COURSE TITLE: BACTERIOLOGY

COURSE CODE: MIPA-219

## RECOMMENDED BOOKS

1. Essentials of Veterinary Microbiology (Fifth Edition)
   By G. R. Carter, M. M. Chengappa and C. A. W. Roberts
2. Veterinary Bacteriology and Virology
   By I. A .Merchant and R. A. Packer
3. Text Book of Veterinary Microbiology
   By S. N. Sharma and S. C. Adlakha
4. Pathogenesis of Bacterial Infections in Animals
   By C. L. Gyles and C. O. Thoen
5. Veterinary Microbiology and Microbial disease
   By P. J. Quinn, B. K. Markey, M. E. Carter, W. J. C. Donnelly, F. C. Leonard
6. Topley and Wilson's Microbiology and Microbial Infections (Volume-3; $9^{th}$ Edition)
   By L. Collier, A. Balows and M.Sussman

**For Practical Class Purposes**

1. Diagnostic Procedures in Veterinary Bacteriology and Mycology
   By G. R. Carter and J. R. Cole, Jr.
2. Color Atlas and Textbook of Diagnostic Microbiology (Fifth Edition)
   By Elmer W. Koneman et.al

# BACTERIAL CLASSIFICATION AND NOMENCLATURE

**Systematic Bacteriology**

Systematic Bacteriology is a branch of Microbiology which embraces the classification and nomenclature of bacteria.

**Taxonomy**

Taxonomy is defined as the science of classification (orderly arrangement of organisms).

**Nomenclature**

Nomenclature is naming an organism by international rules according to its characteristics.

**Identification**

Identification refers -

i. To isolate and distinguish desirable organisms from undesirable ones.

ii. To verify the authenticity or special properties of a culture.

**Isolate**

An isolate is a pure culture derived from a heterogenous, wild population of microorganisms.

**Classification**

Classification can be defined as the arrangement of organisms into taxonomic groups (taxa) on the basis of similarities or relationships. Biochemical, physiologic, genetic and morphologic properties are often necessary for an adequate description of a taxon.

**Nomenclature**

Classical binomial system is used (Linnaean).

Class (al): A class consists of related orders.

Order (ales): Contains a group of related families.

Family (aceae): Closely related genera or tribes.

Tribe (ieae): Closely related genera.

Genus: Contains related species.

Species: Included in the same species are strains of organisms that have many characteristics in common, e.g. different strains of *Escherichia coli* will give substantially the same reactions to many biochemical tests.

Subspecies: Some species may be further subdivided into subspecies on the basis of small but consistent differences. e.g. *Campylobacter fetus* subsp. *fetus*, *Campylobacter fetus* subsp. *intestinalis*, *Campylobacter fetus* subsp. *jejuni*.

**Strain:** A stock of bacteria from a specific source and maintain in successive cultures or animal inoculation.

**Biovar/Biotype:** A term for variants within a species. They are usually distinguished by certain biochemical or physiological characteristics.

**Serotype:** A microorganism determined by the kinds and combinations of constituent antigens present in the cell. (Difference in antigenic properties in the cell)

**Serovar:** Varieties within a species defined by variation in serological reaction.

**Criteria for Classification**

- Microscopic observation.
- Presence or absence of specialized structures such as spores, flagella, capsule.
- Staining procedures such as Gram's stain.
- Biochemical activities.
- Antigenic structure
- ***DNA sequence analysis (gene analysis)***

  Nucleic acid hybridization and homologies: Similarities in base sequences between different organisms are determined by nucleic acid hybridization studies. Hybridization between DNA molecules of *E. coli* strains would be close to 100% but hybridization of *E. coli* with *Salmonella* would be about 45%. The phylogenetic definition of a species generally includes strains with approximately 70% or greater DNA-DNA relatedness.
- ***DNA base compositions***

  The proportions of the four DNA bases in the total DNA of an organism can be assayed. There is a considerable variation in the frequency of adenine-thymine and guanine-cytosine base pairs among various organisms. By convention the base composition of a DNA preparation is expressed as the mole percentage of guanine-cytosine (GC) to the total.

  GC + AT= 100%; if the GC content is 40%, the AT= 60%. Determination of GC% is of value in taxonomy; e.g. all the Enterobacteriaceae from *E. coli* to *Salmonella* have GC% ranging from 50 to 54.
- ***Ribosomal RNA hybridization or gene base sequence comparison***

  RNA exhibits more homology among widely dissimilar organisms than does DNA. Thus it is useful in comparing distantly related organisms.

**Phylogenetic Classification**

Phylogenetic classification is measures of genetic divergence of different phylum.

A eukaryotic species is a biologic group capable of interbreeding to produce viable same kind of off spring. Therefore, the concept of a species - the fundamental unit of eukaryotic phylogenesis has an entirely different meaning when applied to bacteria. A bacterial species is defined as distinct group of organisms that have certain distinguishing features and generally bear a close resemblance to one another in the more essential features of organisms.

**Formal Classification**

| **Formal Rank** | **Example** |
|---|---|
| Kingdom | Prokaryote |
| Division | Gracilicutes (Prokaryotes that have a rigid cell wall containing peptidoglycan and a negative reaction to Gram's stain) |
| Class | Scotobacteria |
| Order | Eubacteriales |
| Family | Enterobacteriaceae |
| Genus | Escherichia |
| Species | *Escherichia coli* |

**Bergey's Manual of Systematic Bacteriology**

Bergey's Manual has evolved since the publication of the first edition in 1923. The manual provides a key that may be used for identification of bacteria.

The possibility that one might draw inferences about phylogenetic relationships among bacteria is reflected in the organization of the latest edition of Bergey's Manual of Systematic Bacteriology, published in 4 volumes from 1984 to 1989. A companion volume published in 1994, Bergey's Manual of Determinative Bacteriology serves as an aid in the identification of those bacteria that have been described and cultured.

Since 1980, valid names of all bacterial species have been published in the 'International Journal of Systematic Bacteriology'.

**Description of the Major Categories and Groups of Bacteria**

Two different groups:

a. Eubacteria: Contain the more common bacteria, that is, those with which most people are familiar.
b. Archaebacteria: Archaebacteria do not produce peptidoglycan, a major difference between them and typical eubacteria. They live in extreme environment (high temp high salts, low $p^H$) and carry out unusual metabolic reactions.

**Four Major Categories of Bacteria are Based on the Character of the Cell Wall**

1. ***Gram negative eubacteria that have cell walls***

   May be phototrophic or non-phototrophic and include aerobic, anaerobic, facultative anaerobic and microaerophilic species.

2. ***Gram positive euabacteria that have cell walls***

   These organisms are generally chemosynthetic heterophils and include aerobic, anaerobic and facultative anaerobic species.

3. ***Eubacteria lacking cell walls***

   These are microorganisms that lack cell walls (commonly called Mycoplasma) and do not synthesize the precursor peptidoglycan.

4. ***The Archaebacteria***

   These prokaryotic organisms are predominantly inhabitant of extreme terrestrial and aquatic environments (High salts, high temp., low $p^H$). Some are symbionts in the digestive tract of animals. The archaebacteria consists of aerobic, anaerobic and facultative anaerobic organisms that chemolithotroph, hetrotrophs and facultative heterotrophs. Some species are mesophils while others are capable of growing at temp at $100^0$C. Archaebacteria can be distinguished from eubacteria in part by their lack of peptidoglycan in cell wall, possession of isoprenoid diether or diglycerol tetraether Multiplication occurs either by binary fission, budding, fragmentation or by unknown mechanisms.

# Streptococcus

**Principal Characteristics**

- Gram positive.
- Non-motile, non-spore forming.
- Occur generally in chain form but may be found in pair or single form.
- Usually facultative anaerobic.
- Catalase negative.

**Habitat**

- Widely distributed in nature.
- Commensals in animals. Potentially pathogenic and non pathogenic species may be present on the skin and on the mucous membranes of the genital, upper respiratory and digestive tracts.
- Fragile bacteria, susceptible to desiccation and survive only for short periods off the host.

**Classification**

Divided into six principal groups based on growth characteristics, type of hemolysis and biochemical activities.

i. Pyogenic streptococci
ii. Oral streptococci
iii. Enterococci
iv. Local streptococci
v. Anaerobic streptococci
vi. Other streptococci

***Lancefield Classification/Lancefield Groups***

This grouping is based on serologic differences in a carbohydrate substance in the cell wall called 'Component C' (Polysaccharide). Lancefield groups are designated by the capital letters from A to U.

Test methods include:

- Ring precipitation test
- Latex agglutination test

***Hemolysis***

Strains are also categorized according to type of hemolysis:

**i. Alpha hemolysis:** Partial or incomplete hemolysis, revealed as a zone of green discoloration around the colony.

**ii. Beta hemolysis:** Clear, colorless zone caused by complete hemolysis.

**iii. Gamma hemolysis:** No detectable hemolysis.

**iv. Alpha prime hemolysis:** A small zone of partially lysed red blood cells lying adjacent to the colony followed by a zone of completely lysed RBCs extending further into the medium.

**Mode of Infection**

- Endogenous.

- Exogenous: Acquired by inhalation or ingestion. Aerosol, direct contact or fomites are the most common modes of spread.

**Streptococcal Metabolites**

1. ***Hyaluronidase:*** Extracellular enzyme. Also called spreading factor because of its solublizing action on the ground substance of connective tissue.
2. ***Hemolysins:*** Two kinds of hemolysins are produced:
   a. Streptolysin O: Oxygen sensitive.
   b. Streptolysin S: Oxygen stable, non antigenic.
   Both produce beta hemolysis, toxic for neutrophils and macrophages.
3. ***Streptokinase (Fibrinolysin):*** Activates plasminogens to become plasmin (protease), leading to digestion of fibrin clots.
4. ***DNase (Streptodornase):*** Reduce the viscosity of fluid containing DNA. Streptococcal pus may be thin because of this enzyme.
5. ***Pyrogenic exotoxins:*** Elaborated by group A streptococci and produce toxic shock syndrome and scarlet fever in man.
6. ***Erythrogenic toxin:*** Some groups produce (Group A, B, C) erythrogenic toxin but it has no clear role in the pathogenesis of invasive streptococcal disease. This toxin is responsible for the rash in scarlet fever.
7. ***NADases:*** Kill phagocytes; produced by group-A streptococci.
8. ***Lipoproteinase, Amylase, Esterase:*** Attack host derive substrates.

**Antigenic Structure**

1. ***Group-specific cell wall antigen:*** This carbohydrate is contained in the cell wall of many streptococci and forms the basis of serologic grouping (Lancefield groups A-U). Extracts of group specific antigen for grouping streptococci may be prepared by extraction of centrifuged culture with hot hydrochloric acid, nitrous acid or formaldehyde; by enzymatic lysis of streptococcal cells. The serologic specificity of the group-specific carbohydrate is determined by an amino sugar. For group A streptococci, this is rhamnose-*N*-acetylglucoseamine; for group B, rhamnose-glucoseamine polysaccharide; for group C, rhamnose-*N*-acetylgalactoseamine; for group D, glycerol teichoic acid containing D-alanine and glucose; for group F, glucopyranosyl-*N*-acetylgalactoseamine.
2. ***M protein:*** M protein appears as hair-like projections of the streptococcal cell wall. It is alcohol soluble protein and is closely associated with virulence.
3. ***T substance and R protein:*** T and R antigens are alcohol insoluble and not associated with virulence. T substance is acid-labile and heat-labile.
4. ***Nucleoproteins:*** Extraction of streptococci with weak alkali yields mixtures of proteins and other substances of little serologic specificity, called P substances, which probably make up most of the streptococcal cell body.

**Pyogenic Infections in General**

A pyogenic infection is one which is characterized by production of pus. Most commonly pus producing bacteria are streptococcus, staphylococcus and corynebacteria. Tissue invasion by pyogenic bacteria evoke an inflammatory response which is characterized by vascular dilation and a marked exudation of plasma and neutrophils. The neutrophils engulf bacteria through phagocytosis. After phagocytosis, bacteria may be digested, but some can multiply within neutrophils due to resistance to lysosomal enzymes. Some produce toxins that kill the phagocytic cells, and the enzymes liberated from dead neutrophils cause partial liquefaction of the dead tissues and phagocytic cells. The liquefied mass turned into thick,

yellow pus. The viscous consistency of pus is due to a considerable amount of deoxyribonucleoprotein from the nuclei of all dead cells.

**Important Streptococci which Produce Disease in Men and Animals**

| Species | Lancefield Group | Haemolysis on Blood Agar | Hosts | Consequences of Infection |
|---|---|---|---|---|
| *Streptococcus pyogenes* | A | β | Man | Scarlet fever, septic sore throat, rheumatic fever |
| *Streptococcus agalactiae* | B | β | Cattle, sheep, goats | Chronic mastitis |
| *Streptococcus dysgalactiae* | C | β (α,γ) | Cattle<br>Lambs | Acute mastitis<br>Polyarthritis |
| *Streptococcus equisimilis* | C | β | Horses | Abscesses, endometritis, mastitis |
| | | | Pigs, cattle, dogs, birds | Suppurative conditions |
| *Streptococcus equi* | C | β | Horses | Strangles, suppurative conditions |
| *Streptococcus zooepidemicus* | C | β | Horses | Mastitis, pneumonia, navel infections |
| | | | Cattle, lambs, pigs, poultry | Suppurative conditions, septicaemia |
| *Enterococcus faecalis* | D | α | Many species | Suppurative conditions following opportunistic invasion |
| *Streptococcus canis* | G | β | Carnivores | Neonatal septicaemia, suppurative conditions, toxic shock syndrome |
| *Streptococcus uberis* | Not assigned (Viridans group) | α | Cattle | Mastitis |

**Additional Groups of streptococci**

***Viridans streptococci***

- Widespread commensals, frequently isolated from clinical specimens.
- α-hemolytic, not soluble in bile, do not usually split esculin and do not grow in 6.5% NaCl.

***Anaerobic streptococci***

- Found as commensals in the alimentary and upper respiratory tracts.
- They may occur as alone or mixed infections associated with operative procedures or wounds involving the gastrointestinal or genitourinary tract.

***Nutritionally Variant streptococci***

- These streptococci require special nutritional requirements.

- Usually α-hemolytic but may be non-hemolytic.
- In humans, these streptococci are commonly recovered from bacterial endocarditis; role in disease process in animals is not clear.

**Diagnostic Procedures**

***Specimen***

Pus (Strangles)

Joint fluid (Arthritis)

Milk (Mastitis)

Blood (septicemia)

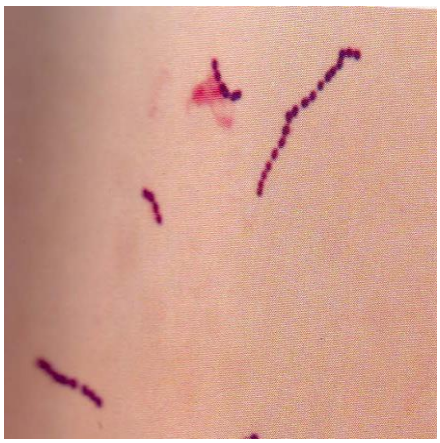
Meningial swabs (*Streptococcus suis* infections)

***Direct Examination***

Organisms can be demonstrated by Gram's stained smear. The organisms give positive reaction to this stain and characteristic chain may be seen.

***Isolation and Identification***

The pathogenic strains grow best on serum or enriched media; blood agar is preferred. Colonies are 1mm diameter, round, smooth, dew drop like. Hemolysis may or may not be present. No growth on MacConkey agar. Give negative reaction to catalase test.



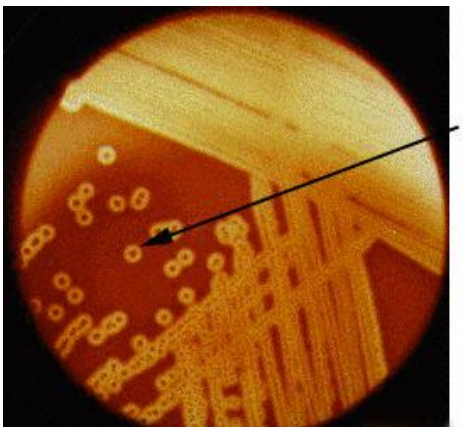



**Fig: *Streptococcus agalactiae***

**Strangles**

- Highly contagious disease of horse.
- Caused by *Streptococcus equi.*
- Febrile disease involving the upper respiratory tract with abscessation of regional lymphnodes.

***Epidemiology***

- Outbreaks of the disease occur most commonly in young horses.
- Assembling horses at sales, shows and race courses increases the risk of acquiring infection.
- Transmission is via purulent exudates from the upper respiratory tract or from discharging abscesses.
- A chronic, convalescent carrier state can develop with bacteria present in the guttural pouch.

***Clinical signs***

- High fever, depression and anorexia followed by an oculonasal discharge that becomes purulent.

- The lymphnodes of the head and neck are swollen and painful and subsequently ruptured.
- The ruptured lymphnode discharge purulent material.
- Sometimes abscessation develops in many organs, termed Bastard strangles.

***Diagnosis***

- Specimen: Pus
- Colonies are usually mucoid, up to 4 mm in diameter, and surrounded by a wide zone of beta-hemolysis.
- Asymptomatic carriers can be detected using the PCR test.

**Bovine Streptococcal Mastitis**

*Streptococcus agalactiae*, *Streptococcus dysgalactiae* and *Streptococcus uberis* are the principal pathogens involved in streptococcal mastitis. *Enterococcus faecalis*, *Streptococcus pyogenes* and *Streptococcus zooepidemicus* are less commonly isolated from cases of mastitis.

**Diagnosis of Mastitis**

1. ***Growth on Edward's media containing blood agar, crystal violet and esculin***

   *Streptococcus agalactiae* form transparent bluish, grey colored colonies and may be hemolytic or non-hemolytic.

   *Streptococcus uberis* produce dark colored colonies surrounded by black or brown zone of coloration due to hemolysis of esculin.

   *Streptococcus dysgalactiae* produce non-hemolytic colonies on the medium.

2. ***Hotis and Miller test***

   The test is carried out by adding 0.5 ml of 0.5 percent solution of bromocresol purple into 9.5 ml of milk. After 24 hours of incubation at 37°C, the test is read. *Streptococcus agalactiae* colonies grow on the walls of the test tube and appear as canary yellow. The yellow color is formed by fermentation of lactose with acid formation which changes bromocresol purple to yellow. *Streptococcus dysgalactiae* and *Streptococcus uberis* also ferment lactose but do not grow as clumps.

3. ***CAMP test (Christie, Atkins and Munch-Petersen)***

   **Principle**

   Ruminants red cell partially lysed by β-toxin (β hemolysin) of staphylococci at 37°C is lysed completely in presence of *Streptococcus agalactiae*. The hemolytic activity of the β hemolysin produced by *Staphylococcus aureus* is enhanced by an extracellular protein produced by group-B streptococci.

   **Procedure**

   i. Draw a line across the centre of blood agar plate.
   ii. Streak the streptococcal culture at right angles across the line.
   iii. Streak the β hemolytic *Staphylococcus aureus* culture across the plate directly over the line.

   **Interpretation**

   The area of increased hemolysis occurs where the β hemolysin secreted by the staphylococcus and the CAMP factor secreted by the group-B streptococcus.

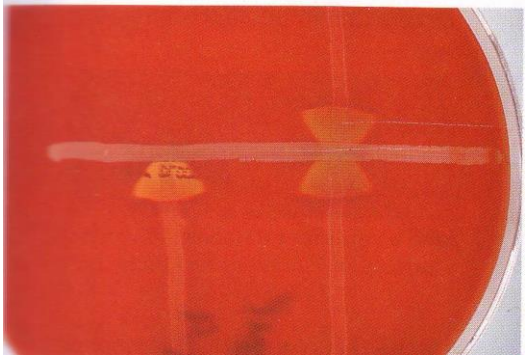


**Fig: CAMP test**

**Differentiation of streptococci which Cause Bovine Mastitis**

| Species | Hemolysis on Blood Agar | CAMP Test | Esculin Hydrolysis (Edwards Medium) | Growth on MacConkey Agar | Lancefield Group |
|---|---|---|---|---|---|
| *Streptococcus agalactiae* | β | + | - | - | B |
| *Streptococcus dysgalactiae* | α | - | - | - | C |
| *Streptococcus uberis* | α | - | + | - | Not assigned |
| *Enterococcus* | α | - | + | + | D |

**Public Health Significance**

- *Streptococcus pyogenes* can be disseminated to humans through consumption of milk from the infected udder of cow.
- *Streptococcus suis* can cause serious infections (meningitis, arthritis, septicemia, diarrhoea, ear infections) in humans directly involved in pig husbandry.

# Pneumococcus

**Species:** *Streptococcus pneumoniae*

**Synonym:** *Diplococcus pneumoniae*

**Principle Characteristics**

- Gram positive, usually occurs in pairs.
- Possessing a polysaccharide capsule, non-motile, and do not produce spore.
- Commensals in the upper respiratory tract of humans and less commonly of animals.

**Distribution**

- Found all over the world associated with infections of human respiratory tract.
- Also isolated from respiratory tracts of healthy animals as well as cases of pneumonia and mastitis in cattle.

**Cultural Properties**

- Aerobic, facultative anaerobic.
- Growth occurs on simple media but presence of blood, serum or glucose and 5-10% $CO_2$ enhances the growth.
- Colonies on serum agar – flat, moist, transparent with slight elevation on edges.
- α-hemolysis is produced on blood agar.

**Antigenic Structure**

Antigenic capsule is composed of complex polysaccharide. The capsular polysaccharide is immunologically distinct for each of the more than 80 types.

The somatic portion of the pneumococcus contains an M protein that is characteristic for each type and a group specific carbohydrate that is common to all pneumococci. The carbohydrate can be precipitated by C-reactive protein, a substance found in the serum of certain patients.

**Pathogenicity**

- Most of the infections are caused by serotypes 12 to 18. The organisms cause bovine mastitis which is acute and affects one, two or all four quarters of udder. There are also systemic disturbances shown by rise in temperature and loss of appetite. In severe cases, death may take place due to septicemia.
- Pneumonia in young calves, horse, dogs, goats.
- Human: Lobar pneumonia, sinusitis, conjunctivitis.

**Diagnosis**

- ***Specimen:*** Sputum, Blood, Milk.
- ***Direct microscopic examination:*** Characteristic gram positive paired cocci.
- ***Isolation and identification:*** Growth on blood agar, serum agar and by different biochemical properties.
- ***Quellung reaction:*** When pneumococci of a certain type are mixed with antipolysaccharide serum of the same type or with polyvalent antiserum on a microscopic slide, the capsule swells markedly. This reaction is useful for rapid identification and for typing of the organisms either in sputum or in cultures.

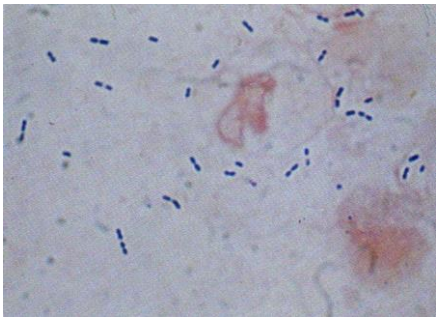


**Fig: Gram's stain of a smear of purulent sputum demonstrating gram positive diplococci, characteristic of *Streptococcus pneumoniae***

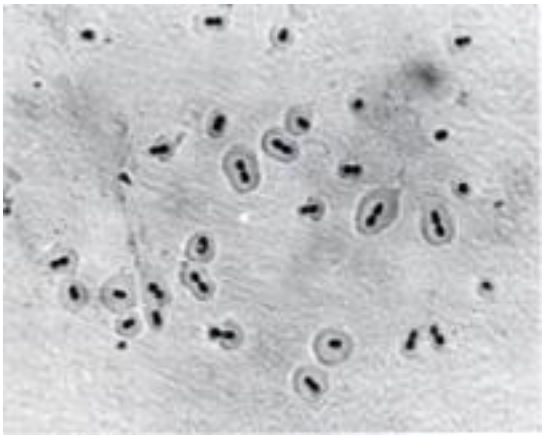


**Fig: Quellung reaction for the identification of *Streptococcus pneumoniae***

# Staphylococcus

**General Characteristics**

Gram positive, generally arranged in grape like clusters, non motile, non spore forming, grow aerobically but are facultative anaerobic, grow well on nutrient broth and nutrient agar, catalase positive.

**Important Species**

*Staphylococcus pyogenes* or *Staphylococcus aureus*: Causes suppurative wound infections in man and animals.

*Staphylococcus epidermidis* or *Staphylococcus albus*: Usually non pathogenic, common commensal of the skin.

