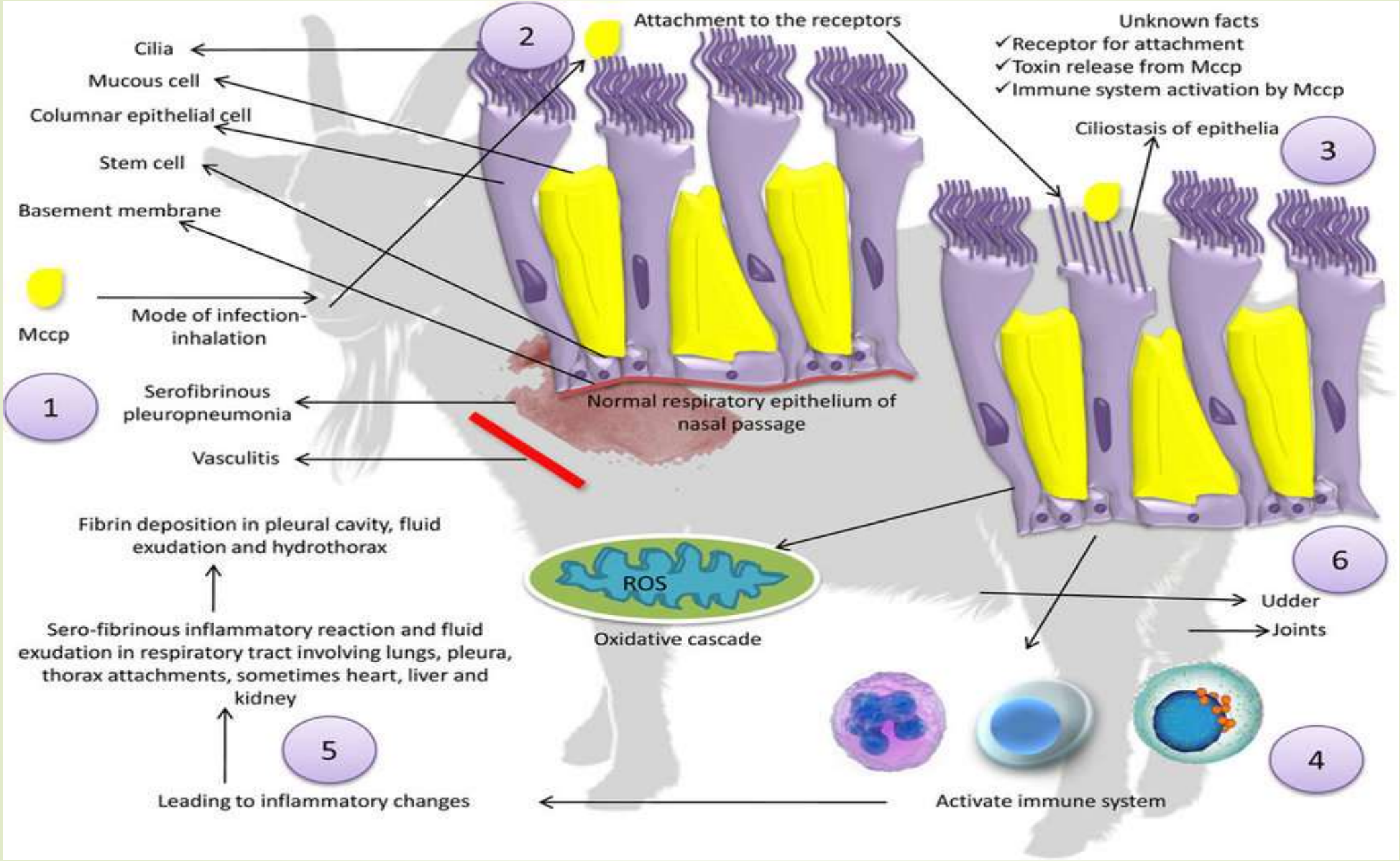
(2) Colonization

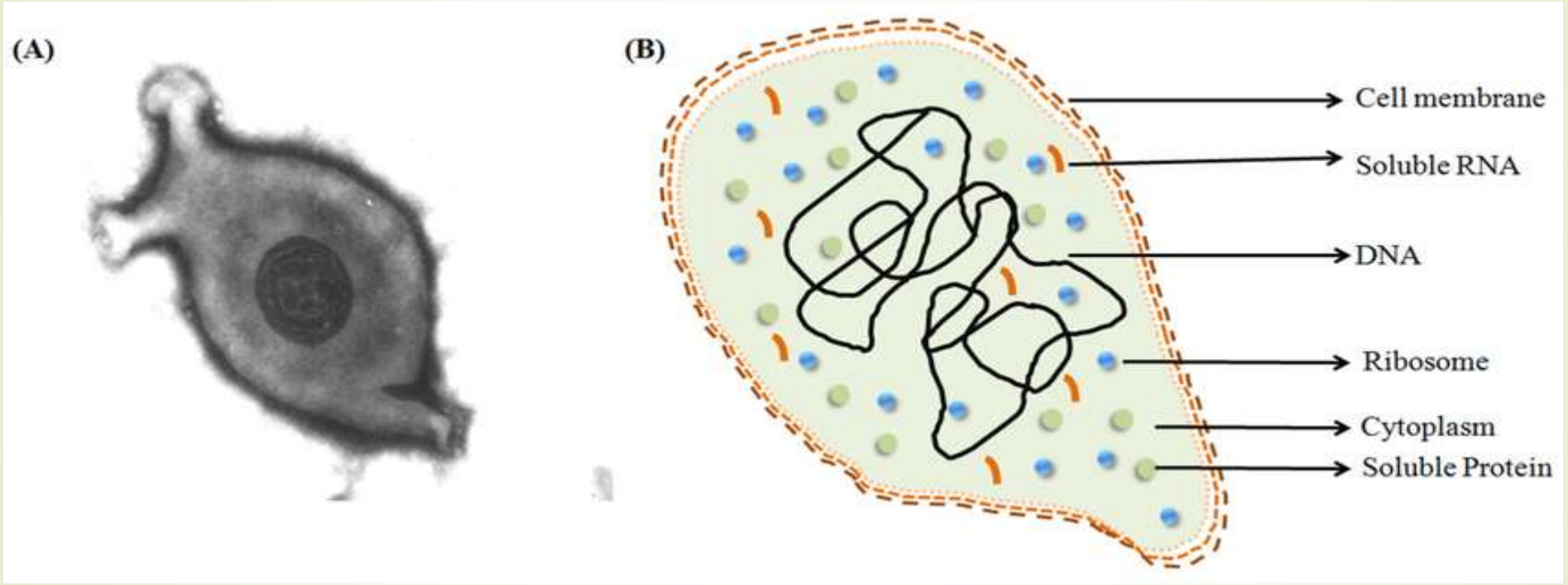
(3) Ciliostasis of epithelia, serofibrinous pleuropneumonia, vasculitis and fibrinocellular exudation.

(4) Mycoplasmal antigens (polysaccharides, galactan, lipoprotein) activate immune system,

