

MOLLICUTES

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- **The mollicutes are members of the order Mycoplasmatales and class Mollicutes (Latin: *mollis*, soft; *cutis*, skin).**
- **The genera *Mycoplasma* and *Ureaplasma* have pathogenic species important in veterinary medicine.**
- **Members of the *Acholeplasma* are sometimes encountered, but usually as contaminants.**

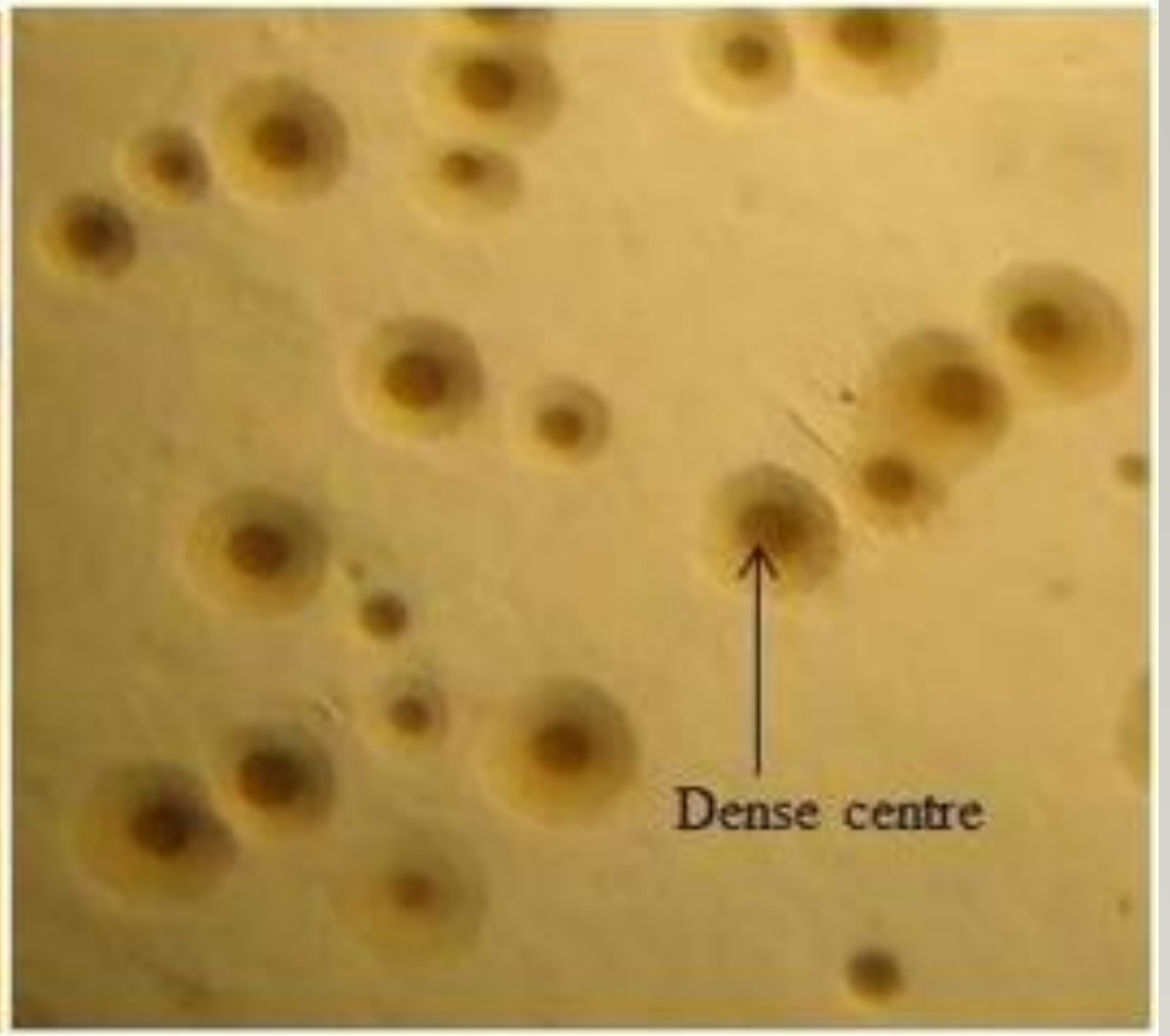
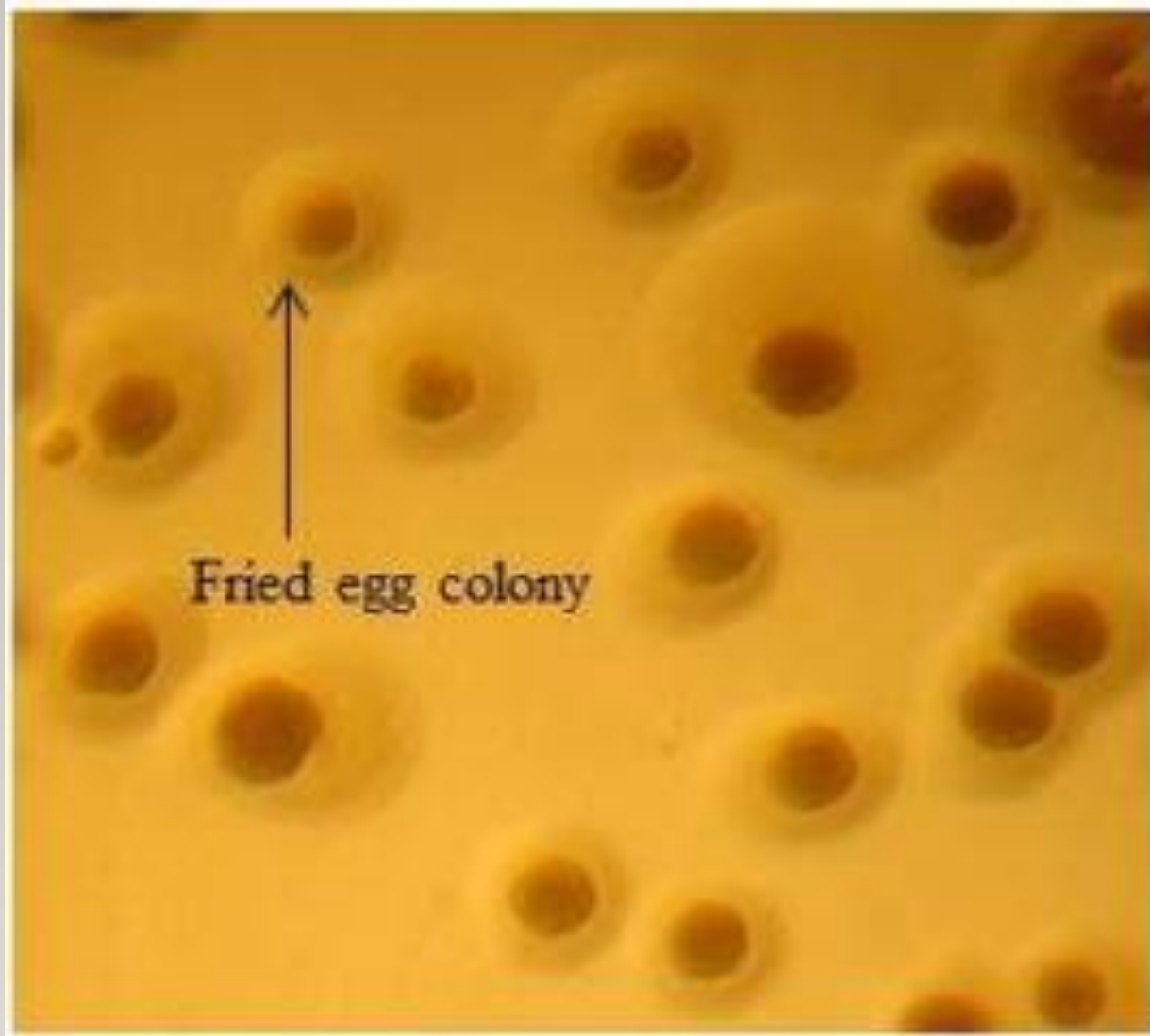