*Staphylococcus intermedius*: Causes pyoderma, otitis externa, suppurative condition in dogs, cats.

*Staphylococcus hyicus*: Occurs on the skin of piglets and causes exudative epidermitis (Greasy pig disease).

**Habitat**

Commensals on the skin and mucous membrane of the upper respiratory and lower urogenital tract.

**Staphylococcal Metabolites**

Some of the substances thought to be involved in the production of staphylococcal infections are listed below; most of them are exotoxins.

1. ***Leucocidin:*** Kills leukocytes; antigenic; non hemolytic; associated with α and δ (delta) toxins.
2. ***Dermonecrotoxin:*** Necrotizing; associated with α toxin.
3. ***Lethal toxin:*** Rapidly lethal for mice and rabbits; associated with α and β hemolysins.
4. ***Hemolysins (Hemotoxins):*** Cause lysis of red blood cells of rabbit, sheep and ox. It also destroys platelets.

   α hemolysin: Responsible for inner clear zone of hemolysis. The toxin causes spasms of smooth muscle and is dermonecrotizing and lethal.

   β hemolysin: The toxin is sphingomyelinase C and is responsible for outer partial zone of hemolysis.

   γ hemolysin: Poorly characterized.

   δ hemolysin: Poorly characterized
5. ***Enterotoxins:*** These toxins are extracellular proteins composed of single polypeptide chains. These are highly heat stable toxins associated with staphylococcal food poisoning in man; produce nausea, abdominal cramps, diarrhoea.
6. ***Coagulase:*** Clotting of plasma; converts prothrombin to thrombin, which converts fibrinogen to fibrin.
7. ***Staphylokinase:*** Degrades fibrin clots by converting plasminogen to the fibrinolytic enzyme plasmin.
8. ***Nuclease:*** It's role in disease production is not clear.
9. ***Hyaluronidase:*** The enzyme is known as 'spreading factor'; it degrades hyaluronic acid.
10. ***Lipase:*** Lipase positive strains tend to cause abscesses of the skin and subcutis; destroys protective fatty acid on skin.
11. Exfoliative toxin: Causes cleavage of desmosomes in the stratum granulosum of the epidermis; causes separation and loss of superficial layers of epidermis.

## *Staphylococcus pyogenes* (or *Staphylococcus aureus*)

**Cultural Characteristics**

Grow well on common laboratory media. Selective media are mannitol salt agar. Blood agar is preferred for primary isolation. Colonies are round, smooth and glistening; may have 'gold' pigmentation. Double zone haemolysis is characteristic.

**Biochemical Properties**

1. Most of the pathogenic strains of *Staphylococcus pyogenes* liquefy gelatin.
2. Produce acid from glucose, maltose, mannitol, lactose, sucrose and glycerol. It does not ferment salicin and raffinose.

**Antigenic Nature**

- The cell wall of *Staphylococcus aureus* has three major components:
  Peptidoglycan
  Teichoic acids: Species specific.
  Protein A: Antiphagocytic; major protein of the cell wall.
- The polysaccharide capsule of some strains of *Staphylococcus aureus* is antiphagocytic. 11 serologic types of *Staphylococcus aureus* have been recognized in animals and humans on the basis of capsular polysaccharide antigens. Capsule type 5 is predominant in bovine milk, whereas capsule type 8 is predominant in caprine and ovine milk.

**Pathogenicity**

Clinical conditions produced by *Staphylococcus aureus*

| | | |
|---|---|---|
| Cattle | : | Mastitis, udder impetigo |
| Sheep | : | Mastitis |
| | | Tick pyaemia (lambs) |
| | | Benign folliculitis (lambs) |
| | | Dermatitis |
| Goats | : | Mastitis |
| | | Dermatitis |
| Pigs | : | Botryomycosis of mammary glands |
| | | Impetigo on mammary gland |
| Horses | : | Scirrhous cord (botryomycosis of the spermatic cord), mastitis |
| Dogs, cats | : | Pyoderma, endometritis, cystitis, otitis externa, and other suppurative conditions |
| Poultry | : | Arthritis and septicaemia in turkeys |
| | | Bumble foot |
| | | Omphalitis in chicks |

**Mastitis**

In cattle, sheep and goats:

Acute staphylococcal mastitis is characterized by fever, loss of appetite, enlargement and hardening of affected quarters. The milk secretion is stopped while blood stained fluid which later becomes thickened with pus is secreted. In fatal cases, animal may die within 3-4 days of onset of symptoms due to toxaemia. In gangrenous mastitis the affected quarter, which becomes cold and blue-black, eventually slaughs. These necrosis is attributed to the alpha toxin which causes contraction and necrosis of smooth muscle in blood vessel walls, impeding blood flow in the affected quarter.

**Diagnosis**

- ***Specimen:*** Pus, milk, affected tissues.
- ***Direct examination:*** Smears disclose clumps of gram positive cocci, non motile, non spore forming.
- ***Isolation and cultivation:*** The organism grows well on nutrient agar. Colonies are soft, low convex, 2 to 3 mm in size. Colonies are sometimes pigmented may vary from white to lemon yellow.
- Coagulase test.

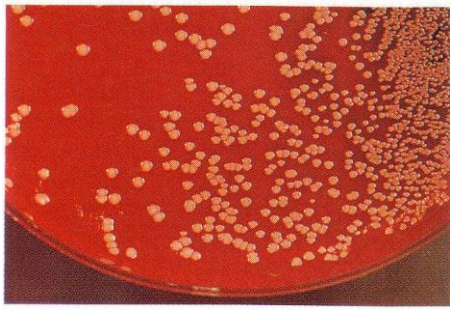


**Fig: Blood agar plate on which are growing round, yellow white, convex, non-hemolytic colonies of *Staphylococcus sp.***

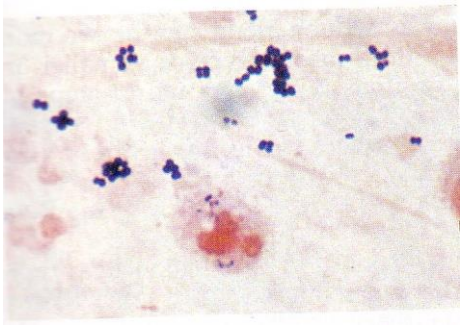


**Fig: Staphylococcus sp. (Gram's stain)**

**Differentiation of Gram-positive Cocci**

| Organism | Appearance in stained smears | Coagulase production | Catalase production | Oxidase production | O-F test[a] | Bacitracin disc |
|---|---|---|---|---|---|---|
| *Staphylococcus spp.* | Irregular clusters | ± | + | - | F | Resistant |
| *Micrococcus spp* | Packets of four | - | + | + | O | Susceptible |
| *Streptococcus and Enterococcus spp* | Chains | - | - | - | F | Resistant |

a. oxidation fermentation test. O = Oxidative, F = Fermentative

**Other species**

*Staphylococcus intermedius*: Dogs: Pyoderma, endometritis, cystitis, otitis externa and other suppurative conditions
Cats: Various pyogenic conditions
Cattle: Mastitis (rare)

*Staphylococcus hyicus*: Pigs: Exudative epidermitis. Arthritis
Cattle: Mastitis (rare)

# Diplococcus

**Species:** *Diplococcus mucosus*

- Produce mucinous colonies on nutrient agar. Microscopically it consists of small diplo and tetra cocci surrounded by capsules.
- Colonies are 1.5-4 mm in diameter, convex, yellowish grey, opaque and mucinous.
- Gelatin is not usually liquefied.
- Most strains seem to be pathogenic for mice. The systematic position of these organisms is still in doubt.

# Neisserea

**Species:** *Neisserea gonorhoeae* (Gonococci)

**Principal Characteristics**

- Gram negative, non motile diplococcus.
- Catalase/oxidase positive.
- Aerobic.
- Ferment only glucose, produce small colonies containing piliated bacteria, appear to be virulent.
- Pili enhance attachment to host cells and resistance to phagocytosis.

**Pathogenicity**

Gonococci attach mucous membranes of the genitourinary tract, eye, rectum and throat, producing acute suppuration that may lead to tissue invasion; this is followed by chronic inflammation and fibrosis. In males there is usually urethritis, with yellow creamy pus and painful urination. The process may extend to the epididymis. As supuration subsides in untreated infection, fibrosis occurs, sometimes leading to urethral strictures, urethral infection in man can be asymptomatic. In females, the primary infection is in the endocervix and extends to the urethra and vagina, giving rise to multipurulent discharge. It may then progress to the uterine tubes, causing sulpingitis, chronic gonococcal cervicitis or proctitis is often asymptomatic.

**Gonococcal opthalmia neonatorum:** An infection of the eye of newborn child is acquired during passes through an infected birth canal. The initial conjunctivitis rapidly progresses and if untreated results in blindness.

**Diagnosis**

- ***Specimen:*** Pus and secretions are taken from the urethra, cervix, rectum, conjunctiva or throat for culture and smear.
- ***Direct examination:*** Gram stained smears of exudates reveal many diplococci within pus cells. This gives a tentative diagnosis.
- ***Isolation and Cultivation:*** Immediately after collection pus or mucus is streak on enriched selection medium (e.g. modified Thayer-Martin medium for *Neisserea gonorrhea*) and incubated in an atmosphere containing 5% $CO_2$ at $37^0$C. The organisms can be quickly identified by their appearance on gram stain smear and by coagulation or immunofluorescence test.

# Bacillus

**Principal Characteristics**

- Gram positive, large rods.
- Aerobic or facultative anaerobic, spore-forming.
- Catalase positive and most are motile.
- A large number of species of this group are ubiquitous, occurring widely in the soil, air, dust and water.

Most species are saprophytes with no pathogenic potential. *Bacillus anthracis* is the only important pathogen of animals and man in the genus. Occasional infections have been attributed to *Bacillus cereus* but animal disease caused by other species is rare.

## *Bacillus anthracis*

**Historical**

- This is the first disease which was found to be caused by a bacterial agent.
- In 1876, Robert Koch cultured *Bacillus anthracis* and reproduced the disease with the culture.
- Pasteur made detail study about the disease in $19^{th}$ century. This is the first disease against which Pasteur made classical study to evolve an attenuated vaccine.

**Morphology**

- Large, cylindrical rods, measuring 1 - 1.5 µm in width and 4 - 8 µm long.
- Capsules are prominent and chains are shorter.
- Non-motile, spore bearing (in animal body, spores are not formed).
- Gram positive.

**Cultural Properties**

- It grows on common laboratory media under aerobic and also partial anaerobic temperature. Optimum temperature is 35 - 37°C. Sporulation occurs in an atmosphere of low pressure of oxygen.
- On agar surface, colonies are dull, opaque, grayish white with an irregular border from which long strands of cells are seen in parallel arrangement resembling a 'Medusa-head'.
- In gelatin stab, the growth pattern is of inverted fir tree. From the line of inoculum a number of fine filaments develop laterally. Those nearer to the surface are longest and become progressively shorter far from the surface.
- On blood agar, produce slight hemolysis.
- In nutrient broth, growth gives rise to turbidity with a flocular growth on the surface which sinks to the bottom in 24 hours.
- Most of the virulence is lost when organisms are incubated at 42°C for two weeks. The colonies of these attenuated strains are smooth, smaller, more convex and smooth in outline. The rough strains of *Bacillus anthracis* are virulent and smooth strains are avirulent.

**Biochemical Properties**

- The organism forms acids but no gas from glucose, sucrose, maltose, fructose, trehalose and dextrin but doesn't ferment lactose, galactose and mannitol.
- Indole and $H_2S$ is not formed and nitrates are reduced to nitrites.

**Antigenic Structure**

The capsular substance of *Bacillus anthracis*, which consists of a polypeptide of high molecular weight composed of D-glutamic acid, is hapten. The bacterial bodies contain protein and a somatic polysaccharide, both of which are antigenic. The capsule protects the organism from phagocytosis.

**Toxins**

- Anthrax toxin is an exotoxin consisting of three protein components: I, II, III.
  - Component I - the edema factor (EF)
  - Component II – the protective antigen (PA)
  - Component III – the lethal factor (LF)
- None of the factors can act individually. Protective antigen transports edema factor and lethal factor to the target cell.
- Edema factor has been shown to be an adenylate cyclase that causes an increase in cAMP, following binding to protective antigen.
- Lethal factor and protective antigen have combined lethal activity. The lethal factor is a $Zn^{++}$ dependent protease that induces cytokine production in macrophages and lymphocytes.
- Component I and component II cause edema with low mortality, however, when component III is included, there is, maximum lethality.

**Resistance**

- The endospores of *Bacillus anthracis* are considerably more resistant to physical influences and chemical disinfectants than vegetative cells. The vegetative form of bacteria can be killed at 60°C for 30 minutes.
- The spores will be destroyed by boiling for 10 minutes and by exposure to dry heat at 140°C for 3 hours.
- Spores are destroyed by 5% phenol in 2 days, by 10 to 20% formalin in 10 minutes and by autoclaving at 121°C for 15 minutes.
- Mercuric chloride 1:1000 added to heat fixed smears kills in 5 minutes.
- Wools, hairs and horse hair, collected from anthrax occurring areas, should be gas sterilized.

**Epidemiology**

- *Bacillus anthracis* is found worldwide where spores are located. The endospores of *Bacillus anthracis* can survive for decades in soil. Anthrax organisms sporulate with greater frequency in low lying marshy areas with soil $p^H$ higher than 6. Animals may become infected from contaminated soil, water, bone meal, oil cake, offal, carrion birds and wild animals.
- Anthrax affects virtually all mammalian species including humans. Ruminants are highly susceptible (Algerian sheep are said to be resistant); pigs and horses are moderately susceptible to infection while canines are comparatively resistant. Birds are almost totally resistant.
- The microorganism is usually acquired by ingestion, inhalation, wounds or skin abrasions.

**Pathogenicity**

- Generally classed as an obligate pathogen. Per-acute, acute, chronic and cutaneous forms of the disease are seen.
- Cattle, sheep: Fatal per-acute or acute septicemic anthrax. The cutaneous form is occasionally seen in cattle following infection of wounds and abrasions.
- Swine: Sub-acute anthrax with edematous swelling in pharyngeal region; an intestinal form with higher mortality is less common.
- Horses: Sub-acute anthrax with localized edema; septicemia with colic and enteritis sometimes occurs.

- Humans: Three main forms of anthrax are recorded.

  Cutaneous anthrax (malignant pustule): result from entering endospores through abraded skin.

  Pulmonary anthrax ('wool sorter's disease'): following inhalation of spores.

  Intestinal anthrax: results from ingestion of infective material.

**Diagnosis**

To prevent sporulation animals should not be opened. Cremation or deep burial (at least 6 ft.) with lime recommended for disposal.

***Specimens***

Septicemic form (cattle, sheep, horse): Swabs from exuded blood or blood taken by syringe. Blood smears may also be submitted.

Localized form (swine): Fluid aspirated from affected lymphnodes.

***Diagnostic Procedures***

a. **Direct Examination:** Smears from tissues or blood are made and stained by the Gram's method or by Giemsa or Wright's stain. The capsule stains a reddish mauve. The finding of large, square ended gram positive rods suggests the possibility of anthrax.

b. Staining by 1% polychrome methylene blue gives a characteristic differentiation between capsule and body of the organism. The capsule material is stained pale pink and bacillary body (somatic cell) takes blue color. This reaction of the dye is known as Mc Fedyean reaction.

c. **Isolation, cultivation and identification:** The organism grows well on all laboratory media.

   Growth characteristics on:

   i. Blood agar: Weak hemolysis may be seen.
   ii. Nutrient agar: Characteristic 'Medusa-headed' colonies.
   iii. Nutrient broth: Cotton wool growth.
   iv. Gelatin stab culture: Inverted fir tree like growth.

d. Subcutaneous inoculation of suspected material into guineapig or mice results in death within 48 hours. Edema and hemorrhage are found at inoculation site and numerous large capsular bacilli are observed in stained smears from the spleen and blood.

e. Ascoli's precipitation test: A piece of tissue weighing 1 – 2 gms is boiled for 5 minutes in about 5 ml saline. The fluid is filtered and constitutes the antigen. 0.5 ml of this fluid is layered over 0.5 ml of anthrax antiserum in a narrow tube. In positive case, a ring will develop within 15 minutes at the junction of the two fluids.

f. FAT

g. PCR

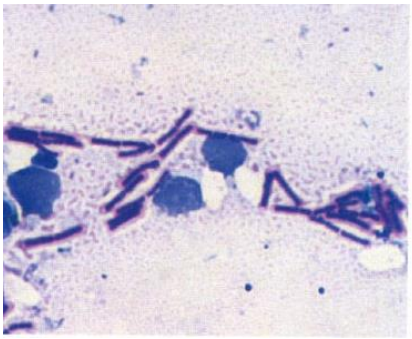


**Fig: *Bacillus anthracis* seen on direct smear**

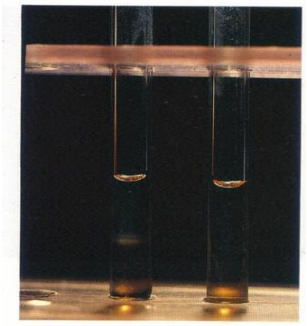


**Fig: Ascoli's precipitation test**
**(Left: Positive, Right: Negative)**

**Control and Prevention**

- Movement of animals, their waste products, feed and bedding from affected and adjacent premises must be prohibited.
- Dead animals, bedding and soil contaminated by discharges should be disposed of either by deep burial in quicklime or by incineration.
- In contact animals should be isolated and kept under close observation for at least 2 weeks.
- Personnel, implementing control measures should wear protective clothing and footwear which must be disinfected before leaving the affected farm.
- Foot-baths containing sporicidal disinfectant (5% formalin or 3% peracetic acid) should be placed at entrances to affected farms.
- Scavenger animals should not be allowed access to suspect carcasses and insect activity should be minimized by application of insecticides on and around carcasses.
- Annual vaccination, particularly of cattle and sheep, is advocated. The Sterne strain spore vaccine should be given about one month before anticipated outbreaks. Protection is afforded in 7 – 10 days and last for about a year. Therefore annual booster vaccination is required. Antibiotics should not be administered to vaccinated healthy animals.
- Chemoprophylaxis employing long-acting penicillin should be considered, when outbreaks threaten valuable livestock.

**Other *Bacillus spp.***

*Bacillus cereus*: Sometimes cause gangrenous bovine mastitis and as well as abortion in cows. *Bacillus cereus* can grow in foods and produce an enterotoxin or an emetic toxin and cause food poisoning (especially after the consumption of Chinese fried rice that has been stored). The organism is also an important cause of eye infections, severe keratitis, endophthalmitis and panophthalmitis.

*Bacillus licheniformis*: An emerging pathogen; cause sporadic abortion in cattle and sheep.

**Differentiation of Some Species of *Bacillus***

| Property | *Bacillus anthracis* | *Bacillus subtilis* | *Bacillus cereus* |
|---|---|---|---|
| Capsule | + | - | - |
| Motility | - | + | Variable |
| Colony characteristics | Medusa headed | Granular round | Variable |
| Gelatin liquefaction | Slow | Rapid | Rapid |
| Urease | - | Variable | Variable |
| Arabinose fermentation | - | + | + |
| Mannitol | - | + | - |

**Public Health Significance**

- Anthrax is a zoonotic disease which constitutes a potential health hazard to persons occupationally exposed to the animals and their products. The disease is most frequently seen in farmers, herdsmen, butchers, veterinarians and tannery workers.
- Cutaneous anthrax accounts for more than 95% of the human disease.

- Sources of spores for humans: Soil, hair, wool (wool sorter's disease); feces, meat (inadequately cooked); blood products.
- Necropsies on animals must be performed with great care.

**Fig: Ring of vesicles surrounding the Developing eschar in anthrax infection**

**Fig: Extensive facial edema around anthrax infection sites**

# Clostridium

**Principal Characteristics**

- Large, gram positive rods.
- Anaerobic.
- Spore forming (produce endospores).
- Most are motile by peritrichous flagella (except *Clostridium perfringens*).
- Catalase and oxidase negative.

**Grouping of Clostridia**

***According to Bergey's Manual***

1. **Spores sub-terminal**
   a. Gelatin not hydrolyzed: Group-I (None associated with animal disease)
   b. Gelatin hydrolyzed: Group-II
      *Clostridium sordelli*
      *Clostridium botulinum*
      *Clostridium novyi*
      *Clostridium chauvoei*
      *Clostridium perfringens*
      *Clostridium haemolyticum*
      *Clostridium septicum*
2. **Spores terminal**
   a. Gelatin not hydrolyzed: Group-III (None associated with animal diseases)
   b. Gelatin hydrolyzed: Group-IV
      *Clostridium tetani*
3. Species with special growth requirements: Group-V (None associated with animal diseases)

***According to the Mode and Sites of Action of Their Potent Exotoxins***

1. **Histotoxic clostridia:** Produce variety of tissue (often muscle) infections and may subsequently cause toxaemia, e.g.
   *Clostridium chauvoei* (Black leg)
   *Clostridium septicum* (Malignant edema)
2. **Hepatotoxic clostridia:** Produce toxins in the liver, e.g.
   *Clostridium haemolyticum* (Bacillary hemoglobinuria)
   *Clostridium novyi* type-B (Black disease)
3. **Enteropathogenic clostridia:** Produce inflammatory lesions in the gastrointestinal tract along with enterotoxaemia, e.g. *Clostridium perfringens* (types A-E) (Enterotoxaemia)
4. **Neurotoxic clostridia:** Produce potent exotoxins (neurotoxins) affecting neuromuscular function without inducing observable tissue damage, e.g.
   *Clostridium tetani* (Tetanus)
   *Clostridium botulinum* (types A-G) (Botulism)

**Ecology**

- Free living saprophytes.
- Distributed widely in soil.
- Some species live in the intestine of animals.

**Mode of Infection**

- Ingestion: *Clostridium chauvoei* (cattle), *Clostridium botulinum, Clostridium perfringens, Clostridium haemolyticum, Clostridium novyi.*
- Wounds: *Clostridium chauvoei* (sheep), *Clostridium septicum, Clostridium tetani.*

**Resistance**

- Endospores of clostridia are very resistant to physical influences and disinfectants.
- It may require 30 minutes boiling or 121°C in autoclave for 20 minutes to kill the spores.

## *Clostridium chauvoei*

Synonym: *Clostridium feseri*

Cause black quarter or black leg in cattle, sheep and other animals.

**Morphology**

- Occur singly or in short chain
- Spore forming; spore oval, sub-terminal
- Motile by peritrichous flagella
- Nonencapsulated
- Gram positive

**Cultural Characteristics**

- On blood agar: Whitish grey irregular colonies of 2 to 4 mm in diameter with a zone of hemolysis.
- On cooked meat medium: Growth is slow; meat is turned pink and has a sour odour.

- In broth: Uniform turbidity forms a flaky white deposit.
- In glucose gelatin shake culture: Colonies appear towards the bottom with a gas bubble and surrounded by a liquefied area.

**Biochemical Properties**

The organism produces acid and gas from glucose, fructose, lactose, sucrose and maltose but does not ferment salicin.

**Toxins**

i. α toxin: Hemolysin, necrotoxin.
ii. β toxin: Deoxyribonuclease.
iii. γ toxin: Hyaluronidase.
iv. δ toxin: Oxygen labile hemolysin.

**Pathogenicity**

- Black leg in ruminants; an acute disease in cattle and sheep.
- In cattle (usually 4 months to 2 years), infection is usually endogenous, the latent spores in muscle becoming activated through traumatic injury.
- The disease may affect the sheep and goat of any age. In many instances, exogenous infection occurs through skin wounds.
- In both cattle and sheep, gangrenous cellulitis and myositis caused by exotoxins produced by the replicating organisms usually lead to rapid death. The muscles of the limbs, back and neck are frequently affected. Skeletal muscle damage is manifested by lameness, swelling and crepitation due to gas accumulation. Lesions in the muscles of the tongue and throat produce dyspnoea. Myocardial and diaphragmatic lesions may cause death.

**Diagnosis**

***Specimen:*** Affected muscles and tissues; exudates from affected parts

***Diagnostic procedures***

i. **Direct examination:** Smears are made from the affected tissues or exudates from affected parts. The finding of gram positive rods, occurring singly or in short chains, sporulated which may be elongated, oval, sub-terminal or terminal suggests the possibility of black quarter.