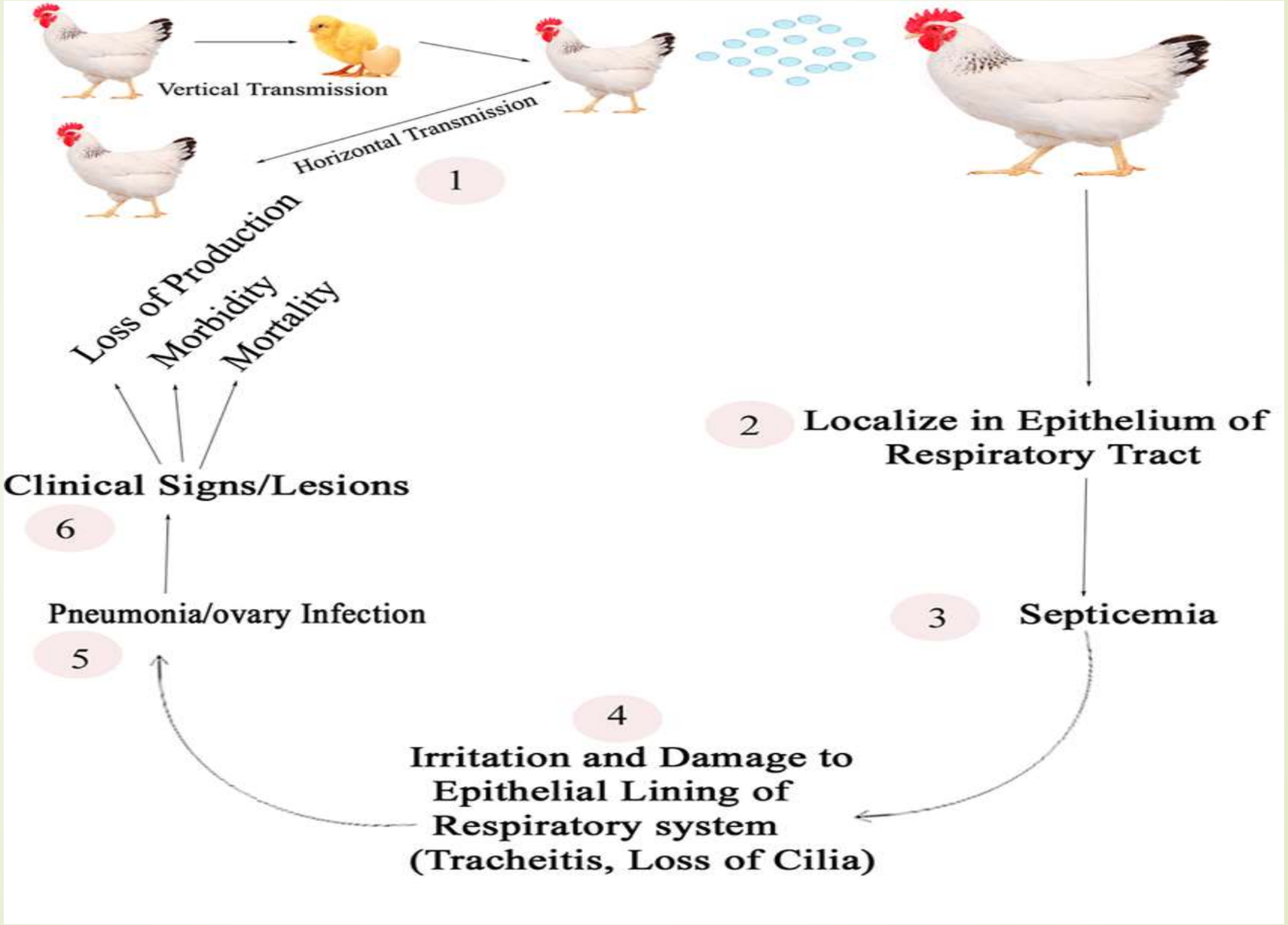
(5) Stimulation of inflammatory and oxidative cascade,

(6) Mycoplasma capricolum subsp. capripneumoniae may also affect other organs like joints, eyes, and udder.


Cilia
Mucous cell
Columnar epithelial cell
Stem cell
Basement membrane
Mccp
Mode of infection-inhalation
2
Attachment to the receptors
Unknown facts
✓Receptor for attachment
✓Toxin release from Mccp
✓Immune system activation by Mccp
Ciliostasis of epithelia
3
1
Serofibrinous pleuropneumonia
Vasculitis
Normal respiratory epithelium of nasal passage
Fibrin deposition in pleural cavity, fluid exudation and hydrothorax
ROS
Oxidative cascade
6
Udder
Joints
Sero-fibrinous inflammatory reaction and fluid exudation in respiratory tract involving lungs, pleura, thorax attachments, sometimes heart, liver and kidney
5
Leading to inflammatory changes
Activate immune system
4



Structure of M. gallisepticum. (A) Negatively stained image of M. gallisepticum


Vertical Transmission
Horizontal Transmission
1
Loss of Production
Morbidity
Mortality
2
Localize in Epithelium of Respiratory Tract
3
Septicemia
4
Irritation and Damage to Epithelial Lining of Respiratory system (Tracheitis, Loss of Cilia)
5
Pneumonia/ovary Infection
6
Clinical Signs/Lesions

How is mycoplasma diagnosed?