- Hemotrophic rickettsial species of the genera *Haemobartonella* and *Eperythrozoon* were reclassified to the genus *Mycoplasma*. They have been given the common name “hemoplasmas.”
- Infections with mollicutes are parasitic in nature. They are most often subclinical to mild but fatal diseases can also be observed.
- Most pathogenic mycoplasmas and ureaplasmas infect the mucosa of the respiratory and/or urogenital tract, conjunctiva, synovial surfaces, and mammary glands.
- The hemoplasmas infect red blood cells and cause hemolytic anemia



MORPHOLOGY AND STAINING

- Cell shapes include spherical, ring-shaped, pear shaped, spiral-shaped, and filamentous forms. Ring forms found in red blood cells infected with hemoplasmas .
- Mollicutes stain poorly by the Gram method due to their lack of a cell wall. Giemsa, Castaneda, Dienes, cresyl-fast violet, orcein, and acridine orange stains are preferred.
- Mollicute colonies are small and difficult to visualize with the unaided eye. When observed with a dissecting microscope, many species exhibit a “fried egg” morphology. *Ureaplasma* species produce smaller colonies than other mollicutes and lack the fried-egg colony morphology.



STRUCTURE AND COMPOSITION

- **The mollicutes lack the genetic capacity to produce a cell wall.**
- **They are bound by a single trilaminar membrane composed of proteins, glycoproteins, lipoproteins, phospholipids, and sterols.**
- **Cholesterol in the membrane provides osmotic stability.**
- **Exogenous sterols are required by mycoplasmas that they acquire from serum.**
- **The mollicutes have a small genome relative to other bacteria and are the smallest self-replicating organisms that have yet been described.**

Nonhemotrophic Mollicutes

- Clinical manifestations include respiratory and urogenital tract infections, conjunctivitis, arthritis, mastitis, septicemia, and otitis media. Most species exhibit a high degree of host specificity
- Unlike the “hemotrophic”, they can be grown *in vitro* on lifeless media.
 - 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.**

Pathogenesis

- The immune response of the host is intimately involved in the pathogenesis of disease (immunopathology).
- The chronicity of mollicute infections suggests that the immune response is not effective at eliminating infection once established, and latent infections are common.
- There is evidence that some *Mycoplasma* and *Ureaplasma* species can penetrate and exist inside nonphagocytic cells, protecting them from the immune system and facilitating their dissemination within the host.

- **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.**
- **Several mycoplasmas readily form biofilms that are a community of individual organisms living in a matrix of extracellular polymeric substance as a means of avoiding the host immune response and antibiotic therapies.**
- **Injury to host tissue may also be induced through other mechanisms. Products of metabolism such as hydrogen peroxide and superoxide radicals in mycoplasmas result in local host cell damage.**



Animal Species	Agent	Common Clinical Manifestations
Cats	<i>M. felis</i>	Conjunctivitis
	<i>M. feliminutum</i>	Respiratory disease
Cattle	<i>M. gatae</i>	Arthritis
	<i>M. alkalescens</i>	Arthritis, mastitis
	<i>M. bovigentialium</i>	Infertility, mastitis, seminal vesiculitis
	<i>M. bovis</i>	Abscesses, arthritis, mastitis, otitis, pneumonia
	<i>M. bovoculi</i>	Keratoconjunctivitis
	<i>M. californicum</i>	Arthritis, mastitis
	<i>M. canadense</i>	Arthritis, mastitis
	<i>M. dispar</i>	Alveolitis, bronchiolitis
	<i>M. diversum</i>	Infertility, pneumonia, vulvovaginitis
	<i>M. mycoides ssp. mycoides (SC)</i>	Arthritis, pleuropneumonia
Chickens	<i>M. wenyonii</i>	Anemia
	<i>M. gallisepticum</i>	Respiratory disease
Dogs	<i>M. synoviae</i>	Airsacculitis, sternal bursitis, synovitis
	<i>M. canis</i>	Urogenital tract disease
	<i>M. cynos</i>	Pneumonia
Felids	<i>M. spumans</i>	Arthritis
	<i>M. felifaucium</i>	Respiratory disease
	<i>M. leocaptivus</i>	Respiratory disease
	<i>M. leopharyngis</i>	Respiratory disease
Goats	<i>M. simbae</i>	Respiratory disease
	<i>M. agalactiae</i>	Agalactiae, arthritis, conjunctivitis
	<i>M. capricolum ssp. capricolum</i>	Arthritis, mastitis, pneumonia, septicemia
	<i>M. capricolum ssp. capripneumoniae</i>	Pleuropneumonia
	<i>M. conjunctivae</i>	Keratoconjunctivitis
	<i>M. mycoides ssp. mycoides (LC)</i>	Abscesses, arthritis, mastitis, septicemia
	<i>M. mycoides ssp. capri</i>	Pneumonia
	<i>M. putrefaciens</i>	Arthritis, mastitis

Laboratory Diagnosis

- **Sample Collection.** The appropriate sample for isolation attempt is determined by the clinical presentation and includes –exudates
 - swabs from
 1. affected sites
 2. affected tissues.
 3. milk.

Direct Examination.

- The variability in microscopic morphology and poor staining with the Gram method make direct examination for most mollicutes unrewarding.
- Immunoperoxidase, immunofluorescent, and immunohistochemistry staining of histopathologic sections .

Isolation of Mycoplasma (Culture) –

1. Semi solid enriched medium containing 20% horse or human serum, yeast extract & DNA. Penicillin & Thallium acetate are selective agents.

(serum – source of cholesterol & other lipids)

1. Incubate aerobically or an aerobically for 7 -12 days

with or without 5–10% CO₂ at 35-37°C. (temp range

22- 41°C, parasites 35- 37°C, saprophytes – lower temp)

3. Typical “fried egg” appearance of colonies

4. Colonies best seen with a hand lens after staining with Diene’s method.



Identification of Isolates

- ★ **Growth Inhibition Test – inhibition of growth around discs impregnated with specific antisera.**
- ★ **Immunofluorescence on colonies transferred to glass slides.**
- ★ **Molecular diagnosis**
 - **PCR-based tests are**
- ★ **being developed and these are expected to be the diagnostic test of choice in the future.**
 - **These should have good sensitivity and be specific**