**ii. Isolation, cultivation and identification**

a. Growth characteristics on different media:
    1. On blood agar: whitish grey irregular colonies with a zone of hemolysis.
    2. On cooked meat medium: meat is turned pink and has a sour odour.
    3. In broth: uniform turbidity forms a flaky white deposit.
    4. In glucose gelatin shake culture: colonies appear towards the bottom with a gas bubble and surrounded by a liquefied area.
b. Study of different biochemical properties
c. Intramuscular inoculation of suspension of infected tissues into guineapig – produces characteristic lesions (48 hours after inoculation) and the organisms can be isolated in pure culture.
d. FAT
e. PCR

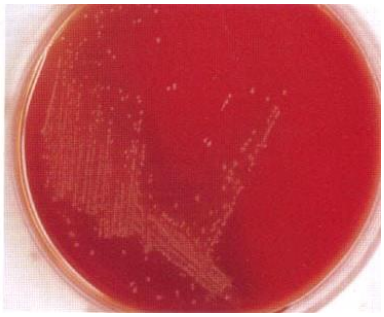


**Fig: Colonies of *Cl. chauvoei* on blood agar**

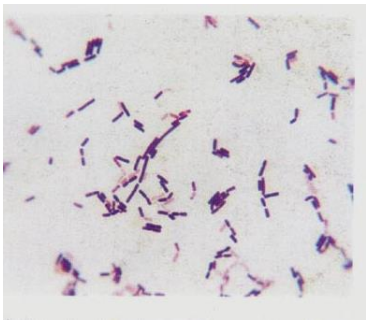


**Fig: *Cl. chauvoei* (Gram's staining)**

**Control and Prevention**

i. Animals that have recovered from an attack of black quarter are immune. Aged and very young animals have considerable immunity.
ii. The dead body should be buried or burnt.
iii. Active immunization is carried out by alum precipitated whole culture vaccine which is formalized.

## *Clostridium septicum*

**Morphology**

- Rod shaped organism with rounded ends
- Occur singly but long chains and filaments are also found
- Spores are oval or subterminal giving cigar shaped form.
- Does not produce capsule and motile due to peritrichous flagella.
- Gram positive

**Cultural Characteristics**

- An obligate anaerobe. Grows well at 37°C in meat infusion media.
- Agar surface: Colonies are granular with filamentous edge
- On the horse blood agar: Produce hemolysis.
- In cooked meat broth: The growth of organism turns meat into pink colored with rancid smell.

**Biochemical Properties**

- Gelatin is slowly liquefied.
- Acid and gas are produced in glucose, fructose, galactose, maltose, lactose and salicin but not from sucrose, mannitol and glycerol.
- Methyl red and Voges Proskauer negative; $H_2S$ positive.

**Toxins**

i. α toxin: Lethal, lecithinase, necrotizing, hemolytic.
ii. β toxin: Deoxyribonuclease, leukocidal.
iii. γ toxin: Hyaluronidase.
iv. δ toxin: Hemolysin, necrotizing factor.

**Pathogenicity**

- Cattle, sheep, horse, pig and occasionally other animals: Malignant edema or gas gangrene. Infection can follow contamination of wounds, parturition injuries or injection sites. There is a large expanding swelling involving skeletal muscles: pits on pressure, gelatinous and red, little gas is formed.
- Sheep: Braxy; associated with the eating of frozen succulent feed; necrotic lesions and hemorrhagic edema of the abomasal and duodenal walls.
- Chickens: Gangrenous dermatitis.

**Diagnosis**

***Specimen:*** Material from wounds, pus, tissue.

***Diagnostic Procedure***

1. **Direct examination:** The presence of gram positive, spore forming rods in gram stained smears suggests malignant edema clostridia.
2. **Isolation and identification of the organism from the lesion**
    a. Growth characteristics on:
        i. Agar surface: Colonies are granular with filamentous edge
        ii. On the horse blood agar: produce hemolysis
        iii. In cooked meat broth: The growth of organism turns meat into pink colored with rancid smell.
    b. Guineapig inoculation: Intramuscular injection of active growing culture into the thigh muscle cause death of guineapig within 24 – 48 hours with an excessive gas gangrene lesion; rabbits are resistant.
3. FAT

**Fig: *Clostridium septicum***

**Control and Prevention**

- Proper cleanliness and hygienic measures will reduce the chance of infection.
- Both surgical and accidental wounds should be properly treated.
- Animals can be immunized by alum precipitated whole culture vaccine which is formalized.

## *Clostridium haemolyticum*

**Synonym:** *Clostridium novyi* type-D

**Disease:** Bacillary haemoglobinuria/Red water/ Infectious icterohaemoglobinuria

**Morphology**

- Rod shaped with rounded ends.
- Occurs singly or in chain form.
- Spores are oval or slightly elongated and situated subterminally.
- Motile by means of peritrichous flagella.
- Capsules are not formed.
- Gram positive.

**Cultural Characteristics**

- The organism is strictly anaerobic.
- On the surface of agar slants: A light growth is present in 24 hours. Colonies at first are homogenous and lenticular, but rapidly develop an eccentric fluff, later appearing as dense, woolly masses with short peripheral filaments.
- In broth: A complete clouding is present.

**Biochemical Properties**

- Produce acid and gas from glucose and fructose; but not from mannose, maltose, lactose and sucrose
- Gelatin is liquefied.
- Methyl red and Voges Proskauer tests are negative.

**Toxins**

β toxin: A lecithinase C which is lethal, necrotizing and causes hemolysis.

**Pathogenicity**

- Causes bacillary hemoglobinuria or red water in cattle and occasionally in sheep.
- Gaining entrance by ingestion, the organism is reaching the liver hematogenously and remains latent until an appropriate anaerobic environment allows its multiplication at this site. The migration of liver flukes plays an important role for the germination of spore. Liver flukes result in infarction of branches of the portal vein; the organism can germinate and grow in the damaged anaerobic tissue, where it produces its toxin.
- Death is apparently brought about by lysis of erythrocytes by the toxin and the animal perishes of anoxia.

**Diagnosis**

- Specimen: Affected liver tissue.
- Direct smears made from the periphery of liver lesions will show a number of gram positive bacilli with subterminal spores.
- Isolation and identification of the organism.
- FAT

**Control and Prevention**

- Elimination of liver fluke through destruction of the snails.
- Active immunity by vaccination (Formalin killed precipitated vaccine).
- Immunity is of relatively short duration and animals at risk should be revaccinated every six months.
- Carcass should be burried or burnt and the premises should be disinfected properly.

## *Clostridium novyi*

**Synonym:** *Clostridium oedematiens*

**Morphology**

- Large rod shaped.
- The edges of the organism are parallel and ends rounded.
- The organism occurs singly or in pairs and in long jointed filaments.
- Spores are oval and situated subterminally.
- Capsules are not produced.
- Motile due to peritrichous flagella. Gram positive.

**Cultural Characteristics**

- The organism does not grow well on ordinary media; glucose and blood enhance the growth.
- On agar plate: The colonies are flat with irregular edges.
- On horse blood agar: An area of hemolysis develops.
- In cooked meat medium: The growth is slow and meat is turned pink.
- In broth: Slight turbidity followed by flocular sediment.

**Biochemical Properties**

- Gelatin is liquefied.
- Ferments glucose and maltose with the production of acid and gas, but does not ferment lactose.
- Indole, methyl red and Voges Proskauer test are negative.

**Toxins**

α toxin: Lethal, necrotizing; causes increased capillary permeability.

β toxin: Lecithinase.

γ toxin: Necrotizing.

δ toxin: Hemolysin.

ε toxin: Hemolytic enzyme.

*Clostridium novyi* is divided into four types: A, B, C and D, on the basis of toxins produced by the organism.

Type-C synthesize five toxins – α, β, γ, δ, ε.

Type-A produces all toxins except β.

Type-B produces α and β toxins.

Strains of A and B are recovered from the liver of normal animals.

**Pathogenicity**

- Type-A: Causes gas gangrene in sheep, cattle and man; found in mixed infection with *Clostridium chauvoei* or *Clostridium septicum*. Also cause "big head" in rams characterized by significant swelling of the head and neck that is edematous in nature. The condition is developed due to fighting.
- Type-B: Causes black disease or infectious necrotic hepatitis in sheep and occasionally in cattle. The mode of infection is oral with the organism being carried to the liver via blood. Due to migration of liver fluke, there is heavy destruction of liver tissues, an anaerobic condition is created and thus the organisms get the opportunity to proliferate and liberate toxin. The toxin (α toxin) produced in the local lesion is absorbed into the circulating blood and eventually producing death. Recovery is rare. Intense congestion of blood vessels of the skin may result in blackening of the skin.
- Type-C: Causes osteomyelitis in water buffaloes.

**Diagnosis**

- Specimen: Affected liver tissue.
- Direct smears made from the periphery of liver lesions will show a number of gram positive bacilli with subterminal spores.
- Isolation and identification of the organism.
- FAT

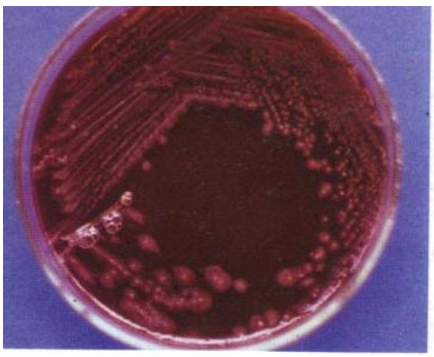


**Fig: Colonies of *Cl. novyi* on blood agar**

**Control and Prevention**

1. Elimination of liver fluke through destruction of snails.
2. Immunizing the animals by giving formalinized whole culture vaccine. The immunity lasts for one year.
3. The outbreaks can be controlled by giving hyperimmune serum.
4. Dead animals should be properly disposed.

## *Clostridium perfringens*

**Synonym:** *Clostridium welchii*

**Morphology**

- Occurs singly but may be found in pairs, short chains.
- Non motile.
- Spores are large, oval, located centrally or subterminally.
- Gram positive.

**Cultural Characteristics**

- Grows on ordinary laboratory media at 37°C. Glucose and blood enhance the growth.
- Colonies on nutrient agar: Round, smooth with entire edge.
- On blood agar: A narrow zone of complete hemolysis and a wider zone of partial hemolysis.
- In cooked meat medium: The meat become pink and is not digested.

**Biochemical Properties**

- Produce acid and gas from glucose, fructose, lactose, sucrose and maltose and not from salicin, mannitol and dulcitol.
- Negative for VP, MR and indole.

**Some Important Toxins and Enzymes of *Clostridium perfringens***

The species is divided into types A to E on the basis of toxins:

***1.*** α (Alpha) toxin: Principal lethal toxin produced in varying amount by all types of *Clostridium perfringens*; hemolytic and necrotizing; possessing the ability to split lecithin or lecithin-protein complexes.

***2.*** β (Beta) toxin: Produced by types B and C; lethal, responsible for inflammation of the intestine.

***3.*** ε (Epsilon) toxin: Produced by types B and D as a protoxin (slightly toxic). Converted to toxin by proteolytic enzyme such as pepsin and trypsin – necrotizing, highly lethal.

***4.*** θ (Theta) toxin: Lethal, hemolytic; produced by types A, B, C, D and E.

***5.*** ι (Iota) toxin: produced only by type E is formed as a protoxin; activated by proteolytic enzyme.

***6.*** κ (Kappa) toxin: Produced by all types. Proteolytic enzyme which breaks down collagen, responsible for softening and pulping of affected muscles.

***7.*** λ (Lambda) toxin: Proteolytic enzyme produced by types B and E and by some strains of type D. It is proteinase and gelatinase.

***8.*** μ (Mu) toxin: Produced by types A and B; hyaluronidase.

***9.*** Nu toxin: Produced by all types except B; Deoxyribonuclease.

**Principal Disease Conditions Caused by *Clostridium perfringens***

| ***Clostridium perfringens*** | **Toxins** | **Disease** |
|---|---|---|
| Type-A | α | Gas gangrene in humans and animals.<br>Enterotoxaemia in lambs (Yellow lamb disease), cattle, goats.<br>Colitis in horse.<br>Necrotic enteritis in chickens.<br>Necrotizing enterocolitis in pigs. |
| | Enterotoxin | Human food poisoning.<br>Canine haemorrhagic gastroenteritis. |
| Type-B | α<br>β<br>ε | Lamb dysentery, occasionally neonatal haemorrhagic enteritis in calves, foals.<br>Haemorrhagic enterotoxaemia in adult sheep and goats. |
| Type-C | α<br>β | Enterotoxaemia (necrotic enteritis) in lambs, goats, cattle.<br>'Struck' in adult sheep.<br>Sudden death in goats and feedlot cattle. |

| | | |
|---|---|---|
| | | Necrotic enteritis in chickens.<br>Haemorrhagic enteritis in neonatal pigs. |
| Type-D | α<br>ε | Pulpy kidney disease (overeating disease) in sheep.<br>Enterotoxaemia in calves, adult goats and kids. |
| Type-E | α<br>ι | Haemorrhagic enteritis in calves.<br>Enteritis in rabbits. |

**Diagnosis**

- Specimens: Fresh, small intestinal contents.
- Direct smears from the mucosa or contents of the small intestine of recently dead animals, which contain large numbers of thick gram positive rods, are consistent with clostridial enterotoxaemia.
- Isolation and identification of the organism.
- All toxigenic strains of *Clostridium perfringens* produce the lethal alpha toxin (lecithinase C) which can be identified by the Nagler reaction. For this reaction, antitoxin with specificity for the alpha toxin is applied to the surface of one half of an egg yolk agar plate and allowed to dry. *Clostridium perfringens* is streaked across the plate which is incubated anaerobically at 37°C for 24 hours. Although the organism grows on both halves of the plate, lecithinase activity is evident only on the half without antitoxin.
- The different types of *Clostridium perfringens* are identified by carrying out neutralization test for lethal or necrotizing activities of the four major lethal toxins namely alpha, beta, epsilon and iota. The lethal toxicity of the intestinal fluid is carried out by collecting intestinal contents from the dead animals immediately after death and chloroform is added as preservative. The sample is diluted with saline and centrifuged. To activate epsilon and iota toxins, the supernatant fluid is heated with 0.05% crystalline trypsin for one hour at 37°C after adjusting $p^H$ 7.0 or slightly alkaline. The fluid, 0.2 ml is injected i/v into the tail of two mice and lethal activity is observed. The death due to toxaemia takes place within 4-6 hours but if not, the mice are observed for several days. When the material is found to be toxic, 0.5 ml of fluid is mixed with 0.2 ml of antitoxin and mixture kept for 1 hour at room temperature. The mixture in 0.3ml volume is injected i/v into mice. The mice are observed for neutralization of lethal activity of the toxin for about 24 hours.

**Toxin neutralization tests, in mice or guinea-pigs, for identifying the types of Clostridium perfringens implicated in enterotoxaemias**

| **Antitoxin (specificity)** | **Test result** | | | | |
|---|---|---|---|---|---|
| | **Toxins identified in intestinal contents** | | | | |
| | **α** | **α, β, ε** | **α, β** | **α, ε** | **α, ι** |
| Type A (anti- α) | - | D | D | D | D |
| Type B (anti- α, β, ε) | - | - | - | - | D |
| Type C (anti- α, β) | - | D | - | D | D |
| Type D (anti- α, ε) | - | D | D | - | D |
| Type E (anti- α, ι) | - | D | D | D | - |

- ELISA

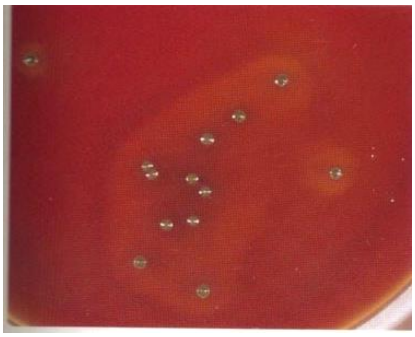


**Fig: Colonies of *Cl. perfringens* on blood agar**

**Control and Prevention**

- Active immunization by formalized alum precipitated vaccine.
- Immunity may not persist for more than 6 to 12 months unless booster injections are given.
- Passive immunization by hyper immune serum is protective and for no longer than 2 to 3 weeks.
- Good management and feeding practices are important in prevention.

## *Clostridium tetani*

**Occurrence**

Normal flora of the soil, intestine of horses and other animals.

**Morphology**

- Long slender rod, end of the organisms are rounded.
- The spores are situated terminally, giving a 'drum stick' appearance.
- Non capsulated.
- Gram positive.

**Cultural Characteristics**

- The organism requires strict anaerobic condition. Grows well in liquid media in which meat particles have been added.
- On agar plate: Colonies are irregular and spreading.
- In gelatin stabs: typical 'fir tree' like growth.

**Biochemical Properties**

- Gelatin is slowly liquefied.
- Carbohydrates are not fermented.

**Toxins**

Two toxic substances are produced:

***1.*** Tetanolysin: is responsible for areas of hemolysis around the colonies of blood agar.

***2.*** Tetanospasmin or neurotoxin: is highly toxic when injected parenterally, however, it is harmless if administered by mouth. Animals vary in their susceptibility to the toxin: horses and man are the most susceptible while cats and poultry are the most resistant. 1 mg of pure toxin contains $10^6$ mouse lethal doses. Toxin is elaborated at the site of infection and passes directly to major nerves and then to the spinal cord. It may also travel via blood and lymph. The important

action of the toxin is the inhibition of post synaptic spinal neuron by blocking the release of inhibitory mediator. This results in hyper reflexia and muscle spasms which may be generalized and is characterized by rigidity in all limbs, dyspnoea, spasms of the masticatory and pharyngeal muscles, elevation of the tail. Opisthotonus and death may result from respiratory failure.

**Pathogenicity**

Spores germinate usually in dirty and neglected wounds with some necrosis. Toxin is elaborated at the wound site after spores germinate. Docking and castration wounds, umbilical infections (tetanus neonatorum), parturition (puerperal tetanus) and dehorning are among the circumstances that can contribute to tetanus.

Tetanus may be ascending or descending based on the movement and distribution of the toxin. Descending tetanus is the most common form seen in horses and humans. The first sign to appear in horses involves protrusion of the nictating membrane followed by involvement of muscles of the fore and hind limbs.

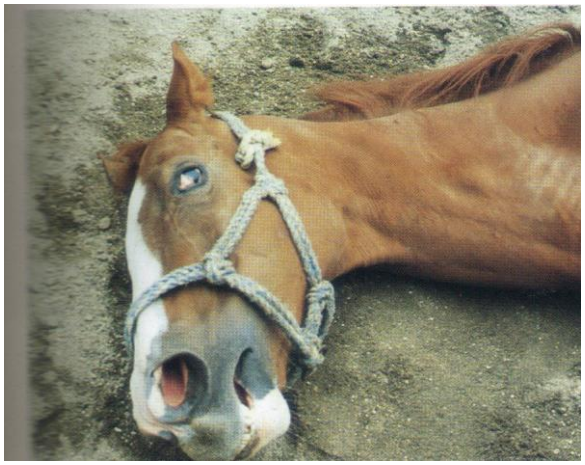


Fig: Diseased horse showing generalized tetany and exposure of the nictating membrane

Fig: Diseased horse showing opisthotonus

**Diagnosis**

1. By clinical symptoms.
2. Specimen: Material from wound site.
3. Growth in gelatin: typical fir tree, carbohydrates are not fermented.
4. Mice inoculation: s/c; i/m; typical symptoms of tetanus are produced.

**Prevention and Control**

1. Tetanus toxoid is given to horses, sheep, cattle including human.
2. Sheep may be given a multicomponent vaccine derived from several species of Clostridia.

## *Clostridium botulinum*

**Morphology**

- Occurs singly or in pairs and sometimes in chains.
- Spores are oval, situated terminally or subterminally.
- Motile due to peritrichous flagella.
- Noncapsulated.
- Gram positive.

**Cultural Properties**

- On solid media: Colonies are large and semitransparent with irregular edge.
- On horse blood agar: Hemolytic.
- Cooked meat medium: Meat is digested and blackened.

**Biochemical Properties**

- Liquefy gelatin.
- Ferment glucose and maltose.

**Toxins**

- Exotoxins of *Clostridium botulinum* are heat labile proteins (100°C for 10 minutes).
- Eight types of neurotoxins (A, B, Cα, Cβ, D, E, F & G) have been identified on the basis of antigenic differences.
- 1 mg of neurotoxin contains more than 120 million mouse lethal doses. Less than 1µg of toxic polypeptide preparation is lethal for men.
- The toxins usually produced in foods are absorbed from the intestinal tract. The toxin is released when the organisms die and undergo lysis. Unlike most other toxins, they are resistant to peptic and tryptic digestion. After absorption, the toxin is transported to susceptible neurons via the blood stream. It appears to be specifically directed to the peripheral nerves and does not affect other body cells. It prevents the passage of impulses from the nerve to the muscle. The action may be concerned with inhibition of release of acetylcholine. Paralysis is ascending and death is caused by circulatory failure and respiratory paralysis, as a result of the action of the toxin on motor nerves.

**Pathogenicity**

Generally botulism is an intoxication resulting from the ingestion of food in which *Clostridium botulinum* has grown and produce toxin. The principal media for the production of botulinum toxins are various spoiled foods, e.g. canned vegetables, meat and fish. The toxin may also be produced in animal carcasses.

Type-C botulism (sometimes type-A and type-E): Occurs in cattle, sheep, turtles, chickens (‘Limberneck’ – flaccid paralysis of neck) and wild fowl that have eaten rotting vegetation.

Type-Cα – Birds and turtles

Type-Cβ – Cattle, sheep and horses

Type-D botulism: Causes ‘Lamziekte’ or ‘Loin disease’ in cattle with pica (phosphorus deficiency) in South Africa, Texas, South America. The toxin is produced in bones and tissues of dead animals as a result of the growth of *Clostridium botulinum* in carcasses. Hungry animals eat toxin-containing bones and tissues.

Type-A, B, E and F have been reported most commonly in humans.

**Toxico infection botulism**: In this case, *Clostridium botulinum* grows in tissues of living animals and produce toxins in there. Such type of condition is produced in foals and horses known as 'Shaker foal syndrome' (gastric ulcer, foci of necrosis in the liver, wound etc.). This disease condition appears to resemble wound botulism in man (Type-A and B).

**Diagnosis**

**Specimen:** Suspected food, meat, urine and serum

- Demonstration of toxin in food staff.
- Materials are macerated in saline. The fluid is centrifuged and injected (2 ml intraperitoneally) into a number of guinea-pigs to determine the presence of toxins. At the same time, a control group is given 2 ml of heated extract (10 minutes at 100°C) intraperitoneally. Guinea pigs in the antitoxin groups receive a mixture of unheated extract and antitoxins of known type. The presence within 5 days of flaccid paralysis followed by death in unprotected animals suggests the presence of botulinal toxin.
- Toxin can be demonstrated by FAT.
- ELISA
- PCR

**Control and Prevention**

- 'Lamziekte' in cattle is prevented by giving to injections of type-C and D toxoid at an interval of seven weeks.
- Access to contaminated feeds and carcasses are to be avoided as far as practicable.
- Cattle and sheep grazing phosphorus deficient pasture should be provided with adequate phosphorus.

**Production, Mode of Action and Effects of the Neurotoxins of *Clostridium tetani* and *Clostridium botulinum***

| Feature of neurotoxin | *Clostridium tetani* | *Clostridium botulinum* |
|---|---|---|
| Site of production | In wounds | In carcasses, decaying vegetation, canned foods. Occasionally in wounds or in intestine (toxico-infections) |
| Genes which regulate production | In plasmids | Usually in genome |
| Antigenic type | One antigenic type (tetanospasmin) | Eight antigenically distinct toxins, types A to G. |
| Mode of action | Synaptic inhibition | Inhibition of neuromuscular transmission |
| Clinical effect | Muscular spasms | Flaccid paralysis |

**Other Clostridia**

*Clostridium bubalorum*: Osteomyelitis in buffaloes.

*Clostridium sordelli*: Gas gangrene and enterotoxaemia in cattle.

*Clostridium colinum*: Acute and chronic ulcerative enteritis of quail.

# Corynebacterium

**Principal Characteristics**

- Small, pleomorphic rods, some species are club shaped.
- Non motile, non-sporeforming.
- Some species contain phosphate granules (metachromatic granules) and stained red purple with methylene blue.
- Gram positive.
- Aerobic or facultative anaerobic and fermentative.
- The cells often remain attached after division which gives an arrangement of 'Chinese letters'.
- Catalase positive, oxidase negative.

## *Corynebacterium renali*

**Morphology**

- Gram positive, pleomorphic.
- Occurs singly but clump formation is common.
- Contain metachromatic granules.
- Non motile, non capsule producing.

**Cultural Characteristics**

- On nutrient agar/Blood serum agar: Colonies are small, dew drop like.
- On blood agar: No hemolysis.
- In broth: Powdery deposit collects on the walls and bottom of the tube.