Culture test: This test looks for growth of mycoplasma from tissue or blood samples. It isn't normally used for respiratory infections but can help diagnose sexually transmitted mycoplasma infections.

Mycoplasma needs special culture media rich in cholesterol such as Eaton agar

They are aerobic or facultative anaerobic microorganisms ,but usually grow better in an aerobic condition supplemented with 5% CO2

They grow slowly on culturing (2–3 weeks incubation at 370C), especially on first isolation. Mycoplasma colonies are small and can only be observed

Serological test

1-Complement fixation test

2-Cold agglutinins

3-ELISA - There is a new ELISA for IgM that has been used for diagnosis of acute infection.

4-PCR

Pathogenic Mycoplasma Species and Their Primary Host(s)

Animal Species	Agent	Common Clinical Manifestations
Cats	M. felis	Conjunctivitis
	M. feliminutum	Respiratory disease
	M. gatae	Arthritis
Cattle	M. alkalescens	Arthritis, mastitis
	M. bovigentialium	Infertility, mastitis, seminal vesiculitis
	M. bovis	Abscesses, arthritis, mastitis, otitis, pneumonia
	M. bovoculi	Keratoconjunctivitis
	M. californicum	Arthritis, mastitis
	M. canadense	Arthritis, mastitis
	M. dispar	Alveolitis, bronchiolitis
	M. diversum	Infertility, pneumonia, vulvovaginitis
	M. mycoides ssp. mycoides (SC)	Arthritis, pleuropneumonia
	M. wenyonii	Anemia
Chickens	M. gallisepticum	Respiratory disease
	M. synoviae	Airsacculitis, sternal bursitis, synovitis
Dogs	M. canis	Urogenital tract disease
	M. cynos	Pneumonia
	M. spumans	Arthritis
Felids	M. felifaucium	Respiratory disease
	M. leocaptivus	Respiratory disease
	M. leopharyngis	Respiratory disease
	M. simbae	Respiratory disease
Goats	M. agalactiae	Agalactiae, arthritis, conjunctivitis
	M. capricolum ssp. capricolum	Arthritis, mastitis, pneumonia, septicemia
	M. capricolum ssp. capripneumoniae	Pleuropneumonia
	M. conjunctivae	Keratoconjunctivitis
	M. mycoides ssp. mycoides (LC)	Abscesses, arthritis, mastitis, septicemia
	M. mycoides ssp. capri	Pneumonia
	M. putrefaciens	Arthritis, mastitis
Horses	M. equigenitalium	Infertility
	M. equirhinis	Respiratory disease (suspected)
	M. fastidiosum	Respiratory disease (suspected)
	M. felis	Pleuritis
	M. subdolum	Infertility
Mice	M. neurolyitcum	Conjunctivitis, neurological disease
	M. pulmonis	Respiratory disease
Rats	M. arthritidis	Arthritis
	M. pulmonis	Respiratory disease, genital tract disease
Sheep	M. agalactiae	Agalactiae
	M. conjunctivae	Keratoconjunctivitis
	M. ovipneumoniae	Pneumonia
Swine	M. hyopneumoniae	Enzootic pneumonia
	M. hyorhinis	Arthritis, pneumonia, polyserositis
	M. hyosynoviae	Arthritis
Turkeys	M. gallisepticum	Sinusitis, respiratory disease
	M. iowae	Embryo mortality, leg deformities
	M. meleagridis	Airsacculitis, decreased egg hatchability, perosis
	M. synoviae	Sternal bursitis, synovitis