**Biochemical Properties**

- Glucose is fermented with production of acid.
- Indole is not formed, does not reduce nitrates to nitrites and does not form $H_2S$.
- Methyl red and Voges Proskauer tests are negative.

**Habitat**

*Corynebacterium renali* has been recovered from the normal bovine female and male genital tracts.

**Mode of Infection**

Infection is transmitted venerally and by contaminated urine. The adherence of *Corynebacterium renali* and probably of *Corynebacterium pilosum* and *Corynebacterium cystitidis*, to the mucous membrane of the urogenital tract may be facilitated by pili.

***Corynebacterium renali* Group**

Three immunologic types based on the recognition of different surface antigens by agar diffusion precipitin test.

Type-I: *Corynebacterium renali*

Type-II: *Corynebacterium pilosum*

Type-III: *Corynebacterium cystitidis*

**Differentiation of Bacteria in The *Corynebacterium renali* Group**

| Feature | *Corynebacterium renali* (type-I) | *Corynebacterium pilosum* (type-II) | *Corynebacterium cystitidis* (type-III) |
|---|---|---|---|
| Colour of colony | Pale yellow | Yellow | White |
| Growth in broth at pH 5.4 | + | - | - |
| Nitrate reduction | - | + | - |
| Acid from xylose | - | - | + |
| Acid from starch | - | + | + |
| Casein digestion | + | - | - |
| Hydrolysis of Tween 80 | - | - | + |

**Pathogenicity**

- The organism causes pyelonephritis, ureteritis and cystitis in cattle (minor- sheep, horse, dog, human etc.). Infection is not hematogenously but is ascending from the ureter and urethra. The organism has a predilection site in the kidneys because of high urease activity. The urease is nephrotoxic and produces pyelonephritis. In affected cows there is frequent passage of turbid or blood stained urine. Urine contains RBC, pus cells, albumen. Kidneys are enlarged and contain areas of necrosis and suppuration in medulla.

  The infection is ascending and involves the bladder, ureter and one or both kidneys in a severe pyogenic inflammatory process.
- **Sheep/Goat:** Ulcerative balanoposthitis (posthitis is thought to result from the inflammation caused by the ammonia resulting from urease produced by *Corynebacterium renali*).
- **Swine:** Kidney abscessation.

**Diagnosis**

- ***Specimen:*** A mid-stream sample of urine from live animal; affected kidney, ureter, bladder and urethra from necropsied animal.
- ***Direct Examination:*** Gram-stained urine smears from purulent urine disclose clumps of short, pleomorphic, gram positive rods.
- ***Isolation and Cultivation:*** Urine samples are usually plated on blood agar and incubated aerobically at 37°C. Young colonies are initially small which subsequently become opaque and ivory colored as they enlarge.
- Absence of growth on MacConkey agar

## *Corynebacterium pseudotuberculosis*

**Synonym**: *Corynebacterium ovis*

**Habitat**

The organism occurs on normal skin and mucous membrane.

**Mode of Infection**

The organism most commonly enters abrasions resulting from shearing injuries. Occasionally, infections occur as a result of inhalation or ingestion.

**Pathogenicity**

*Corynebacterium ovis* causes caseous lymphadenitis in sheep and goats, abscess and chronic lymphadenitis in wild ruminants, camels and ulcerating lymphangitis in horses. Folliculitis and pectoral abscess in horses and purulent arthritis in lambs have been reported.

In horse, the organism causes ulcerative lymphangitis. The animal shows signs of pain and swelling of hind limbs. The lymphatic vessels and lymphnodes enlarge and ulceration occurs. In severe cases, the disease spreads to abdomen, forelegs and neck, causing death of animal.

**Diagnosis**

- ***Specimen:*** Pus
- Gram stained smears from specimens may reveal gram positive, pleomorphic rods.
- ***Isolation and Cultivation:*** The organism grows well on blood agar aerobically at 37°C. Colonies are initially small, later large, become dry and crumbly and turn cream to orange in color. Complete haemolysis is seen on blood agar. On blood agar, *Corynebacterium ovis* inhibits staphylococcal β toxin but results in a synergistic haemolysis with *Rhodococcus equi*.

**Other Corynebacteria**

*Corynebacterium suis (Eubacterium suis)*: Cause Pyelonephritis and cystitis in pigs

*Corynebacterium bovis*: Mild mastitis in cattle.

*Corynebacterium haemolyticum*: Pharyngitis, skin infections in humans.

*Corynebacterium diphtheriae*: Diphtheria in children.

*Corynebacterium ulcerans*: Mastitis in cattle.

# Rhodococcus

**Principal Characteristics**

- Gram positive, weakly acid fast organisms that assume coccoid and bacillary forms.
- Capsule producing and non-motile.
- Grow aerobically and non-fermentative.
- Catalase and urease positive, and oxidase negative.

# *Rhodococcus equi*

**Synonym**: *Corynebacterium equi*

**Habitat**

- Inhabitant of both soil and intestinal tract of animals.
- Persists for long periods in the manure and litter of stables.

**Mode of Infection**

Infection may result from direct contact with, or inhalation, of contaminated soil, manure, infectious secretions or feces.

**Cultural Characteristics**

- On nutrient agar: Colonies are large, moist and pale pink in color.
- In nutrient broth: Heavy turbidity and little sediment.
- Blood agar: No hemolysis.

**Biochemical Properties**

- Does not ferment sugars.
- Reduces nitrates to nitrites.
- Urease positive.
- Does not liquefy gelatin; Indole, Methyl Red and Voges Proskauer tests are negative.

**Pathogenicity**

- ***Foals of 1 to 4 months of age:*** Produces suppurative bronchopneumonia characterized by large, pulmonary abscesses; abscesses may also be found in the mediastinal lymphglands and lymphglands of peritoneal cavity.
- ***Horses:*** Superficial abscessation.
- ***Swine:*** Submandibular and cervical lymphadenitis.
- ***Pigs, cattle:*** Cervical lymphadenopathy.
- ***Cats:*** Subcutaneous abscesses, mediastinal granulomas.

**Diagnosis**

- ***Specimen:*** Tracheal aspirates, portions of lung and lymphnodes containing abscesses or swabs from excised abscesses, nasal swab
- ***Direct Examination:*** Gram positive, usually coccoid, occasionally rod shaped organisms are seen in pus.
- ***Isolation and cultivation:*** The organism grows well on blood agar aerobically at 37°C. In 48 hours colonies are smooth, mucoid, translucent. On some media, colonies have a salmon to red pigment, but on blood agar colonies appear grayish-white.
- CAMP test positive. *Rhodococcus equi* produces a factor which completely lyses the red cells previously damaged by the β haemolysin of *Staphylococcus aureus*, producing a spade shaped pattern of complete haemolysis which extends across the streak of *Staphylococcus aureus*.
- Absence of growth on MacConkey agar.

**Control**

- No commercial vaccines are available.

- Foal manure should be removed from pastures at frequent intervals.
- Foals and their dams should be moved regularly to fresh pasture.
- Hyperimmune serum from the dam, administered to the foal in the first month of life, is claimed to reduce the prevalence of disease.

**Public Health Significance**

- *Rhodococcus equi* cause infrequent infections in immunocompromised adults (AIDS related) with deficient cell mediated immunity.
- Necrotizing pneumonia, abscesses, osteomyelitis, peritonitis and septicemia have been reported.

# Listeria

**Species**

*Listeria monocytogenes*

**Principal Characteristics**

- Small rod shaped with rounded ends; may appear in chain.
- Motile with peritrichous flagella.
- Non spore-forming, generally non-capsulated.
- Gram positive.
- Catalase-positive, oxidase-negative

**Cultural Properties**

- Facultative anaerobic.
- On blood agar- small colonies surrounded by a narrow zone of ß hemolysis.
- On tryptose agar- colonies of the organism have fine texture surface and distinct blue green color.
- In broth - a slight turbidity is developed and granular sediment is formed.

**Biochemical Properties**

- *Listeria monocytogenes* produces acid from glucose, ramnose and salicin within 24 hours after inoculation.
- Produces acid in sucrose, maltose, lactose, and starch in 7 to 12 days.
- Does not produce indole, does not reduce nitrates or form $H_2S$.
- Does not liquefy gelatin.

**Antigenecity**

- On the basis of somatic (O) and flagellar (H) antigens, 4 principal serologic groups and 11 serotypes have been identified. Somatic antigens are heat stable and flagellar antigens are heat labile.

**Habitat**

- Soil, feces, genital secretions and nasal mucus of apparently healthy animals and in silage.
- Raw and processed foods, including meat, sea food, vegetables and dairy products.

**Mode of Infection**

- Neural: Infection is via branches of the trigeminal nerve or probably via the eye, nose and oropharynx.
- Visceral: Through ingestion.

**Resistance**

- Pasteurization (62°C for 30 min; 71.6°C for 15 to 30 sec) destroys *Listeria monocytogenes*.
- Susceptible to common disinfectants.

**Pathogenicity**

*Listeria monocytogenes* produces natural infection in a variety of host – cattle, sheep, goat, buffaloes, swine, chickens, horses, man and others. The organism has predilection for certain tissues like brain, gravid uterus, liver and spleen. In adult sheep, cattle and pigs, lesions are confined to central nervous system where marked meningitis is produced. In young animals it may be septicemia. Infection of fetus results in death of fetus and abortion.

In cattle and sheep, the first signs of encephalitis are stiffness of neck and head and head is held on one side, incordination of limbs and animals moves in circles. For this reason, neural form of listeriosis is sometimes called circling disease.

In chickens, extensive necrosis of myocardium is produced. In man, meningitis and abortion is produced.

**Diagnosis**

***Specimens***

Neural form: Cerebrospinal fluid (CSF) and tissue from the pons and medulla.

Visceral form: Portions of affected organs.

Abortion: Cotyledons, foetal abomasal contents and uterine discharges.

***Isolation and Cultivation***

1. Small, Gram positive rods occurring singly, in pairs or in short chains are seen in stained smears (smears from liver lesions or cotyledons).
2. Material is streaked on the solid media (Blood agar). Smooth colonies are rounded, glistening and bluish green by transmitted light; narrow zones of ß hemolysis are evident.
3. A cold enrichment procedure is necessary for isolating the organism from brain tissue. Small pieces of medulla are homogenized and a 10% suspension is made in nutrient broth. The suspension is held at 4°C in a refrigerator and subcultured weekly onto blood agar for up to 12 weeks. The mechanism of enhancing effect at 4°C is not understood. Cultural procedures may be indicated by the finding of microscopic lesion in the brain characteristics of listeriosis.
4. A polyvalent fluorescent labeled antibody is available for the identification of *Listeria monocytogenes.*
5. CAMP test is positive with *Staphylococcus aureus*.
6. Isolates of animal origin which are virulent can be confirmed by animal inoculation. Instillation of a drop of broth culture into the eye of a rabbit induces keratoconjunctivitis (Anton test).

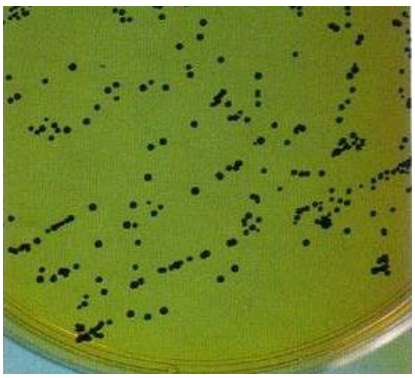


**Fig: Colonies of *Listeria monocytogenes***

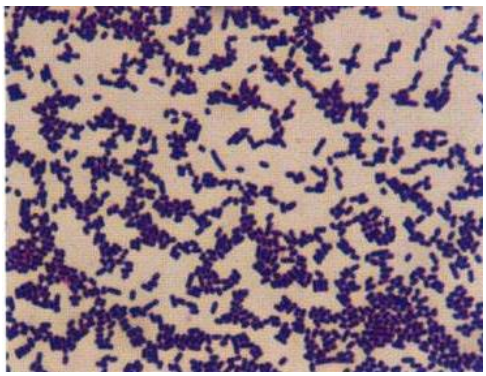


**Fig: *Listeria monocytogenes* (Gram staining)**

**Control**

- Poor-quality silage should not be fed to pregnant ruminants. Silage feeding should be discontinued if an outbreak of listeriosis is confirmed.
- Feeding methods which minimize direct ocular contact with silage should be implemented.
- Vaccination with killed vaccines, which do not induce an effective cell mediated response, is not protective because *Listeria monocytegenes* is an intracellular pathogen. Live, attenuated vaccines, which are available in some countries, are reported to reduce the prevalence of listeriosis in sheep.

**Public Health Significance**

- Infections in humans are frequently opportunistic, involving mainly immunocompromised and immunologically immature individuals.
- In humans, causes meningitis and encephalitis, uterine infection with abortion, stillbirth and a neonatal septic form called granulomatosis infantiseptica, valvular endocarditis, febrile pharyngitis and septicaemia.
- The possible sources of human infections are soil, animals, contaminated milk, cheese, meat, some vegetables and human carriers.
- Several cases of bovine mastitis caused by this organism have been reported. Unpasteurized cow's milk yielding the organism is a potential source of human infection.

**Other species**

*Listeria ivanovii*: Causes abortion in sheep, cattle.

# Erysipelothrix

**Species:** *Erysipelothrix rhusiopathiae*
**Synonym:** *Erysipelothrix insidiosa*

## *Erysipelothrix rhusiopathiae*

**Principal Characteristics**

- Gram positive.
- Small rods (smooth form) or filaments (rough form).
- Non motile, non spore-forming.
- Catalase-negative.

**Habitat**

- Mucous membrane of normal swine and some other animals.
- Slime on the bodies of fresh water and salt water fish.
- Multiply during warm months in alkaline soil and survive for several weeks in soil.
- Carrier pigs are the primary reservoir of the organism.

**Mode of Transmission**

- Direct contact with infected pigs and fomites.
- By ingestion of contaminated food and water.
- Through fish meal.

**Cultural Characteristics**

- Facultatively anaerobic.
- The organism grows on nutrient agar. Growth of this organism is enhanced by addition of serum or blood.
- On blood agar- non hemolytic, pin point colonies appear after incubation for 24 hours and after 48 hours, a narrow zone of greenish, incomplete hemolysis develops around the colonies.
- In broth uniform turbidity with no pellicle and little sediment is formed.
- In gelatin stab culture, bottle brush type of growth is found.

**Biochemical Properties**

- Produce acid fermenting lactose, glucose, fructose and galactose.
- Does not form indole, negative to Voges Proskauer and Methyl Red tests.

**Antigenic Nature**

- On the basis of difference in somatic antigens, three types A, B, N.
- Type A: Principal cause of acute disease in swine. Type B/Type N: associated with chronic diseases.
- Based on peptidoglycan antigens 23 different serotypes have been recognized. In affected pigs, the most common serotypes are 1a, 1b and 2.

**Resistance**

- Resistant and survives for about a year in putrefying meat.
- Killed at $70^0$C in 5-10 minutes.
- Resistant to alcohol, $H_2O_2$, formaldehyde and phenol.
- Susceptible to caustic soda and bichloride of mercury.

**Pathogenicity**

***Pigs:*** Swine erysipelas

- Age range: 3 to 18 months; but animals of all ages are susceptible.
- The disease occurs in four forms-

  **Acute:** In acute septicemic form- course is short and the mortality is high. Area of haemorrhage appears on the base of ears, surface of abdomen and inside the thigh.

  **Skin or urticarial form:** Raised diamond or square- shaped red to purple areas; hence called 'diamond skin disease'. This lesion later becomes gangrenous resulting in large scabs and sloughing takes place.

  **Arthritic form:** Lameness; stifle and hock joints often involved; progresses to a marked periarticular fibrosis.

  **Cardiac form:** Characterized by valvular endocarditis. Progressive and accompanied by dyspnea, passive congestion with resulting skin discoloration of extremities, debilitation, stunted growth and sudden death due to heart failure.

***Sheep:*** Non suppurative polyarthritis in lambs; the organisms gain entry through unhealed navel and wounds; post dipping lameness, pneumonia, valvular endocarditis.

***Turkeys:*** Septicemia, arthritis, valvular endocarditis.

***Dog:*** Valvular endocarditis.

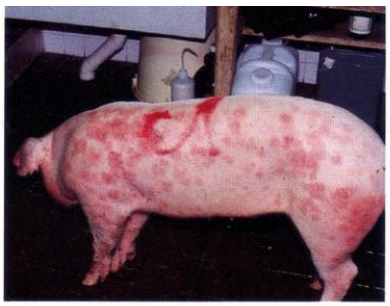


**Fig: Diamond skin lesions on the skin of the affected pig**

**Diagnosis**

***Specimens***

1. Acute or septicemic form: Blood and blood smears from live animal; liver spleen, heart blood from necropsied animals.
2. Arthritic and cardiac form: Swabs from affected joints and affected tissues.

***Isolation and Cultivation***

- The organism grows readily on media enriched with serum or blood. 5-10% $CO_2$ stimulates growth.
- Two kinds of colonies are seen: smooth colonies are small, smooth and round; rough colonies are larger with irregular borders. α-hemolysis is usually seen around young colonies.
- Gram stained smears from smooth colonies reveal slender gram positive rods. Smears from rough colonies disclose highly pleomorphic and filamentous forms.
- Biochemical reactions:
  - Catalase negative
  - Coagulase positive
  - $H_2S$ production is detected by a thin, black central line in TSI agar.
- Animal inoculation: Mice are susceptible to infection and die within 4 days after intraperitoneal inoculation of 0.1-0.5 ml of broth culture.
- A PCR based method for the detection of virulent *Erysipelothrix rhusiopathiae* isolates has been developed.

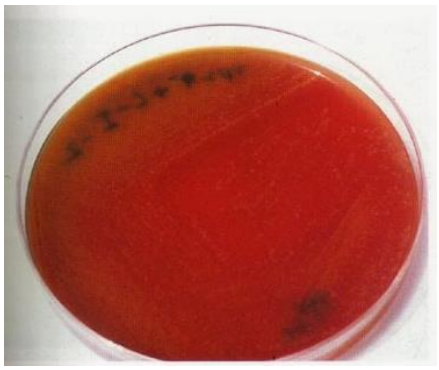


**Fig: Isolated colonies of *E. rhusiopathiae* on blood agar**

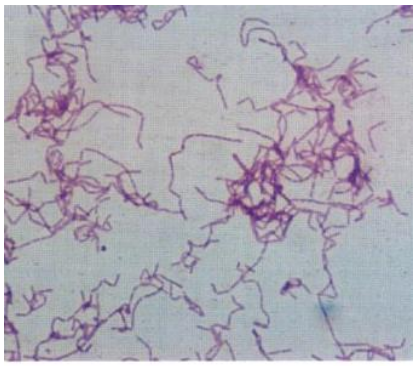


**Fig: *Erysipelothrix rhusiopathiae* (Gram staining)**

**Control**

- Live and killed vaccines are of value and are widely used.
- Hyperimmune serum can be used for treatment and passive protection.

**Public Health Significance**

Human infections with *Erysipelothrix rhusiopathiae* is termed Erysipeloid; usually an occupational disease of veterinarians, butchers and others. The organism usually enters via the skin and after 1-5 days of incubation there is an erythematous swelling at the site of entry. Commonly the infection is localized and most frequently involves the hand and fingers. Cases of septicemia, valvular endocarditis and septic arthritis have been reported.

**Other Species**

*Erysipelothrix tonsillarum*: non pathogenic for pigs, commensal of the tonsils of normal swine; causes endocarditis in dogs.

# Actinomycetes

**General Characteristics**

- Actinomycetes include a group of gram positive bacteria, which tend to grow slowly and produce branching filaments. These organisms were regarded as fungi because of filament formation and granulomatous response to tissue invasion.
- They are sometimes called 'higher bacteria' because of having some of the cultural and morphological characteristics of the fungi. These include filamentation, branching, production of aerial hyphae with asexual spores (conidia).
- Some produce club shaped cells and acid fast elements bearing a resemblance to the corynebacteria and mycobacteria.
- The actinomycetes, causing disease in domestic animals belong to the genera:

  ***Actinomyces:*** *Actinomyces bovis, Actinomyces viscosus*

  ***Actinobaculum:*** *Actinobaculum suis*

  ***Arcanobacterium:*** *Arcanobacterium pyogenes*

  ***Nocardia:*** *Nocardia asteroides*

  ***Dermatophilus:*** *Dermatophilus congolensis*

# Actinomyces

**Principal Characteristics**

- Gram positive.
- Varying from short rods to branching filaments; branching is not septed.
- Anaerobic (except *Actinomyces viscosus*).
- Non spore forming, non motile.
- Non acid fast.

## *Actinomyces bovis*

**Cultural Characteristics**

- The organism grows well on blood agar, brain heart infusion agar and thioglycollate broth. Anaerobic atmosphere containing 5 - 10% $CO_2$ is preferred. Colonies are white, rough, and nodular which are difficult to remove from the media.
- Growth in thioglycollate broth is typically soft and diffuse; some strains produce soft mulberry like granules in broth medium, which are easily disintegrated upon shaking.

**Biochemical Properties**

- Ferment a wide range of CHO producing formic, acetic, lactic and succinic acids.
- Does not reduce nitrates and form $H_2S$.
- Indole, Voges Proskauer and Methyl Red tests are negative.

**Habitat**

- Commensal in the oral cavity of cattle.

**Mode of Infection**

- Wounds of the mucous membrane of the upper digestive tract .

**Resistance**

- Destroyed at $60^0$C in 20 minutes.
- Resistant of penicillin, tetracycline.

**Pathogenicity**

- *Actinomyces bovis* causes a subacute or chronic progressive disease principally of cattle characterized by the development of indurated, granulomatous, suppurative lesions involving bone and soft tissue.
- Cattle: Causes osteomyelitis of the mandible resulting in a swelling of the jaw, often referred to as 'Lumpy jaw'. The disease spreads by contiguity and manifests the formation of sinus, presence of sulfur granules in the pus. Seen most commonly are orchitis, mastitis and lesion of liver and other internal organs.
- Horse: The organisms may be recovered from 'fistulous withers' and 'poll evil' along with other organisms.

**Fig: Formation of swelling tumor at lower jaw**

**Diagnosis**

- ***Specimen:*** Pus
- ***Direct examination:*** A small amount of pus is placed in a Petri dish and washed to expose the small 1-3 mm sulfur granules. A granule is transferred to a slide and a drop of 10% NaOH is added. A coverslip is placed on the granule and it is crushed by gentle pressure. In actinomycosis, the characteristic 'ray fungi' with the club shaped margins can be seen under low power.

  The cover slip is removed and the material spread to make a smear. This is dried, fixed and stained by Gram's method. Intertwined, branching, gram positive filaments are seen in case of actinomycosis.
- ***Isolation and cultivation:*** The organism grows well on blood agar, brain heart infusion agar and thioglycollate broth. An anaerobic atmosphere containing 5 - 10% $CO_2$ is preferred. Colonies are white, rough, and nodular which are difficult to remove from the media. Small cottony colonies may be seen in thioglycollate broth.
- Gram stained smears from growth on solid or fluid media disclose masses of gram positive rods and slightly branched filaments.

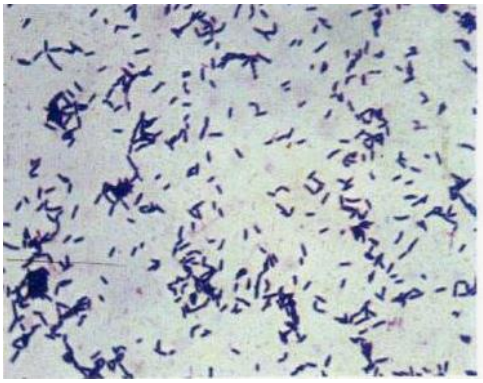


**Fig: *Actinomyces bovis* (Gram's staining)**

### *Actinomyces viscosus*

- The organism grows aerobically.
- Commensal in the oral cavity of dog.
- Disease conditions produced by *Actinomyces viscosus* in domestic animals:

  Dogs: Canine actinomycosis

  - Cutaneous pyogranulomas
  - Pyothorax and proliferative pyogranulomas pleural lesions

  Horses: Cutaneous pustules

  Cattle: Abortion

## Arcanobacterium

**Species:** *Arcanobacterium pyogenes*

**Synonym:** *Actinomyces pyogenes*

*Corynebacterium pyogenes*

- Common commensal on the mucous membrane of the nasopharynx of cattle, sheep and swine.
- Infections arise when the organisms gain entrance to tissue as a result of various injuries and other infections.
- Frequently found in mixed infections; e.g. with *Fusobacterium necrophorum* in bovine liver abscesses.

**Pathogenicity**

- Causes bovine mastitis most frequently in heifers and dry cows in summer month which is known as summer mastitis. The affected quarter of the udder becomes enlarged, firm and suppuration takes place in some cases. The mortality rate is high and among the survivors, the affected quarter is permanently damaged.
- Purulent disease possesses in cattle, pigs, sheep, goats and other animals: Suppurative pneumonia (abscess in lungs).
- Pyometra, endometritis in cattle, develop on calving and there is retention of placenta.
- Seminal vesiculitis in bulls and boars.

**Diagnosis**

- ***Specimen:*** Pus
- ***Direct Examination:*** Gram stained smears of pus disclose small, slender, gram positive pleomorphic rods which may be somewhat curved and clubbed at one ends.
- ***Growth Characteristics:***
  - On blood agar: pin point, glistening, β hemolytic colonies in 48 hours. With age, colonies become opaque and dry.
  - On serum agar: Dew drop like colony.
- ***Biochemical Properties:***
  - Acid but no gas from glucose, maltose and lactose; do not ferment salicin and mannitol.
  - Gelatinase positive.
  - Indole, Voges Proskauer and Methyl Red tests are negative.

# Nocardia

**Principal Characteristics**

- Non motile, non spore forming.
- Form mycelia that fragment into rod shaped and coccoid elements.
- Gram positive.
- May be partially acid fast. Acid fastness of *Nocardia spp.* is often pronounced in clinical than cultural material.
- When cultured, these organisms produce aerial filaments which may form spores.

**Important species**

*Nocardia asteroides*: Domestic animals and man.

*Nocardia caviae*: Guinea pig; cause of bovine mastitis.

*Nocardia brasiliensis*: Nocardiosis in human.

*Nocardia farcinica*: Cause of bovine farcy.

## *Nocardia asteroides*

**Cultural Characteristics**

- Grows on unenriched media, on blood agar and Sabouraud agar at 25°C - 35°C. Growth is evident in 4 or 5 days.
- On blood agar: Colonies are irregularly folded, raised and smooth or granular; colonies are non hemolytic. The color varies from white through yellow to deep orange.

**Biochemical Properties**

- Catalase positive.
- Hydrolyze oesculin.
- Reduce nitrate to nitrite.

**Mode of Infection**

- Inhalation
- Wounds (hands and feet of agricultural laborers)

**Resistance**

- Quite resistant to heat and desiccation.
- Destroyed at 70°C for 10-15 minutes.

**Pathogenicity**

- Nocardiosis is usually a chronic progressive disease characterized by suppurating, granulomatous lesions.
- Cattle: Acute or chronic mastitis with granulomatous lesions and draining fistulous tracts.
- Dogs and cats: Localized form with subcutaneous lesions (mycetomas) and/or lymphnode involvement. There is ganulomatous pleuritis and/or peritonitis with the accumulation of pleural, pericardial and peritoneal fluid. Pulmonary infection in dogs less than a year old is the usual form of nocardiosis. Abscess may be found in the heart, brain, liver and kidneys as well.
- Pigs: Abortion
- Sheep, goat, horses: Wound infections, mastitis, pneumonia, and other pyogranulomatous conditions.

**Diagnosis**

- ***Specimen:*** Pus, exudates, mastitic milk, aspirates.
- ***Direct Examination:*** Smears of exudates should be stained by the Gram and MZN methods. Gram stained smears reveal gram positive branching filaments with or without clubs. *Nocardia asteroidses* is MZN-positive.
- ***Isolation and Cultivation:***
  - The organism can be cultured on blood agar and Sabouraud agar at 37°C or 25°C. Colonies on blood agar are white, powdery and firmly adherent to the agar. Colonies are variably haemolytic and odourless.
  - Subculture onto Sabouraud dextrose agar yields dry, wrinkled, orange colored colonies after incubation for up to 5 days.

## *Nocardia farcinica*

**Pathogenicity**

The organism causes bovine farcy (bovine nocardiosis) or lymphadenitis. It is a chronic infection of superficial lymphatic vessels and lymphnodes. Early lesions consist of small cutaneous nodules, often on the medial aspect of the legs and on the neck. The subcutaneous lymphnodes, particularly the prescapular and popletial are grossly enlarged due to abscessation and eventually rupture and ulcer.

**Differentiation of *Actinomyces bovis* and *Nocardia asteroides***

| Organisms | $O_2$ Requirement | Acid fast | Aerial filament production | Growth on Sabouraud Agar | Catalase | Lactose fermentation |
|---|---|---|---|---|---|---|
| *Actinomyces bovis* | Anaerobic | - | - | - | - | + |
| *Nocardia asteroides* | Aerobic | Partial | + | + | + | - |

## Dermatophilus

**Species:** *Dermatophilus congolensis*

**Principal Characteristics**

- Gram positive, branching, filamentous rod.
- Aerobic, non spore forming.
- Produces motile coccal zoospores.

**Pathogenicity**

- Disease produced by this organism is called Streptothricosis or Dermatophilosis.
- Dermatophilosis has been encountered in horses, cattle, sheep, goats, dog, cat and human.
- Involves superficial layers of the skin and is characterized by the formation of crusts or scabs.
- In sheep, the disease is referred to as mycotic dermatitis and is seen in three forms - **1**. Dermatitis of the wool covered areas or 'lumpy wool'. **2**. Dermatitis of the face and scrotum. **3**. Dermatitis of the lower leg and foot which may result in a severe ulcerative dermatitis referred to as 'strawberry foot rot'.

**Diagnosis**

- ***Specimen:*** Scab, crusts and plucked hair are collected; skin biopsies taken after removal of scabs.
- Smears are made from scabs softened with distilled water and then stained by the Giemsa or Gram method - Gram positive, branching filaments are seen.
- ***Growth characteristics:*** Grows well on blood agar, tryptose agar. Small, rough, grayish white colonies appear in 24 to 48 hours. No growth occurs on Sabouraud dextrose agar.

## Actinobaculum

**Species:** *Actinobaculum suis*

**Disease Conditions:** Cystitis, pyelonephritis in pigs.

**Comparative Features of Actinomycetes of Veterinary Importance**

| Feature | *Actinomyces spp.* | *Arcanobacterium pyogenes* | *Actinobaculum suis* | *Nocardia spp.* | *Dermatophilus congolensis* |
|---|---|---|---|---|---|
| Atmospheric growth requirements | Anaerobic or facultatively anaerobic and capnophilic | Facultatively anaerobic and capnophilic | Anaerobic | Aerobic | Aerobic and capnophilic |
| Aerial filament production | - | - | - | + | - |
| Modified Ziehl-Neelsen staining | - | - | - | + | - |
| Growth on Sabouraud dextrose agar | - | - | - | + | - |
| Usual habitat | Nasopharyngeal and oral mucosae | Nasopharyngeal mucosa of cattle, sheep and pigs | Prepuce and prepucial diverticulum of boars | Soil | Skin of carrier animals, scabs from lesions |
| Site of lesions | Many tissues including bone | Soft tissues | Urinary tract of sows | Thoracic cavity, skin and other tissues | Skin |

# Mycobacterium

The cell wall of mycobacteria contains N-glycolylmuramic acid in place of N-acetylmuramic acid and has high lipid content. This renders their surface hydrophobic and makes mycobacteria difficult to stain with commonly used aniline dyes at room temperature. The bacteria take up the stain with dyes by prolonged application or by heating. When once the bacteria are stained, they resist decolourization with 1% hydrochloric acid and also with 95% ethanol. For this reason they are sometimes referred as acid-fast or acid-alcohol-fast organisms.

**Morphology**

Rod shaped, obligate aerobes, acid fast, non-spore forming, non-motile, Gram positive, grow slowly than most of the pathogenic bacteria.

**Members of the Genus**

1. **Classic Species**

   *Mycobacterium bovis*

   *Mycobacterium tuberculosis*

   *Mycobacterium paratuberculosis*

   *Mycobacterium avium*

   *Mycobacterium leprae*

2. **Runyon Groups**

   Previously called the atypical or anonymous mycobacteria; include species that occur widely in feces and in nature.

   A classification of mycobacteria of environmental origin which infrequently produce opportunistic infections:

   - *Photochromogens:* Nonpigmented when grown in the dark; yellow pigment formed when grown in the light; slow growing.

     *Mycobacterium kansasii*

     *Mycobacterium marinum*

   - *Scotochromogens:* Red-orange pigment formed when grown in the dark or light; slow growing.

     *Mycobacterium scrofulaceum*

   - *Nonchromogens:* Nonpigmented in dark or light; slow growing.

     *Mycobacterium avium* complex

   - *Rapid growers:* may be pigmented or nonpigmented.

     *Mycobacterium fortuitum*

     *Mycobacterium phlei*

3. **Additional Species**

   *Mycobacterium microti*

   *Mycobacterium lepraemurium*

**Important Species**

*Mycobacterium tuberculosis*: Man (Natural host), Primates, Cattle, Swine, Elephant

*Mycobacterium bovis*: Cattle (Natural host), Swine, Dogs, Horse, Sheep

*Mycobacterium avium*: Birds (Natural host), Swine.

*Mycobacterium leprae*: Man

*Mycobacterium paratuberculosis*: Cattle, Sheep, Goat, Deer.

*Mycobacterium fortuitum*: Dog, Cattle, Swine

*Mycobacterium cansasii*: Monkey, Cattle, Swine.

**Mycobacterial Cell Constituents**

1. No exotoxin is produced by any of the members. They have a high concentration of lipids, 20 to 40% dry weight. The thick cell wall of mycobacteria is rich in mycolic acid and other complex lipids.
2. Some cell constituents of mycobacteria are –
   a. **Mycolic acids:** Responsible for acid fastness. Muramyl dipeptide (a component of peptidoglycan) complexed with mycolic acids can cause granuloma formation.
   b. **Phospholipids:** Induce caseation necrosis.
   c. **Protein and Wax D:** Induce tuberculin sensitivity.
   d. **Glycolipids:** They result in toxicity, granulomatous response and enhanced survival of phagocytosed mycobacteria. Cord factor, a glycolipid, is responsible for the characteristic colonial growth of virulent mycobacteria.

**Mode of Infection**

- ***Mycobacterium bovis:*** The organisms leave the host in respiratory discharges, feces, milk, urine, semen and genital discharges. Infection is by inhalation. In calves, mode of infection may be by ingestion.
- ***Mycobacterium avium:*** Shed in feces; acquired mainly by ingestion of contaminated food, water and soil.
- ***Mycobacterium tuberculosis:*** Shed in the sputum and respiratory discharges. Direct spread by droplet infection and fomites.

**Pathogenicity**

Disease results from establishment and proliferation of virulent organisms and interaction with the host. The local manifestation depends upon the route of invasion.

Inhalation: Lungs and tracheobronchial lymphnodes.

Ingestion: Mesenteric lymphnodes and intestinal wall and to the liver via portal system. Organisms from nodes may reach the thoracic duct with general dissemination. In the tissues of the infected animals and man, the tubercle bacillus causes the production of a characteristic and unique lesion- tubercle. In it's typical form the tubercle shows a peripheral zone of fibroblast and lymphocytes and inner zone of macrophages and in it's center one or more giant cell. As it increases in size, necrosis of the central portion occurs. The necrotic material is not normally digested; consequently it has a cheesy appearance in consistency. The necrotic changes found in the tubercle are clearly the result of tuberculoprotein hypersensitivity, which develops during the course of infection. They are almost certainly due to the action of toxic lymphokines liberated from sensitive lymphocytes following exposure to tuberculoprotein.

**Tissue/Organ Involvement**

| ***M. bovis*** | ***M. tuberculosis*** | ***M. avium*** |
|---|---|---|
| Cattle: Pulmonary tuberculosis with involvement of associated lymphnodes, infection of viscera and bones in humans especially from milk. | Frequently pulmonary and lymphnodes. | Lesion may be found anywhere usually involving intestine, liver, spleen and bone marrow; infrequently lung. |

**Cultural Characteristics**

| *Mycobacterium bovis* | *Mycobacterium avium* | *Mycobacterium tuberculosis* |
|---|---|---|
| Dry, sparse, delicate, nonluxuriant. Growth on solid media incubated at 37°C usually appears within 3-8 weeks. | Moist, slimy, glistening, luxuriant, frequently yellow or gray. Growth on solid media incubated at 40°-42°C usually appears within 2-6 weeks. | Dry, crumbly, luxuriant; colonies are usually yellowish with roughened surfaces. Growth on solid media incubated at 37°C usually appears within 3-8 weeks. |

**Clinical Significance, Growth Characteristics and Biochemical Differentiation of Pathogenic Mycobacteria**

| | *M. tuberculosis* | *M. bovis* | *M. avium complex* |
|---|---|---|---|
| Significance of infection | Important in man and occasionally in dogs. | Important in cattle and occasionally in other domestic animals and man. | Important in free-range domestic poultry, opportunistic infections in man and domestic animals |
| **Cultural characteristics and requirements** | | | |
| Growth rate | Slow (3 - 8 weeks) | Slow (3 - 8 weeks) | Slow (2 - 6 weeks) |
| Optimal incubation temperature | $37^0$C | $37^0$C | 37-43°C |
| Colonial features | Rough, buff, difficult to break apart | Cream-colored, raised with central roughness, break apart easily | Sticky, off-white, break apart easily |
| Effect of added glycerol | Enhanced growth | Growth inhibited | Enhanced growth |
| Effect of added sodium pyruvate | No effect | Enhanced growth | No effect |
| **Biochemical differentiation** | | | |
| Niacin accumulation | + | - | - |
| Pyrazinamidase production | + | - | + |
| Nitrate reduction | + | - | - |
| Susceptibility to TCH* (10 µg/ml)[a] | Resistant | Susceptible | Resistant |
| Guinea pig inoculation | ++ | ++ | Developed only localized lesion |
| Rabbit inoculation | Slightly susceptible | ++ | Developed only localized lesion |
| Chicken inoculation | - | - | +++ |

* TCH, Thiophen-2 carboxylic acid hydrazine

**Diagnosis**

- ***Specimen:*** Lymphnodes, sputum, milk etc.
- ***Direct Examination:*** The organism can be demonstrated in smears from lesions employing Ziehl-Neelsen method. The organisms are small, straight or slightly curved and they occur singly or in clumps. They stain red by Ziehl-Neelsen acid-fast stain.
- ***Isolation and Cultivation:***
  - Decontamination of specimens to eliminate fast growing contaminating bacteria. Ground-up specimens are treated for up to 30 minutes with 2-4% NaOH, followed by neutralization with 2N HCl for a maximum of 30 minutes. Centrifugation is used to concentrate the mycobacteria and the supernatant is discarded.
  - Slants of Lowenstein-Jensen medium, without glycerol and containing 0.4% sodium pyruvate, are inoculated with the centrifuged deposit and incubated aerobically at 37°C for up to 8 weeks.
- ***Identification Criteria for Isolates***
  - Growth rate and colonial appearance.
  - Positive Z-N staining of bacilli in smears from colonies.
  - Biochemical profile.
- **Tuberculin Test:** Tuberculin as originally prepared by Koch, is an extract of the soluble bacterial products of the bacilli, which have been grown in glycerinated veal broth. After being allowed to grow for 8 weeks, the bacilli are killed by streaming steam, they are removed from the broth by filtration (filtered through fine mash copper gauze). The filtrate is evaporated to one-tenth its original volume. This represents Koch's old tuberculin.

  PPD (Purified Protein Derivative) is prepared by growing organisms in synthetic medium and killing them with steam and filtering. PPD tuberculin is precipitated from this filtrate with trichloroacetic acid or ammonium sulfate, washed and finally resuspended in buffer ready for use.

  Three different tuberculin tests can be used in the diagnosis of bovine tuberculosis-

  **Subcutaneous:** A dose of 2 ml Koch's old tuberculin is administered s/c. Previous to the injection the temperature of the animal is taken 3 times at two hours interval. In positive reaction is noted by an increase in temperature of at least 2°F after 8 to 18 hours injection.

  **Intradermal:** 0.1 ml of tuberculin is injected into the dermal layer in caudal fold. A positive reaction is noted by a small or large, firm, swelling at the point of injection within 72 hours.

  **Ophthalmic:** Tuberculin is inserted into the conjunctival sac. A positive reaction is characterized by marked inflammation of the conjunctiva, profuse lacrimation and mucopurulent exudates.

  **Comparative intradermal test:** 0.1ml of avian PPD and 0.1 ml of bovine PPD are injected intradermally into separate clipped sites on the side of the neck about 12 cm apart. Skin thickness at the injection sites is measured before injection of tuberculin and after 72 hours. An increase in skin thickness at the injection site of bovine PPD which exceeds that at the avian PPD injection site by 4 mm or more is interpreted as evidence of infection and the animal is termed a reactor.

**Fig: Tuberculin test (Positive reactor)**

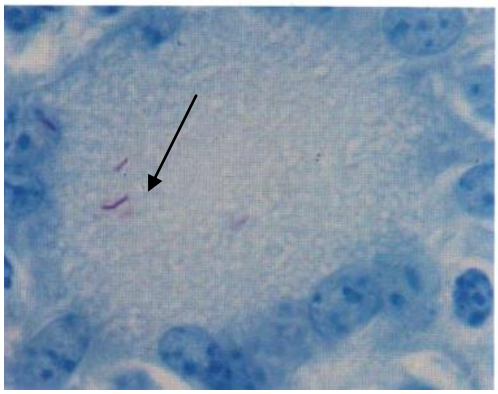


**Fig: Acid fast bacilli (arrow)**

**Prevention and Control**

1. The tuberculosis eradication programme is based upon the detection and slaughter of infected animals as determine by the series of tuberculin test.
2. A living vaccine comprising of an attenuated strain of bovine type known as BCG (bacilli Calmette and Guerin) is used to confer immunity among human being but degree of immunity which develop in cattle is uncertain.
3. Newly purchased animals should be tested before allowing them to mix with the rest of the animals in the herd.
4. Premises inhabited by the sick animals should be properly disinfected.
5. Farm personnel should be periodically tested for tuberculosis.

## *Mycobacterium paratuberculosis*

Originally known as *Mycobacterium johnei* which was first observed by Johne and Frothingham in Germany in 1895.

**Disease**

Paratuberculosis/Johne's Disease; characterized by chronic contagious enteritis in cattle, sheep, goats, camels and buffaloes.

**Morphology**

Short rod; on artificial medium the organism tends to shorter; in smears from infected mucous membrane the organism is found in clumps but single organism is also present, acid fast.

**Cultural Characteristics**

Egg yolk agar containing glycerin and mycobactin (extract of *Mycobacterium phlei*) is recommended for isolation of *Mycobacterium paratuberculosis*. Primary cultures require 4-8 weeks incubation. Minute grayish white, friable irregular colonies appear, as the culture ages the colonies increase in size and become pale yellow in color. In glycerin broth, containing mycobactin the bacillus form a thin pellicle which become thick and folded as the culture ages.

**Epidemiology**

- Infection is acquired by calves at an early age through ingestion of organisms shed in the feces of infected animals.
- The organism may remain viable in the environment for up to one year under suitable conditions.
- The organism is shedding through milk and isolated from genital organs and semen of infected bulls.

- Calves under one month of age are particularly susceptible to infection. The incubation period is long and variable.
- Clinical disease is rarely encountered in cattle under 2 years of age.

**Pathogenicity**

Chronic enteritis with often severe diarrhoea in cattle. Diarrhoea in sheep, goats and other ruminants-less severe. Incubation period may be year or more. Calves are susceptible but do not show signs until adult. The disease is usually progressive leading to emaciation due to the malabsorbtion of amino acids and the loss of protein in the intestine ( protein loss enteropathy). The ileum and colon are usually involved and the infection may extend to the rectum in advanced cases. The mucous membranes become corrugated and thickened due to epitheloid and giant cells both of which contain many organisms.

**Diagnosis**

- ***Specimen:*** Intestinal mucosa of dead animals particularly ileum, colon and caeco-colic junction.
- ***Direct Examination:*** A small piece of rectum in living animals washing with saline and then making a smear-stain by Ziehl-Neelsen method. Examine under microscope looking for acid fast bacteria. A number of samples necessary for examination.
- Isolation of *M. paratuberculosis* from feces or tissues is a sensitive diagnostic procedure but it is difficult and time consuming. After decontamination of the specimen with 0.3% benzalkonium chloride and concentration by centrifugation, slants of Herrold's egg yolk medium with and without mycobactin are inoculated aerobically at 37°C for up to 16 weeks and examined weekly for evidence of growth.
- **Johnin Test:** Johnin is prepared from the extract of *Mycobacterium paratuberculosis*. 0.2 ml of Johnin is injected into the skin of a clipped area on the neck. After 48 hours the test is read. A positive reaction consists of an increase in thickness of 3 mm or greater than the original thickness; an increase of 3 to 2 mm is considered suspicious and less than 2 mm is negative.

  Single intradermal (SID) may give negative result in animals suffering from clinical disease. An intravenous Johnin test is positive in this case and may be a preferable alternative to the SID test. In this test, the antigen is administered intravenously and the animal's temperature is noted at intervals. A rise in temperature of 1°C or a neutrophilia after 6 hours is considered a positive result.
- Complement Fixation Test
- ELISA
- AGIDT

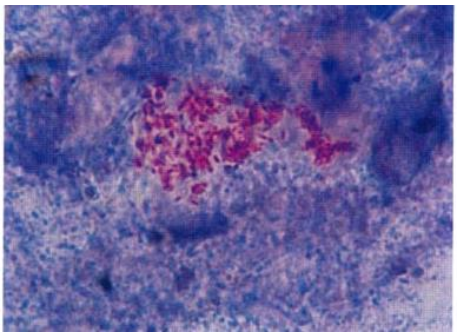


**Fig: Clusters of acid fast bacilli seen on direct smear of diarrhoeal feces**

**Prevention and Control**

1. A live vaccine is practiced in some countries which consists non pathogenic strain of organism. The vaccine is inoculated in calves soon after birth.
2. Animals with persistent diarrhoea or chronic weight loss should be isolated.
3. Clean and disinfect the premises after the removal of infected animals.
4. Removing Johnin positive animal from the herd for slaughter. Purchase only Johnin negative animals from herd with no history of disease.
5. Culture the feces from all animals 2 years old or older every 6 months and remove and slaughter animals whose cultures are positive.
6. If artificial insemination is used, semen should come from culturally negative bulls.

### *Mycobacterium lepraemurium*

An identical organism causes feline leprosy but the organism get uncultivated but it has been suggested that cats acquired the infection via rat bites and the disease is characterized by the formation of single and multiple granulomatous nodules of the skin, 1-3 mm in diameter. They are painless.

## Actinobacillus

**Species**

*Actinobacillus lignieresii*

**Morphology**

- Gram negative.
- Rods to coccobacillary form.
- Non motile, non spore forming, non capsulated.
- They may show bipolar staining.

**Cultural Characteristics**

- *Actinobacillus lignieresii* grows well on blood agar. Colonies of fresh isolates are rough in appearance but sticky in nature and non-haemolytic. On repeated sub-cultivation, the organism loss their stickiness.
- Grows on MacConkey agar. Colonies are initially pale, turning pink after 48 hours.
- Produces uniform turbidity with little sediment in broth.

**Biochemical Properties**

- *Actinobacillus lignieresii* produces acid without gas promptly in maltose, mannitol and sucrose but delayed reaction in lactose.
- $H_2S$ is produced, MR negative, VP variable.
- Hydrolysis of oesculin is negative.

**Habitat**

- Commensals on mucous membranes of animals in the upper respiratory tract and oral cavity.

**Mode of Infection**

- The organism gains entrance to the oral mucosa through injuries to the tissue.
- Carrier animals play a major role in transmission.

**Pathogenicity**

Actinobacillosis - a disease that most often affects soft tissues and lymphnodes, although bony structures also may be involved by direct extension. In cattle, classical actinobacillosis usually affects the tongue accompanied by swelling and hardening; often referred to as "Wooden tongue". Rumen, reticulum and skeletal muscles are less frequently affected.
In sheep affects the facial skin, lip, lymphnodes which are much more purulent than those in cattle. Tongue is not involved.

**Diagnosis**

- Specimen: Pus or necrotic material from early lesions.
- Direct examination: Small, Gram negative rods are demonstrable within granules. The granules are washed and transferred to a slide and a drop of 10% NaOH is added. The granules are smeared and stained. The granules are small less than 1 mm, grey and white. Gram negative rods or coccobacilli are seen.
- Isolation and Cultivation: The organism can be recovered consistently if clinical material is seeded onto serum or blood agar and incubated at $37^0$C; 10% $CO_2$ increases the growth.

Small, translucent, smooth and glistening colonies are evident in 24 - 48 hours. Stained smears disclose small Gram negative rods or coccobacilli.

**Other Pathogenic Species**

*Actinobacillus equuli*: In foals it causes an acute septicaemia with enteritis in which death usually occurs within the first three days of life. Affected animals often appear semi-comatose at birth. Hence the disease is called 'Sleepy Foal Disease'. Foals can be infected *in utero* and after birth via umbilicus. Occasionally cause abortion, septicaemia and peritonitis in adult horses.

*Actinobacillus suis*: Septicaemia, pneumonia, arthritis in swine.
*Actinobacillus actinoides*: Pneumonia in calves.
*Actinobacillus seminis*: Epididymitis in rams, polyarthritis in lambs.
*Actinobacillus pleuropneumoniae*: Pleuropneumonia in pigs.

## Enterobacteriaceae

**Principal Characteristics**

Gram negative, facultative anaerobic, medium-sized rods, oxidase negative, catalase positive, non spore-forming, fermentative, usually motile.

**Important genus and species**

| Genus | Species |
|---|---|
| *Escherichia* | *coli* |
| *Shigella* | *dysenteriae* |
| | *flexneri* |
| | *boydii* |
| | *sonnei* |
| *Salmonella* | *typhi* |
| | *paratyphi* |
| | *typhimurium* |
| | *enteritidis* |
| | *pullorum* |
| | *gallinarum* |
| | *choleraesuis* |
| | *dublin* |
| *Klebsiella* | *pneumoniae* |
| *Enterobacter* | *aerogenes* |
| *Serratia* | *marcescens* |
| *Proteus* | *mirabilis* |
| | *vulgaris* |
| *Yersinia* | *pestis* |
| | *pseudotuberculosis* |
| | *enterocolitica* |

**Other (not important) Genera**

*Edwardsiella*

*Arizona*

*Citrobacter*

*Providencia*

*Erwinia*

*Pectobacterium*

# Escherichia

Isolated first Theodor Escherich in 1885

**Species:** *Escherichia coli*

**Morphology**

Motile due to peritrichous flagella. Some strains produce polysaccharide capsules; mucoid growth.

**Cultural Characteristics**

- Grows well on ordinary media: Colonies are 2-3 mm in diameter

- On nutrient agar: Colonies are circular, low convex, smooth, and colorless.
- In MacConkey agar: Large pink colored colony.
- Blood agar is discolored around the growth; may be haemolysis.
- Nutrient broth: Diffuse cloudiness, heavy sediment.
- Eosin-Methylene Blue (EMB) agar: The colonies have a metallic sheen, characteristic to *E. coli*.

**Antigenic Properties**

- Complex.
- Somatic or O antigens: The somatic antigens are lipopolysaccharide in nature and located at the surface of the cell wall. There are 159 serotypes on the basis of this antigen. Somatic antigens are designated by Arabic numerals, e.g. O133. O78, O1, O35 and O11 are important for chicken.
- K (surface or envelope) antigens: Capsular antigens are composed of polysaccharides. K antigens are of three types A, B and M.

  A → Thermostable → lost its property → 121°C → 2 hours.

  B → 100°C → 1 hour → lost property.

  M → Mucoid strains → Thermolabile.

  There are more than 100K antigens. They are designated by the letter with an Arabic number, e.g. K4.
- H or flagellar antigens: These antigens are protein in nature and are of 53 types. These antigens are designated by H followed by an Arabic number, e.g. H2.
- Fimbrial antigens: Proteinaceous fimbrial antigens act as adhesins facilitating attachment to mucosal surfaces, e.g. K88 (F4), K99 (F5), 987P (F6), F41. The most common adhesin present in strains of *E. coli* infecting pigs is K88. The k99 and F41 adhesins occur in calves and K99 in lambs.
- An example of a complete designation is O111: K4: H2.
- Colicins: Bacterial products and protein in nature having bactericidal activities.

**Differentiation of Enterobacteriaceae**

| **Property** | ***Escherichia*** | ***Klebsiella*** | ***Salmonella*** | ***Shigella*** | ***Proteus*** | ***Enterobacter*** |
|---|---|---|---|---|---|---|
| Citrate | - | + | ± | - | - | + |
| Gelatin liquefaction | - | - | V | - | + | - |
| $H_2S$ | - | - | ± | - | + | - |
| Indole | + | ± | - | + | ± | - |
| VP | - | + | - | - | - | + |
| MR | + | - | + | + | + | - |
| Urease | - | ± | - | - | + | - |
| Motility | V | - | + | - | + | + |
| Glucose | + | + | + | + | + | + |
| Lactose | + | + | - | - | - | + |
| Sucrose | ± | + | - | - | ± | + |
| Mannitol | + | - | + | V | V | - |

**Pathogenicity**

- Opportunistic pathogen
- Some pathogenic serotypes have fimbrial antigen k88-piglets, k99-calves
- Septicemia and diarrhoea in man, animals and birds.
- Calves: Deprived of colostrums or have too little immunoglobulin in the blood or other predisposing factors.
  Septicaemia: Polyarthritis (tenderness and swelling of joints), Pneumonia
  Diarrhoea: "White scour" → Greyish white feces with bad odour.
  Cattle: Acute mastitis
- Sheep/goats: Enteritis, Septicemia, polyarthritis
- Horses: Joint ill, naval ill, polyarthritis
- Poultry: ▪ Colysepticaemia: Airsacculiis
  Pericarditis
  Perihepatitis
  - ▪ Panophthalmitis
  - ▪ Arthritis, osteomyelitis
  - ▪ Salpingitis
  - ▪ Coligranuloma (Hjarre's disease) → mainly cecum, spleen, liver.
  - ▪ Enteritis: diarrhoea, feces become yellow colored.

# Salmonella

- Usually motile, produce acid and gas from glucose, maltose, mannitol and sorbitol, do not ferment lactose, sucrose or salicin, do not form indole, coagulate milk or liquefy gelatin.
- Species mainly cause enteric infection except *Salmonella abortivoequina* and *Salmonella abortusovis*.
- Food products of animal origin may cause extensive out breaks of enteric infection.

**Differential Metabolic Characteristics of Important Salmonella spp.**

| Species | Xylose | Arabinose | Trehalose | Inositol | Maltose | $H_2S$ production |
|---|---|---|---|---|---|---|
| *Salmonella paratyphi* | - | AG* | AG | - | AG | - |
| *Salmonella typhi* | V | V | A* | - | A | + |
| *Salmonella typhimurium* | AG | AG | AG | AG | AG | + |
| *Salmonella. abortivoequina* | AG | AG | - | - | AG | V |
| *Salmonella. abortusovis* | AG | AG | - | - | AG | V |
| *Salmonella choleraesuis* | AG | - | - | - | AG | V |
| *Salmonella enteritidis* | AG | AG | AG | - | AG | + |
| *Salmonella pullorum* | AG | AG | AG | - | V | + |
| *Salmonella gallinarun* | A | A | A | - | A | V |
| *Salmonella anatis* | AG | AG | AG | - | AG | + |

AG* = Acid/Gas; A*= Acid

**Antigenic Properties**

- Somatic or O antigens: designated by Arabic numerals; group classification is based on several of these antigens. Preparation of O antigen: Heating the bacterial suspension at 80-100$^0$C for an hour. The various O antigens are designated by numerals, 2, 3, 4 etc. and upon the basis of close relationships the species of the *Salmonella* are placed into groups designated types A, B, C etc.

  e.g. Type D- *Sal. typhi* - 9, 12
  *Sal. enteritidis* - 1, 9, 12
  *Sal. dublin* - 1, 9, 12
  *Sal. gallinarum* - 1, 9, 12
  *Sal. pullorum* - 9, 12

- Flagellar or H antigens: Diphasic -
  1. Specific phase: Those antigenic components which are specific for the species or strain. These are designated as a, b, c etc.
  2. Nonspecific phase: Antigens shared by other species in other group types and are designated 1, 2, 3, 4 etc.

  *Salmonella pullorum* and *Salmonella gallinarum* have no H antigens.
- K antigens (capsular or envelope): 'Vi' antigen, 'M' antigen, and so forth. These antigens may interfere with agglutinability of O antisera.
- An example of a complete designation is *Salmonella typhimurium*, 1, 4, [5], 12: i: 1, 2.
- S→R dissociation results in the loss certain components of the cell essential for complete O- antigen.
- Serological diagnosis: By agglutination test.

**The Isolation of *Salmonella***

- Isolation form intestinal tract presents a more complex problem than isolation of *Salmonella* affecting lung, liver kidney or muscle.
- *Proteus* spp: Swarming type of growth on agar plate.
- Need selective media for *Salmonella* are: (i) SS agar (ii) Bismuth Sulfite Agar (iii) MacConkey brilliant green agar (iv) Desoxycholate citrate agar (v) Brilliant green- phenol red agar.

## *Salmonella typhimurium*

- Host: Numerous spp. of animals and birds
- Sources: Food of animal origin, animal bi- products, contaminated water supply.

**Biochemical Properties**: As mentioned in the comparative chart.

**Pathogenicity**

Causes acute and fatal intestinal infections of mice, rat, guinea pigs, sheep, calves, horses, chickens, ducks etc. The symptoms of the disease are characterized by increased temperature, severe diarrhoea and marked weakness. Postmortem examination includes intestinal mucous membrane- hyperemia; acute cases- hemorrhage.

Outbreaks in man occur from animal sources.

**Diagnosis**

- Isolation and Identification of the causal agent
- Serological test: Agglutination test.
-

## *Salmonella pullorum*

- Antigenically identical with *Salmonella gallinarum*. But the two organisms are identified by different biochemical tests.

**Distribution and Transmission**

- Through feed and water
- Carrier birds
- Through eggs
- Through hatched infected chicks.

**Morphology**

It does not possess flagella. The normal shape of the organism is short and plump.

**Growth Requirements and Characteristics**

The organism is isolated in pure culture by opening the infected chick with aseptic precautions and streaking blood or liver and spleen pulp upon the surface of meat infusion agar. The organism produces smooth, glistening, opalescent colonies on nutrient agar.

**Biochemical Properties**

Reduces nitrates to nitrites.

**Antigenic Structure**

- Presence of only somatic antigen.
- The complete antigenic formula of the organism is considered to be 9, 12 ($12_{1\text{-}3}$).

**Pathogenicity**

*Salmonella pullorum* produces an acute disease in chicks during their first few days of life and is characterized by a severe enteritis and bacteremia. The fecal material is white in color and pasty in consistency termed bacillary white diarrhea. But the appropriate term is pullorum disease/Salmonellosis/Paratyphoid.

In mature hens, chronic infection is characterized by a shrunken and misshaped ovary.

**Diagnosis**

- Isolation and identification
- Agglutination test: Macroscopic tube test and macroscopic plate test.

## *Salmonella anatum*

Produces disease mainly in ducklings

**Morphology:** Similar to *Salmonella pullorum*.

### *Salmonella gallinarum*

**Morphology**

Usually occurs singly, but short chains are sometimes observed.

**Growth Characteristics**

- Colony on the agar plate: Small, blue-grey, moist, circular.
- In broth: Uniform turbidity, granular sediment; some strains produce thin and filmy pellicle.

**Antigens**

O1, O9, O12.

**Pathogenicity**

Acute intestinal and generalized infection of fowls (usually few weeks of life).

**Diagnosis**

- Isolation and identification
- Agglutination test

**Other *Salmonella* species**

*Salmonella abortivoequina*: Causes abortion in mares.

*Salmonella abortusovis*: Causes abortion in ewes.

*Salmonella choleraesuis*: Causes acute infectious enteritis in pigs.

*Salmonella enteritidis*: Produces enteritis in rodents and in man.

*Salmonella Dublin*: Causes calf diarrhea.

*Salmonella typhi*: Causes typhoid in man.

*Salmonella paratyphi* (A, B, C): Paratyphoid in man.

Typhoid + Paratyphoid = Enteric fever and characterized by a generalized infection of the reticuloendothelial system and intestinal lymphoid tissue accompanied by sustained fever and bacteremia.

Typhoid: more severe

Paratyphoid: less severe (milder)

But typhoid is far more common than paratyphoid. The global burden of typhoid is estimated as some 1,60,00,000 cases and 6,00,000 deaths each year (1995).

## Klebsiella

*Klebsiella pneumoniae*: Pneumonia and suppurative infections in foals, cervicitis and metritis in mares, mastitis in cows, wound infections and septicemia and pneumonia in the dog.

## Proteus

*Proteus mirabilis*: Variety of sporadic infections of dogs, cattle and fowl. Cystitis and urinary infections are the most common, particularly in dogs.

## Serratia

*Serratia marcescens*: Infrequent cases of bovine mastitis and other uncommon sporadic infections.

## Shigella

*Shigella dysenteriae, Shigella flexneri, Shigella boydii, Shigella sonnei*: Cause bacillary dysentery in man.

- Enteroinvasive: invade the mucosa of the large intestine.
- Faecal-oral transmission
- The defining clinical feature of *Shigella* infection is passage of bloody, small volume stools with urgency and with rectal tenesmus.

## Yersinia

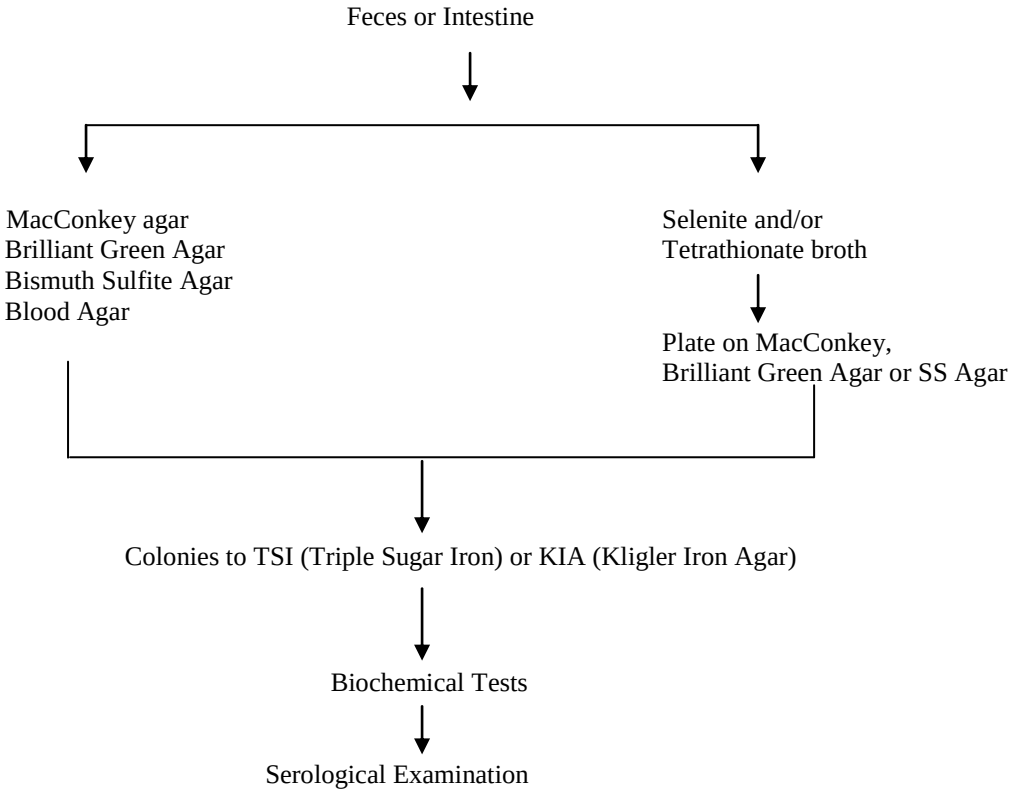
*Yersinia pestis*: is a cause of plague (Black Death). Plague is fundamentally a disease of rats and wild rodents. Man is considered an accidental host. The organism is transmitted to humans via infected fleas. Lymph nodes associated with the flea bite enlarge to form a bubo (bubonic plague). The bubonic form can give rise to the pneumonic form which is highly contagious and usually fatal if not treated.

*Yersinia pseudotuberculosis*: Produces pseudotuberculosis in various rodents, guinea pigs, cats and turkeys, epididymo-orchitis of rams.

Humans: Mesenteric adenitis, rarely septicaemia.

*Yersinia enterocolitica*: Causes diarrhoel disease, terminal ileitis, mesenteric adenitis, may be septicaemia, polyarthritis in humans but rarely in animals (mares, deer, rabbit, dog, pigs, avian spp., goats, cattle, and sheep). Animals generally act as reservoir hosts.

**Procedures for the Isolation and Identification of Enterobacteria from Feces or Intestine**



**Appearance of Important Enterobacteria on Selective Media**

| Organisms | Brilliant Green Agar | Salmonella-Shigella (S.S) Agar | MacConkey Agar |
|---|---|---|---|
| *E. coli*<br>*Enterobacter*<br>*Klebsiella* | Inhibited. If present are yellowish-green. | Inhibited. If present are red. | Grow and are red. *Enterobacter* and *Klebsiella* may be larger and mucoid. |
| *Salmonella* | Grow. Red due to peptone hydrolysis. | Grow; colorless. $H_2S$ producers: dark centers. | Grow; colorless. |
| *Proteus* | Grow; don't spread; yellowish-green. Sucrose negative: strains are colorless. | Grow; don't spread, colorless. $H_2S$ producers: dark centers. | Grow and spread. Colorless. |

**Sequence of Procedures for the Isolation and Identification of Enterobacteria**

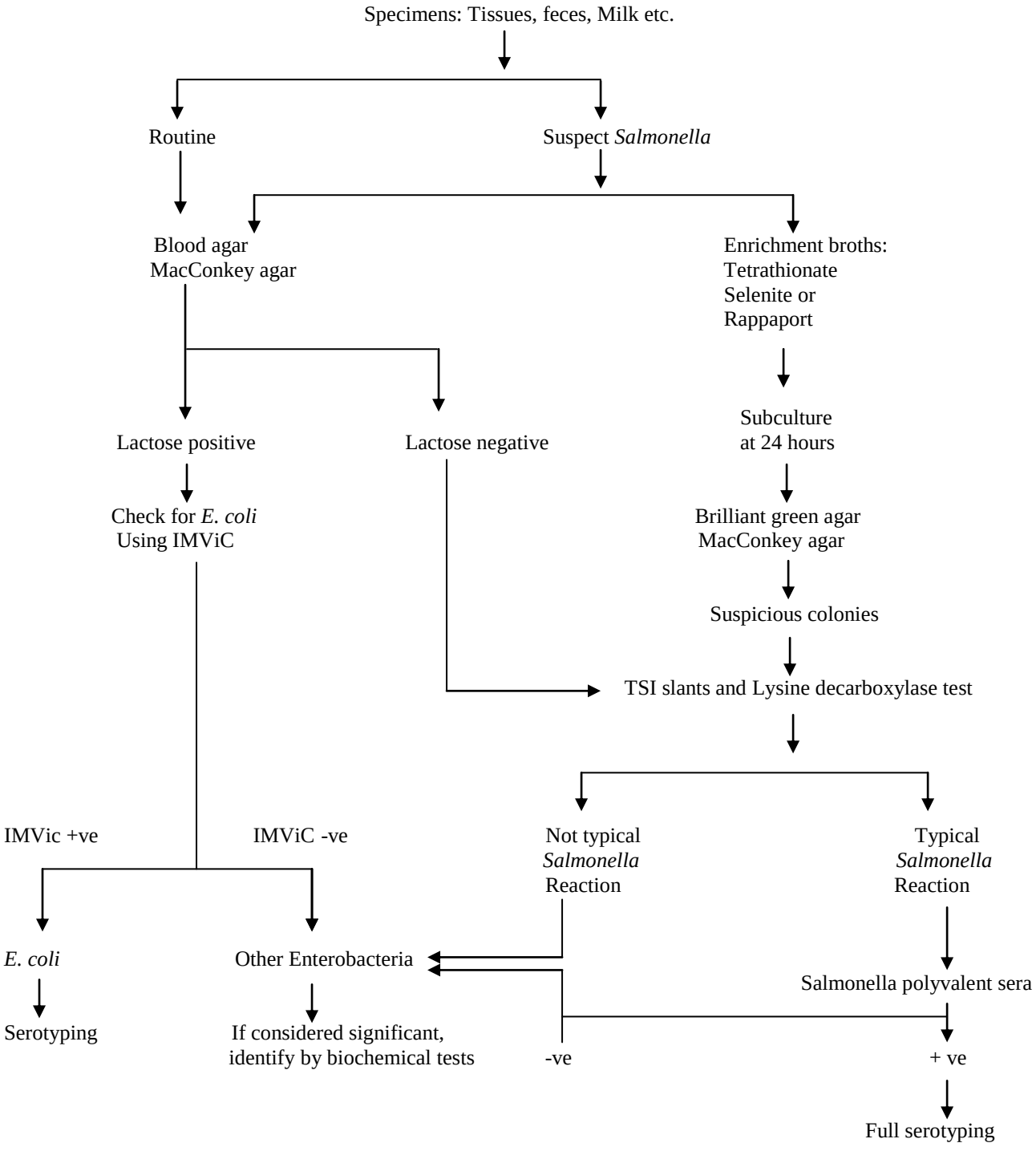


**Fig: Sequence of procedures for the isolation and identification of enterobacteria**

# Pseudomonas

**Principal Characteristics**

- Gram negative medium-sized rods
- Aerobic
- Motile by one or several polar flagella (except *Pseudomonas mallei* / *Burkholderia mallei*)
- Catalase and oxidase positive

*The classification of Pseudomonads is based on rRNA/DNA homology.

**Important Species**

1. *Pseudomonas aeruginosa* – Causes pyogenic infection. Found on the skin and mucous membrane and in feces. Natural habitat – Water, soil and decaying vegetation.
2. *Burkholderia pseudomallei* (Formerly *Pseudomonas pseudomallei*) – Causes Melioidosis.
3. *Burkholderia mallei* (Formerly *Pseudomonas mallei*) – Causes Glanders. Obligate parasite.

## *Pseudomonas aeruginosa*

**Morphology**

- Gram negative medium-sized rods.
- Motile due to 3 polar flagella.
- Does not produce spore.

**Cultural Characteristics**

- Grows well on ordinary nutrient media. Colonies are irregular spreading and may show bluish metallic sheen.
- Beta-hemolysis is usually observed around the colonies growing on blood agar.
- A distinctive aromatic odor is usually apparent.
- An abundant growth occurs in broth with the formation of a thick pellicle and dense turbidity.
- Produce two types of pigments, both of which are water soluble-
    a. Pyocyanin – Bluish green → Chloroform soluble ('Blue pus bacillus').
    b. Fluorescein or Pyoverdin – Yellowish green → not chloroform soluble.

    Occasionally a strain produces a dark red pigment (pyorubin) or a brown-black pigment (pyomelanin).

**Biochemical Properties**

- Acid without gas is produced in glucose.
- Gelatin is liquefied.

**Pathogenicity**

It is an opportunist in weakened tissues as for example burns, wounds, individual with malignancy, an immunodeficiencies and young animals. The organism is associated to the development of abscesses and wound infections characterized by formation of greenish yellow pus.

Some diseases or conditions with which *Pseudomonas aeruginosa* has been associated are-

Horse: Abortion, Guttural pouch infections.

Cattle: Mastitis, Abortion, Infertility.

Sheep: Mastitis, fleece-rot, pneumonia, otitis media.

Pigs: Respiratory infections, otitis

Dogs/Cats: Prostatitis, Cystitis, Dermatitis.

Fowl: Septicemia, Respiratory infections.

Wound infection and abscesses in all animals.

**Diagnosis**

- Specimens: Urine, pus, affected tissues and swabs from infected tissue surfaces.
- Isolation and Cultivation: All strains grow well on ordinary nutrient media. Colonies are irregular, spreading, translucent and may show bluish-metallic sheen. Beta-hemolysis is usually observed around colonies growing on blood agar plates. A distinctive aromatic odor is usually apparent.
- Medium-sized, Gram negative rods are seen in smears.

## ***Burkholderia mallei***

**Synonym**

*Actinobacillus mallei*

*Pfeifferella mallei*

*Malleomyces mallei*

*Loefflerella mallei*

**Morphology**

- Slender rods with rounded ends.
- After repeated subculture, rough, pleomorphic form may develop as filaments, some of which may be branched. The coccoid forms are also seen.
- Non capsulated and non spore-forming.

**Cultural Characteristics**

- Aerobic and facultative anaerobic; optimum $p^H$ 6.6.
- Grows well on most common bacteriological media, but the addition of serum and glycerin yields more rapid and abundant growth.
- The growth of the organism on solid media is slimy.
- In broth, the organism produces light turbidity and forms a thin pellicle.

**Biochemical Properties**

- The organism gives negative reaction to the indole, methyl red and Voges Proskauer tests.

**Pathogenicity**

The clinical form of the disease is commonly classified into three types:

a. Pulmonary form
b. Nasal form
c. Cutaneous glanders and farci

**a. Pulmonary form**

This form is characterized by the formation of round, grayish, firm, encapsulated nodules embedded through the lung tissue. Many of the nodules contain yellowish cheesy pus surrounded by a zone of inflammation.

**b. Nasal form**

The nasal form develops initially as a reddening of nasal mucosa and mucoid discharge from one or both nostrils. The nodules develop on the nasal septum which eventually ruptured and liberate mucopurulent exudates.

**c. Cutaneous form**

The cutaneous form develops nodules or farci buds from along the lymph vessels between affected lymphnodes. The nodules rupture and discharge yellowish pus and form into ulcers which heal slowly.

Human infection which can be fatal usually begins as an ulcer of the skin or mucous membranes followed by lymphadenitis and abscessation.

**Diagnosis**

- Specimen: Materials from infected areas, urine, pus etc.
- Isolation and Cultivation: It grows on blood and serum agar. The growth of the organism on solid media is slimy. In broth, it produces light turbidity and forms a thin pellicle.
- **Mallein Test:** Mallein test is most commonly used for the diagnosis of glanders. Mallein is an autoclaved whole culture of *Pseudomonas mallei*. In the subcutaneous test 2.5 ml of mallein is inoculated. The positive reaction is characterized by a local swelling and febrile reaction. In intradermal palpebral test, 0.1 ml of mallein is inoculated into the skin of lower eyelid. The positive reaction is characterized by swelling of eyelid with congestion of conjunctiva and mucus discharge from the eye. The reaction begins 9 to 10 hours after inoculation and reaches its highest between 24 to 36 hours.

***Burkholderia pseudomallei***

- *Burkholderia pseudomallei* causes melioidosis, an endemic glander like disease of animals and humans, primarily in Southeast Asia and Northern Australia.
- The organism is a natural saprophyte found in soil, water and vegetables.
- Infection originates from these sources by contamination of skin abrasions and by ingestion or by inhalation.
- Maleioidosis may manifest itself as acute, subacute or chronic infection. The localized infection may lead to the acute septecemic form of infection with involvement of many organs. Sometimes develop chronic suppurative infection with abscesses in skin, brain, lung, liver, bone and other sites.
- A Gram stain of appropriate specimen will show small gram negative bacilli; bipolar staining is seen with Wright's stain or methylene blue stain.

# Bordetella

**Important Species**

*Bordetella bronchiseptica*: Natural host – Animals; causes respiratory disease.

*Bordetella pertussis*: Natural host- Man; causes whooping cough in children.

*Bordetella parapertussis*: Natural host - Man; causes parapertussis- a mild form of whooping cough.

*Bordetella avium*: causes rhinotracheitis (Coryza) of turkey poults.

## *Bordetella bronchiseptica*

**Synonym**

*Brucella bronchiseptica / Alcaligenes bronchiseptica*

**Morphology**

- Coccoid bacilli, pleomorphic.
- Usually occurs singly, but pairs are found and in fluid media chains may be observed.
- Motile due to peritrichous flagella.
- Capsules in culture, do not produce spore.
- Gram negative, but may show bipolar staining.

**Cultural Characteristics**

- Aerobic, grows well on blood or serum agar, MacConkey agar with 1% glucose; may be β-haemolytic.
- Small circular dew drops colonies in 48 hours.
- In broth, produces uniform turbidity with granular sediment.

**Biochemical Properties**

- Does not ferment CHO.
- Does not produce indole, $H_2S$.
- Grows on citrate media.
- Urease positive.

**Antigens**

- Flagellar (H) antigens
- Surface (K) antigens: heat labile
- Surface (O) antigens: heat stable
- Fimbrial antigens

**Ecology**

Commensal in the upper respiratory tract of dogs, cats, swine, rabbits, horses, guinea pigs and possibly other animals.

**Mode of Infection and Transmission**

- Endogenous
- Inhalation

- Direct contact
- Fomites

**Pathogenicity**

- Secondary invader in the pneumonia of canine distemper (caused by a virus). Sometimes involved in the etiology of kennel cough (canine infectious tracheobronchitis) below 6 months in dogs. This organism spreads very rapidly particularly in a confined place like kennel or veterinary hospitals.
- Swine pneumonia: Tendency to be chronic atrophic rhinitis of swine (due to dermonecrotoxic toxin: it impairs the ability of osteoblasts to differentiate) characterized by sneezing followed by atrophy of turbinate bones which may be accompanied by distortion of the nasal septum and shortening and twisting of the upper jaw.
- Horses: Respiratory infections.

**Diagnosis**

- Specimen: Nasal swab, tracheal aspirates and exudates.
- Isolation and cultivation: The organism can be cultured on blood agar and MacConkey agar and plates are incubated aerobically at 37°C for 24 to 48 hours. Colonies are small, circular, dew drop like.

**Whooping Cough**

- Caused by *Bordetella pertussis*
- *Bordetella pertussis* toxin in the trachea and bronchi →Irritate surface cells causing coughing → Obstruction of the smaller → bronchioles by mucus plugs → Results diminished oxygenation of blood → Convulsion, whooping cough, sometimes vomiting and cyanosis.

**Prevention**

- Vaccination under 1 year children.
- 3 doses of injection of killed organism in association with DTP.

## *Bordetella avium*

- Synonym: *Alcaligenes faecalis*
- Cause rhinotrachitis in turkeys (turkey coryza).
- The disease is highly contagious characterized by oculonasal discharge, sneezing, dyspnea, decreased weight gain and tracheal collapse.
- The organism colonizes ciliated tracheal epithelium leading to inflammation and destruction of the epithelium and tracheal rings.

**Virulence factors of *Bordetella bronchiseptica* and *Bordetella avium***

| Virulence Factor | Activity | *Bordetella bronchiseptica* | *Bordetella avium* |
|---|---|---|---|
| Filamentous haemagglutinin | Binds to cilia | + | - |
| Pertactin | Binds to cells | + | - |
| Fimbrae | Mediate attachment to cells | + | + |
| Adenylate cyclase-hemolysin | Interferes with phagocytic cell function | + | - |
| Tracheal cytotoxin | Inhibits ciliary action, kills ciliated | + | + |

| | | | |
|---|---|---|---|
| | cells | | |
| Dermonecrotoxic toxin | Induce skin necrosis, impairs osteogenesis | + | + |
| Osteotoxin | Toxic for osteoblasts | + | + |

# Moraxella

**Species**

*Moraxella bovis*

**Morphology**

- Short, plump Gram negative rods, or occasionally, cocci (typically occur in pairs).
- Non-motile, non-sporeforming.
- Capsule is present in young cultures.

**Cultural Characteristics**

- On blood agar, the colonies are surrounded by a narrow zone of β hemolysis.
- Colonies are translucent, grayish white and 1-2 mm in diameter on the blood agar.
- The organism grows slowly in nutrient broth, forming a light turbidity and coarse sediment.

**Biochemical Properties**

- *Moraxella bovis* does not produce acid in any of the carbohydrates.
- Gelatin is liquefied slowly.
- Indole is not formed.
- Catalase and oxidase positive.

**Pathogenicity**

*M. bovis* is commensal on the conjunctiva or in the nasopharynx of cattle, causes infectious bovine keratoconjunctivitis, referred to as 'pink eye' leading to ulceration to cornea. Both young and adult cattle are susceptible. The organisms possess pili that help in adherence and colonization to the cornea, circumventing the protective effects of lacrimal secretions and blinking.

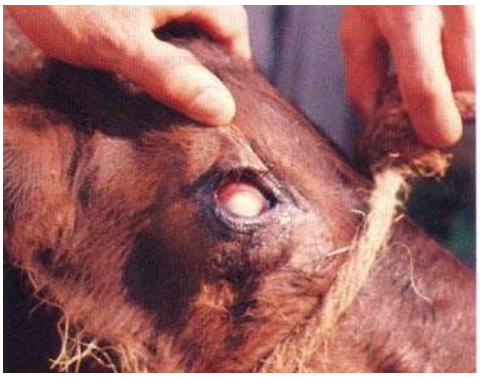


**Fig: Severe cloudiness with elevation of cornea**

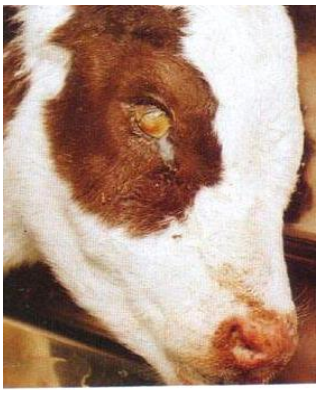


**Fig: Yellow whitish turbid cornea**

**Diagnosis**

- Specimen: Lacrimal secretion. For transportation, swabs of lacrimal secretions should be placed in 1 to 2 ml of sterile water.
- Isolation and cultivation: Specimens should be cultured on blood agar and incubated aerobically at 37°C for 48 to 72 hours. Colonies will appear as round, small, shiny and friable. Virulent strains are surrounded by a zone of complete haemolysis.
- Smears from colonies reveal short Gram negative rods in pairs.
- A fluorescent antibody test is frequently used for rapid identification of *Moraxella bovis* in tears or from cultures.

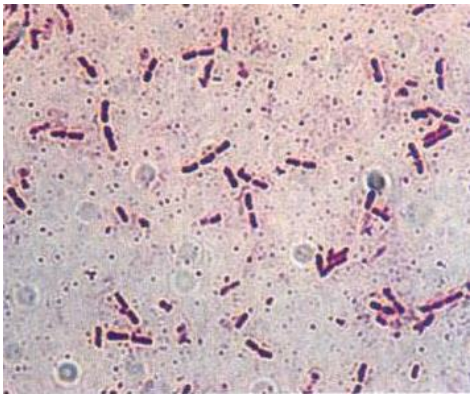


**Fig: *Moxella bovis* (Gram's staining)**

# Brucella

**Principal Characteristics**

- Small, coccobacilli.
- Non-motile, non-sporeforming.
- Catalase and urease positive.
- Gram negative.

**Cultural Characteristics**

- Growth requires an aerobic environment and enriched media such as trypticase soy or blood agar.
- On serum dextrose agar, colonies appear small and delicate which are translucent.
- The normal colony is smooth (S) and other types are mucoid (M) and rough (R).
- In broth, the organism produces turbidity with fine granular deposit

**Biochemical Properties**

- The organisms do not produce indole, do not liquefy gelatin.
- Voges Proskauer and Methyl red reactions are negative.

**Historical**

- *Brucella melitensis* was identified by Bruce in Malta in 1887.
- *Brucella abortus* was first recognized by Bang in 1897.
- *Brucella suis* was discovered by Traum in 1914.

**Pathogenic Species**

| Species | Host | Biotype |
|---|---|---|
| *Brucella abortus* | Bovidae | 1 – 9 |
| *Brucella melitensis* | Goat, sheep | 1 – 3 |
| *Brucella suis* | Swine | 1 – 4 |
| *Brucella canis* | Dogs | - |
| *Brucella ovis* | Sheep | - |
| *Brucella neotomae* | Rat | - |

**Antigenicity**

- Different species of brucellae contain surface protein-lipopolysaccharide surface antigens designated A (abortus) and M (melitensis) in different proportions. In addition a superficial L antigen has been demonstrated.
- The A and M antigens of *Brucella abortus* occur in a ratio of 20:1, whereas in *Brucella melitensis* the proportion is 1:20.
- *Brucella canis* and *Brucella ovis* are antigenically rough and do not possess the A and M antigens. Both possess an R surface antigen.

**Resistance**

- The organisms are killed at 60°C in 10 minutes.
- In milk, the organisms are killed by pasteurization.
- The organisms are susceptible to direct sunlight, acid $p^H$ and disinfectant.

**Pathogenicity**

The organism is highly infectious and usually gains entrance to the body as a result of: **1.** Ingestion of food, water and milk contaminated with uterine discharges, urine or feces of an infected animal **2.** Penetration of the skin **3.** Service by an infected bull.

The incubation period is 30 to 60 days. In the cow, the infection localizes usually after bacteremia, in the placenta of the gravid uterus (placentitis). If the animal is not pregnant, there is usually localization in the udder and adjacent lymphnodes. It may also localize in the liver, lungs, lymphnodes or spleen, where it produces granulomatous foci. Cows may remain infected for years.

The predilection that brucellae have for the gravid uterus, fetal fluids, and testes of the bull, ram and boar is attributed to erythritol. It is not present in the human placenta.

The sequelae of the bovine disease are loss of calves as a result of abortion at 6 months or later and sterility or infertility of either the male or female.

In the bull, infection may localize in the testicle, epididymis or seminal vesicle and abscessation is a common sequelae. *Brucella* organisms may be discharged in the semen.

***Brucella melitensis*:** Causes abortion in sheep, goats; epididymitis and orchitis in rams.

***Brucella suis*:** Causes abortion, sterility, orchitis and high piglet mortality.

In human, *Brucella* causes undulant fever or Malta fever characterized by an acute bacteremic phase followed by a chronic stage that may extend over many years and may involve many tissues. *Brucella melitensis* infection is more acute and severe. *Brucella abortus* usually causes mild disease, *Brucella suis* infection tends to be chronic and *Brucella*

*canis* cause mild disease. The incubation period is 1 – 6 weeks. The disease starts with malaise, fever, weakness, aches and sweats. The fever usually rises in the afternoon and falls during the night.

**Diagnosis**

- Specimen: Fetal tissues and stomach contents, placental cotyledons, vaginal discharges, colostrums, milk, semen, testes, lymphnodes.
- Direct Examination: Stained smears reveal characteristic organism which occur in clumps extracellularly or intracellularly.
- Isolation and Cultivation: Good growth is obtained on tryptose, liver infusion and blood agar. Colonies are round, smooth, glistening and translucent. *Brucella abortus* requires 10% $CO_2$ for initial isolation. Plates should be incubated as long as 30 days.

  Small rods, single or in pairs or short chains are seen in smears from colonies.
- Animal inoculation: The guinea pigs are inoculated subcutaneously with the specimen. The animals are killed 4 – 6 weeks later. The spleen and lymphnodes are cultured and the serum tested for antibodies. The male guinea pigs develop orchitis.
- **Serological test**

  **Milk Ring Test:** Milk ring tests are widely used for testing herds or individual animal. In milk ring test, one drop (0.03ml) of stained brucella antigen (standardized against International Standard Serum) is added to 1 ml of milk that has been kept in refrigerator overnight. The test is read after incubation for 1 hour at 37°C. A positive reaction is indicated by a stained cream layer over white column of milk.

  **Rose Bengal Plate test:** The rose Bengal plate test was introduced when agglutunins produced as a result of vaccination were most acid labile than those arising from field infection. The antigen is stained with Rose Bengal Dye and suspended in buffer at $p^H$ 3.65 (At this $p^H$ the activity of Ig M is reduced and is mediated through the Ig G). Blood serum is added and positive reaction is noted by agglutination.

  **Serum Agglutination Test:** The serum agglutination test is widely used for detection of infection in cattle by using standard antigen. With antigen prepared at the Central Veterinary Laboratory, Weybridge, titre of 50% agglutination at 1:40 or more indicative of infection. A titre of 1:20 is doubtful and animal should be tested again after a few weeks.
- CFT
- ELISA

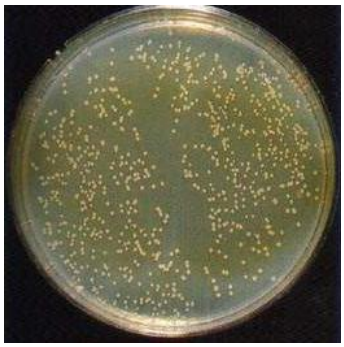


**Fig: Round, transparent small colonies of *Brucella***

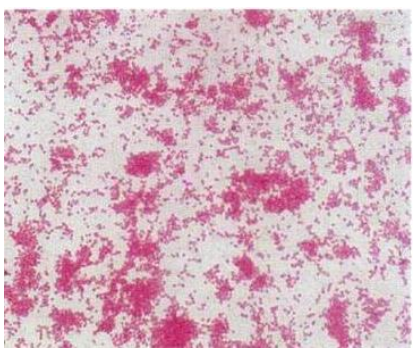


**Fig: *Brucella abortus***

**Control and Prevention**

- Where it is desired to eradicate the disease, test and slaughter method will be the most rational approach. But, this is not commercially feasible in countries like us due to financial embargo on the owner.
- Hygienic disposal of uterine discharges, foetus, foetal membranes.
- The calving should be attended with all hygienic precautions.
- All newly purchased animals are to be kept in strict isolation and tested and the positive reactors must be disposed off.
- Attenuated live vaccine: *Brucella abortus* strain 19 is used in female calves between 4 – 8 months of age. The dose is 5 ml given s/c. The vaccine is not advocated to male calves, because it causes infertility in male calves.

**Differentiation of Important *Brucella* Species**

| Species | $CO_2$ Requirement | Urease | $H_2S$ Production | Agglutination | | Growth in presence of | |
|---|---|---|---|---|---|---|---|
| | | | | A | M | Basic Fuchsin 1:100,000 | Thionin 1: 100,000 |
| *Brucella melitensis* | - | + (slow) | - | - | + | + | + |
| *Brucella abortus* | + | + (slow) | Moderate (2-3 days) | + | - | + (except biotype 2) | - (except biotypes 1, 2 & 4) |
| *Brucella suis* | - | + (rapid) | Heavy (4-5 days) | + | - | - (except biotypes 3 & 4) | + |
| *Brucella canis* | - | + (rapid) | - | - | - | - | + |

# Haemophilus

**Principal Characteristics**

- Small, Gram negative rods, often appear coccobacillary and occasionally may form short filaments.
- The organisms require enriched media, usually blood or its derivatives for isolation.
- On sub cultivation - pleomorphic forms may develop.
- Non-motile, non spore-forming.

**Cultural Characteristics**

- Aerobic or facultative anaerobic.
- Require one or both growth factors termed:

  X factor: Requirement for iron porphyrin, hemin; supplied by blood agar or chocolate agar.

  V factor: Nicotinamide adenine dinucleotide (NAD) or one of its riboside precursors; supplied by fresh yeast

  extract, staphylococcal growth, or chocolate agar.
- Produce 1-2 mm translucent colonies after overnight incubation.

**Habitat**

- Commensals on the mucous membrane of the upper respiratory tract.
- Susceptible to desiccation and do not survive for long periods away from their host.

**Mode of Infection**

- Inhalation
- Fomites
- Endogenous or exogenous

**The Host and X and V Factor Requirements of Some Important Species**

| Organisms | Host | Requirement for | |
|---|---|---|---|
| | | X-Factor | V-Factor |
| *Haemophilus influenzae* | Man | + | + |
| *Haemophilus parainfluenzae* | Man, cat; probably cattle, sheep, fowl | - | + |
| *Haemophilus parasuis* | Swine | - | + |
| *Haemophilus paragallinarum* | Fowl | + | + |
| *Haemophilus parahaemolyticus* | Man and swine | - | + |
| *Haemophilus haemoglobinophilus* | Dog | + | - |

**Antigenicity**

- Encapsulated *Haemophilus influenzae* contains capsular polysaccharides of 6 types.
- *Haemophilus influenzae* can be typed by capsule swelling test with specific antiserum.
- Four distinct types of *Haemophilus suis* have been distinguished.

**Pathogenicity**

***Haemophilus influenzae*:** The organism gains entrance to tissues via the respiratory tract with usually an initial nasopharyngitis. If this infection is not checked it may lead to sinusitis, otitis media and pneumonia. If bacteremia develops, joint infections and meningitis may follow.

***Haemophilus parasuis*:** The organism is a cause of disease in young pig known as 'Glasser's disease'; characterized by serofibrinous pleuirtis, pericarditis and arthritis.

***Haemophilus paragallinarum*:** Causes 'infectious coryza' in poultry; an acute or subacute respiratory disease of worldwide distribution characterized by nasal discharge, sneezing and swelling of the face under the eyes.

***Haemophilus somnus***

- Cannot be classed as true *Haemophilus* as this organism does not require either X or V factor.
- *Haemophilus somnus* infection of cattle is manifested by four principal syndromes-

  1. Respiratory involvement with pneumonia and bacteremia 2. Localization in the CNS with thromboembolic meningoencephalitis 3. Joint infection accompanied by arthritis 4. Reproductive failure

***Haemophilus ducreyi*:** Causes 'chancroid'; a sexually transmitted disease of humans whose consists of a ragged ulcer on the genitalia, with marked swelling tenderness. The regional lymphnodes are enlarged and painful.

**Diagnosis**

- Specimens: Depend on the clinical condition and type of lesions. *Haemophilus* species are fragile and neither refrigeration nor transport media maintain viability. Specimens should be frozen in dry ice and delivered to the laboratory within 24 hours of collection.
- Isolation and Identification: *Haemophilus* species grow on blood or chocolate agar with a *Staphylococcus* streak providing the V factor. Blood agar supplies sufficient hemin, chocolate agar supplies both X and V factors. Plates are incubated for 24 to 48 hours. Small dew drop colonies will appear after 24 hours of incubation. If the V factor is required, the small colonies will appear near the *Staphylococcus* streak (satellite growth).
- Tests for X and V factor requirements: The disc method can be used for determining the requirement for X and V growth factors. Isolates of *Haemophilus* species are spread over nutrient agar and discs containing X, V, and X & V factors are placed on the inoculated media. After incubation in 10% $CO_2$ at 37°C for 3 days, colonies of *Haemophilus* species grow around the discs supplying the growth factor required by the particular isolate.
- Capsule swelling test for *Haemophilus influenzae.*

# Taylorella

**Species:** *Taylorella equigenitalis*

**Synonym:** *Haemophilus equigenitalis*

The organism causes 'contagious equine metritis' (CEM), an acute highly contagious venereal disease of mares and female ponies characterized by a metritis, cervicitis and copious, purulent vaginal discharge. Stallions are infected and spread the disease during coitus but show no clinical signs. Abortion may occur within first 60 days. Spread may also by contaminated equipment and attendants.

# Pasteurella

**Principal Characteristics**

- Short ovoid rods.
- After repeated culture the organism tends to form longer rods and becomes more pleomorphic.
- Non-motile, non spore-forming.
- Gram negative.
- In smears from infected tissues stained by the Giemsa method, pasteurellae exhibit bipolar staining. (Also with methylene blue or Leishman's stain).

**Habitat**

- Commensals on the mucosae of the upper respiratory tract of animals. Their survival in the environment is short.

**Antigenic Nature**

- The types (or serogroups) of *Pasteurella multocida* are identified on the basis of differences in capsular substances (polysaccharides) and are designated A, B, D, E and F.

- Capsular types may be subdivided further into somatic types on the basis of serologic differences in lipopolysaccharides (somatic or O antigens). A serotype is designated by the capsular type, followed by the number representing the somatic type, e.g. serotype B: 2 is the cause of epidemic haemorrhagic septicaemia.
- Three biotypes or subspecies of *Pasteurella multocida* are recognized, namely *Pasteurella multocida* subspecies *multocida, Pasteurella multocida* subspecies *septica* and *Pasteurella multocida* subspecies *gallicida*.
- Two different biotypes of *Pasteurella haemolytica* have been identified: biotype A and biotype T. *Pasteurella trehalosi* is now used to denote *Pasteurella haemolytica* biotype T isolates while biotype A isolates of *Pasteurella haemolytica* have been allocated a new genus and renamed *Mannheimia haemolytica*.
- Seventeen serotypes of *Mannheimia haemolytica/Pasteurella trehalosi* are recognized on the basis of extractable surface antigens. Serotypes 3, 4, 10 and 15 are classified as *Pasteurella trehalosi*; the remaining serotypes are classified as *Mannheimia haemolytica*.

**Host Range, Disease of Principal *Pasteurella* spp.**

| Species/Serotype | Major Host(s) | Disease | Distribution |
|---|---|---|---|
| *Pasteurella multocida* | Cattle | Shipping fever, Mastitis | Global |
| type – A | Deer | Septicaemia | Global |
| | Sheep | Pneumonia, Mastitis | Global |
| | Poultry | Fowl cholera | Global |
| | Swine | Pneumonia, atrophic rhinitis | Global |
| *Pasteurella multocida* | Camel | Haemorrhagic septicaemia | Sudan |
| type – B | | | |
| type B:3 | Deer | Haemorrhagic septicaemia | U.K. |
| type B:6 | Buffalo, cattle | Haemorrhagic septicaemia | Asia |
| type D | Swine | Atrophic rhinitis, pneumonia | Global |
| | Poultry | Fowl cholera | Global |
| type E | Buffalo, cattle | Haemorrhagic septicaemia | Central Africa, Europe, Russia |
| *Mannheimia haemolytica* | | | |
| biotype A1 | Cattle | Shipping fever, Fibrinous pneumonia, Mastitis | Global |
| biotype A2 | Sheep | Pneumonia | Global |
| biotype T (*P. trehalosi*) | Sheep | Septicaemia | Global |
| *Pasteurella pneumotropica* | Rodents | Pneumonia | Global |

**Pathogenicity**

- The diseases associated with *Pasteurella multocida* infection include haemorrhagic septicaemia in ruminants, porcine atrophic rhinitis, fowl cholera and bovine pneumonic pasteurellosis.
- Haemorrhagic septicaemia: An acute, fatal septicaemia particularly virulent for buffaloes; less virulent for cattle, pigs, sheep, goat and others. *Pasteurella multocida* serogroup B: 6 is more prevalent in Asia. Predisposing factors such as overwork, poor body condition and monsoon rains are important in its development.

  Sudden onset of high fever, respiratory distress and a characteristic edema of the laryngeal region, which may extend to the throat and to the brisket are features of the disease.

  Gross pathological changes may include widespread petechial haemorrhages, enlarged haemorrhagic lymphnodes and blood-tinged fluid in the pleural cavity and the pericardial sac.
- Fibrinous pneumonia is accompanied by thickening of interlobular septa and fibrinous pleurisy.
- Bovine pneumonic pasteurellosis (shipping fever): The condition is commonly associated with *Mannheimia haemolytica*, although, *Pasteurella multocida* has also been isolated from lungs of affected cattle. The principal serotype of *Mannheimia haemolytica* associated with the disease is A1.

The disease is characterized by severe bronchopneumonia and pleurisy occurs most commonly in young cattle, particularly when the animals are subjected to severe stress such as transportation, close confinement etc.

- Fowl cholera: Avian pasteurellosis caused by *Pasteurella multocida* capsular type A affecting both wild and domestic birds. The disease is seen in peracute, acute and chronic forms.

  Clinically: discharge from beak, nostrils; diarrhoea; bright yellow or green, sometimes blood stained.

  Post mortem lesions: Acute - serofibrinous pericarditis, haemorrhagic enteritis (mainly duodenum).

  Sub acute or chronic: nasal catarrh or sinusitis, swelling and edema of wattles, lameness due to arthritis.

**Diagnosis**

- Specimen: Blood, tracheobronchial aspirates, nasal swabs, saliva, mastitic milk, faeces, urine, subcutaneous tissue fluids and others.
- Direct examination: Large number of coccobacilli exhibiting bipolar organisms can be demonstrated in blood smears in septicemias; stained by Giemsa or methylene blue staining.
- Isolation and Cultivation: Good primary growth requires media enriched with serum or blood. Colonies appear after incubation for 24 hours at 37°C in air or in atmosphere of 6 to 8% $CO_2$. They are usually of moderate size, round and grayish and non-haemolytic. Some strains produce large mucoid colonies. Colonies have a characteristic sweetish odour. Smears reveal small, Gram negative rods and coccobacilli.
- Identification: Non-motility, indole production, lack of hemolysis and production of oxidase are of special significance.

**Fig: Colonies of *P. multocida* on blood agar**

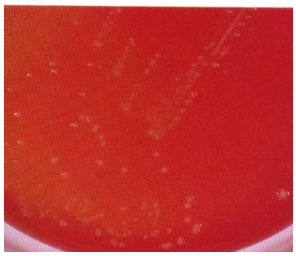


**Fig: Colonies *P. haemolytica* on blood agar**

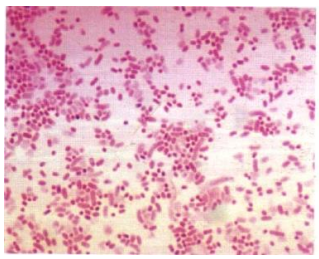


**Fig: *Pasteurella sp*. (Gram staining)**

**Differential Characteristics**

| Characteristics | *Pasteurella multocida* | *Mannheimia haemolytica* | *Pasteurella pneumotropica* |
|---|---|---|---|
| Glucose fermentation | + | + | + |
| Maltose fermentation | V | + | + |
| Sucrose fermentation | + | + | + |
| Lactose fermentation | - | + | - |
| Indole production | + | - | + |
| Growth on MacConkey agar | - | + | - |
| Urease activity | - | - | + |
| Oxidase activity | + | + | + |
| Catalase activity | + | + | + |

**Prevention**

- Vaccination is routinely used; especially before the beginning of the rainy season
- Vaccines

  i. Formalize inactivated strains of *Pasteurella multocida* isolated from cases of haemorrhagic septicaemia. HS vaccine is produced by LRI, Mohakhali. The inactivated organisms are incorporated in an oil emulsion adjuvant. Dose: Cattle & buffalo: 2 ml and Sheep/Goat: 1 ml; s/c at the neck region and the booster dose on 2 - 4 weeks after the 1st injection and immunity persists for one year, and thus it is necessary to revaccinate at year interval.

  ii. Fowl cholera vaccine (LRI, Mohakhali): 0.5 ml/bird s/c of more than 2 month and the booster dose should be given on 21 days after the first injection. Immunity persists for 6 months and thus it is necessary to revaccinate in every 6 months.

# Francisella

**Species:** *Francisella tularensis*

Produce tularemia in rabbits and wild animals and in humans.

Humans: sudden onset of flue like symptoms with fever, chills, prostration and regional lymphadenitis.

# Campylobacter

**General Characteristics**

- Thin, spirally curved (one or more spirals), Gram negative rods.
- Motile due to single polar flagellum.
- Microaerophilic (5 – 10% $O_2$) to anaerobic.
- Oxidase and catalase positive.

**Important *Campylobacter* species**

| Species | Host | Habitat | Diseases |
|---|---|---|---|
| *Campylobacter fetus* subspecies *venerealis* | Cattle | Bovine reproductive tract | Metritis, early embryonic death, abortion |
| *Campylobacter fetus* subspecies *fetus* | Cattle, sheep, goat, human and others | Intestinal tract | Abortion, stillbirths in sheep and goats, sporadic abortion in cattle |
| *Campylobacter jejuni* | Birds and mammals | Intestinal tract | Abortion in sheep, enteritis in dogs, avian hepatitis, enterocolitis in humans |
| *Campylobacter coli* | Pigs | Intestine | Diarrhoea |
| *Campylobacter fecalis* | Sheep | Intestine | Diarrhoea |

## *Campylobacter fetus*

Strains of *Campylobacter fetus* comprise two subspecies, *Campylobacter fetus* subspecies *fetus* and *Campylobacter fetus* subspecies *venerealis*. Subspecies *venerealis* can further be separated on the basis of $H_2S$ production into two biotypes, *venerealis* and *intermedius.*

**Morphology**

- In young culture the organism is comma shaped and S shaped.
- In old culture, spiral forms are seen in which the organisms cling together.
- Motile by single polar flagellum.
- Gram negative.

**Cultural Characteristics**

- *Campylobacter* species are strictly microaerophilic, requiring an atmosphere of 5 to 10% oxygen and $CO_2$ for growth.
- A selective enriched medium such as Skirrow agar is used for primary isolation. Colonies are small, round, smooth and translucent with a dewdrop appearance.
- It can also be isolated on blood agar and serum agar but thiol media is superior to either media. On blood agar, colonies are fine, pin pointed bluish areas, 1 to 3 mm in diameter; raised above the surface of the media.

**Biochemical Properties**

- It does not ferment sugar.
- It does not form indole.

**Mode of Transmission**

- From bull to cow and cow to bull during coitus.
- Through A.I. where infected semen is used.
- From bull to bull through contaminated semen collecting apparatus.
- Cow to cow when improperly cleaned instruments used for the treatment of genital tract of cows.

**Pathogenicity**

*Campylobacter fetus* subspecies *venerealis*, is the principal cause of bovine genital campylobacteriosis, is transmitted during coitus to susceptible cows by asymptomatic carrier bulls. The bacteria survive in the glandular crypts of the prepuce and may remain infected indefinitely. The disease is characterized by temporary infertility, early embryonic mortality, and abortion.

**Diagnosis**

- History of infertility and abortion.
- Specimen: Uterine exudates, cervical mucus, prepucial secretion, placenta, stomach contents of aborted fetus. Specimens of mucus should be placed in special transport medium.
- Direct examination: Smears stained by Gram method disclose Gram negative thin, curved bacillus. Short forms (comma-shaped), medium forms (S-shaped), and long forms (helical with several spirals) may be observed.

- Isolation and Cultivation: Grows best in microaerophilic condition containing 5-10% $O_2$. Colonies of *C. fetus* usually appear in the recommended media (Skirrow agar) after 2–5 days. Growth may be slow, particularly in the presence of contaminating bacteria in the samples. To prevent overgrowth of specific colonies by contaminants, it is recommended to check the media daily and to subculture colonies suspected of being *C. fetus*. After 3–5 days of incubation, colonies measure 1–3 mm in diameter. They are slightly grey-pink, round, convex, smooth and shiny, with a regular edge. Cultures should be incubated for a minimum of 6 days.
- Agglutination test: The antibodies in the vaginal mucus are present for 2 – 12 months or even longer after infection. The mucus is collected in pipettes and mixed with saline. The antibodies are extracted from the mucus and test is carried out in tubes adding specific antigen.
- PCR for rapid screening test in bull's semen.

**Control and Prevention**

- Abortions in cattle can be reduced by antibiotic therapy.
- Killed vaccine may reduce the incidence of diseases in a herd but do not eradicate the infection.

**Public Health Significance**

Veterinarians, farmers, packing house workers and others associated with cattle and sheep occasionally sustain infections due to *Campylobacter fetus*. Diseases attributed to these infections are abortions, enteritis, endocarditis and fever with bacteremia.

### *Campylobacter jejuni*

- Commensal in the intestinal tract of many species of domestic and wild animals, including birds and poultry.
- Dogs and cats frequently shed the organism in their feces, cause febrile enteritis with diarrhoea in these animals.
- Cause abortion in bitches and does, and mastitis in cows.
- Cause enteritis in humans; signs include fever, abdominal pain, nausea, vomiting, blood in stool and diarrhoea.
- In chicken: Avian infectious hepatitis (Vibrionic hepatitis).

## Helicobacter

**Species**

- *Helicobacter pylori*: Isolated from humans with chronic gastritis and duodenal ulcer.
- *Helicobacter felis*: Infects cat and cause gastritis.
- The organisms are highly prolific producers of urease; have ability to colonize the stomach by providing an alkaline environment.

# Vibrio

**Principal Characteristics**

- The vibrios are among the most common bacteria in surface waters worldwide.
- Facultative anaerobe.
- Gram negative, straight or curved rods.
- Motile, oxidase positive.
- Fermentative; ferment glucose without gas.

**Species**

*Vibrio cholerae*

*Vibrio cholerae* serotype Ol and other related *Vibrio* causes cholera in human which is characterized by profuse watery diarrhoea that can rapidly lead to dehydration and death.

**Other species**

- *Vibrio parahaemolyticus*: Causes acute gastroenteritis following ingestion of contaminated sea food such as raw fish or shellfish.
- *Vibrio vulnificus*: Causes severe wound infection.
- *Vibrio anguillarum*: Produces vibriosis in fish, characterized by haemorrhages and ulcerations in skin, fin, tail and haemorrhages and degenerative changes of internal organs.

# Fusobacterium

**Species:** *Fusobacterium necrophorum*

**Synonym:** *Sphaerophorus necrophorus*

**Morphology**

- Cylinder, pleomorphic organism, varying from coccoid to filamentous forms.
- In young culture, the filamentous forms are most prevalent, but as the culture ages these filaments appear to segment and to break up into numerous coccoid and bacillary forms.
- Numerous of the short rods contain granules.
- Gram negative, non motile, non spore forming.

**Growth Characteristics**

- **Strictly anaerobic** (Obligate anaerobes), grows at temperature between 30°C and 40°C, grows abundantly in medium which is slightly alkaline but growth occurs between $p^H$ 6.0 to 8.4.
- Pure cultures are more easily obtained from contaminated material by inoculating a rabbit s/c with the necrotic material. Subcutaneous abscesses develop within a week and in many cases isolated abscesses are formed in the liver. Necrotic material is transferred from the abscess to a number of test tubes of Rosenow's dextrose brain broth.
- In broth, the organism produces a uniform turbidity and the formation of a slight, fine, dirty white sediment. As the culture ages, the medium clears and the sediment increases in quantity and becomes darker in color.
- On serum agar, incubated under anaerobic conditions, the organism forms small, round, opaque, circular colonies.
- A small zone of ß hemolysis is formed around the colony on blood agar.

**Biochemical Properties**

- The organism produces acid and gas from glucose, maltose and glycerol; slight fermentation of lactose, galactose, fructose, arabinose and mannitol.
- Does not liquefy gelatin, does not reduce nitrates.
- $H_2S$ is produced.
- The organism gives positive reaction to the indole test and negative reaction to the methyl red and Voges Proskauer tests.

**Mode of Infection**

- Commensal in the alimentary tract and on mucous membranes.
- Infections are endogenous.

**Antigens**

Three biotypes of *Fusobacterium necrophorum*:

a. Biotype A: Designated *Fusobacterium necrophorum* subspecies *necrophorum*; most frequently isolated in pure culture from bovine liver abscess. More virulent than biotype B and has greater haemolytic activity.
b. Biotype B: Designated *Fusobacterium necrophorum* subspecies *fundaliforme*; predominates in ruminal contents and ruminal lesions, usually isolated in mixed infections from liver abscesses.
c. Biotype C: Reclassified as *Fusobacterium pseudonecrophorum*; avirulent species.

**Pathogenicity**

- The organism is responsible for necrotic and gangrenous lesions in a variety of species of animals; characterized by a necrosis of tissues with subsequent accumulation of tissue debris. The organisms do not progress far from the localized lesion but in some cases metastasis occurs and abscesses are formed within internal organs such as the liver.
- In all animals, the condition produced by the organism is called necrobacillosis.
- In calf, it produces calf diphtheria- an infectious disease of calves affecting the larynx (necrotic laryngitis), oral cavity (necrotic stomatitis) or pharynx (necrotic pharyngitis) characterized by fever, ulceration and swelling of the affected structures.
- Hepatic abscessation in cattle, most commonly encountered in feedlot animals.
- Sheep: The organism causes foot rot, ulcers on the lips, necrotic vulvitis.
- Goat: Causes necrotic stomatitis.
- Horse: Usually involved in the infectious process called “Thrush”- involving frog of the hoof; characterized by a foul-smelling discharge from the hoof.
- Swine: “Bull nose” characterized by suppuration and necrosis of the snout.
- **Foot rot**

  **Synonym:** Interdigital necrobacillosis

  The disease is characterized by symmetrical edema and erythema of the interdigital region, severe lameness, elevated body temperature and lactation may cease. Eventually, necrosis occurs and longitudinal fissure appears which reveals a purulent, foul smelling discharge and central mass of necrotic tissue.

**Diagnosis**

- Specimen: Affected tissue, pus from abscesses. Specimens should be cultured immediately or precautions must be taken to prevent exposure to oxygen.
- Direct Examination: Gram stained smears of affected tissue reveals gram negative rods of variable length and long characteristically beaded filaments.
- Isolation and Cultivation: The organism is a strict anaerobe and grows best on enriched media. Several days of incubation are required. Many strains produce some L forms on initial isolation. Colonies are small, smooth, convex and whitish yellow in color with a narrow zone of α or ß hemolysis. Initially cultures may be pleomorphic, short rods, long filaments may be seen.
- Animal Inoculation: Rabbit inoculation and isolation; mice are susceptible but guinea pigs are somewhat resistant.

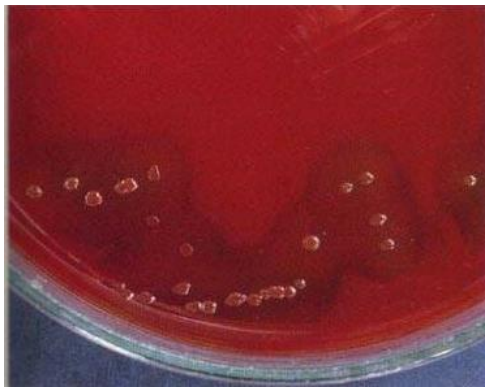


**Fig: Colonies *F. necrophorum* on blood agar**

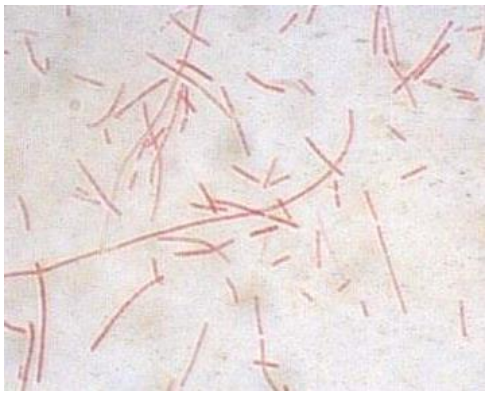


**Fig*: F. necrophorum* (Gram staining)**

# Bacteroides

**Morphology**

- Rod shaped, Gram negative organism.
- Non motile, non spore and non capsule forming.
- Strict anaerobe.

**Species**

- *Bacteroides nodosus*

  Synonym: *Fusiformis nodosus*

  Current nomenclature: *Dichelobacter nodosus*

  Causes foot rot in sheep and cattle.
- *Bacteroides melaninogenicus*

  Current nomenclature: *Prevotella melaninogenica*

  Frequently associated with *Fusobacterium necrophorum* in foot rot of cattle.
- *Bacteroides asaccharoliticus*

  Current nomenclature: *Porphyromonas asaccharolytica*

  Causes periodontal disease in animals; characterized by periodonitis and gingivitis. This is the main reason for teeth loss in dogs.
- *Bacteroides fragilis* – Causes of foal diarrhea.

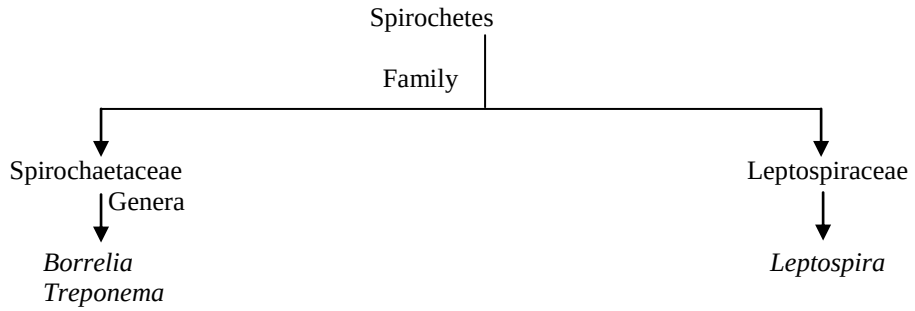
# Alkaligenes

- **Species:** *Alkaligenes faecalis*
- Motile by peritrichous flagella, does not ferment any carbohydrate.
- Alkaline reaction in litmus milk.
- Commensal of intestinal tract of man and animals.
- Although no pathogenic significance, some scientist reported that the organism might cause abortion in cattle.

**Foot Conditions in Farm Animals Associated with Mixed Infections including Anaerobic Non-Spore-Forming Bacteria**

| Species | Disease Condition | Bacteria Implicated |
|---|---|---|
| Sheep | Interdigital dermatitis | *Fusobacterium necrophorum*<br>*Dichelobacter nodosus* |
| | Footrot | *Fusobacterium necrophorum*<br>*Dichelobacter nodosus*<br>*Arcanobacterium pyogenes* |
| Cattle | Interdigital necrobacillosis (Foul-in-the-Foot) | *Fusobacterium necrophorum*<br>*Porphyromonas levii* |
| | Interdigital dermatitis | *Fusobacterium necrophorum*<br>*Dichelobacter nodosus*<br>*Prevotella spp.* |
| Pigs | Foot abscess in young pigs | Mixed anaerobes |

# Spirochetes




* Majority are saprophytes, a few are commensals and some are pathogenic, causing disease in both humans and animals.
* Spirochetes are inactive biochemically and hence differentiation is difficult by this means. Identification is based on antigenic properties.
* Although spirochetes are cytochemically Gram negative but they stain poorly. They may be demonstrated by special procedures-
    a. Giemsa or Wright Stain
    b. India ink or Nigrosin (Negative stain)

c. Silver Impregnation

d. Darkfield Microscopy

e. Immunofluorescence

# Leptospira

**Morphology**

- Tightly coil, thin, flexible spirochetes.
- Closely spiraled with one end commonly bent into the shape of a hook.
- Active rotational motion but no flagellum has been reported. Electron micrographs show a thin axial filament and a delicate membrane.
- Barely be seen by the usual microscopic methods, but easily visualized by darkfield microscope.
- It does not stain normally with conventional coalter dyes; but can be impregnated with silver salts using a technique described by Fontana.

**Growth Characteristics**

- Grows best under aerobic conditions at 29°C to 32°C in protein rich semisolid media (Fletcher's media) where they produce round colonies 1 to 3 mm in diameter in 6 to 10 days.
- *Leptospira* also grows in chorioallantoic membrane (CAM) of embryonated eggs.
- *Leptospira* derive energy from long chain fatty acids and cannot use amino acids or carbohydrates as a major source of energy. Ammonium salts are main sources of nitrogen.

**Species**

Two species –

*a. Leptospira interrogans*

*b. Leptospira biflexa*

*Leptospira interrogans* cause disease in man and animals whereas *Leptospira biflexa* is free living saprophyte. There are more than 180 serovars and 19 serogroups.

**Principal Leptospirae associated with infection in animals**

| Host | Species |
|---|---|
| Cattle | *Leptospira interrogans* serovar *pomona* |
| | *Leptospira interrogans* serovar *grippotyphosa* |
| | *Leptospira interrogans* serovar *canicola* |
| | *Leptospira interrogans* serovar *icterohaemorrhagiae* |
| | *Leptospira interrogans* serovar *hardjo* |
| Horse | *Leptospira interrogans* serovar *pomona* |
| | *Leptospira interrogans* serovar *canicola* |
| | *Leptospira interrogans* serovar *grippotyphosa* |
| | *Leptospira interrogans* serovar *icterohaemorrhagiae* |
| Sheep & Goat | *Leptospira interrogans* serovar *pomona* |
| | *Leptospira interrogans* serovar *grippotyphosa* |
| Pig | *Leptospira interrogans* serovar *pomona* |
| | *Leptospira interrogans* serovar *canicola* |

| | |
|---|---|
| | *Leptospira interrogans* serovar *icterohaemorrhagiae* |
| Dog | *Leptospira interrogans* serovar *canicola*<br>*Leptospira interrogans* serovar *icterohaemorrhagiae*<br>*Leptospira interrogans* serovar *pomona* |

**Mode of Infection**

- Direct contact with the urine of infected animals or ingestion of urine contaminated with water or food. Transmission is even possible through drinking water contaminated by urine of rodents or wild animals.
- The organisms may enter the animal's body through abraded skin, mucous membrane or conjunctiva.
- Transplacental infection can occur in cattle, pig and men.
- Calves can acquire infection through milk.

**Pathogenicity**

| | |
|---|---|
| Bovine Leptospirosis | ➢ Principally caused by *Leptospira interrogans* serovar *pomona. Leptospira interrogans* serovar *hardjo* causes fewer abortions but results in infertility. Serovar *grippotyphosa*, *canicola* or *icterohaemorrhagiae* is involved occasionally.<br>➢ Sources of the organisms are urine. Cattle may shed the organism for 3 months. Outbreaks are associated with heavy rainfall.<br>➢ Red water (discolored urine from pink to dark brown, including acute nephritis) is seen in calves and young animals, accompanied by anemia and jaundice. Encephalitis may occur. Death from renal or hepatic failure may occur in 3 to 10 days, while survivors may develop chronic nephritis with poor renal function and excrete leptospirae. An outcome in pregnant cows is abortion or stillbirth, one to three weeks after the acute illness.<br>➢ Lesions include – Jaundiced appearance of carcass, congestion of kidney with pinpoint hemorrhages, enlargement of spleen. |
| Canine Leptospirosis | ➢ Primarily caused by *Leptospira interrogans* serovar *canicola* and less frequently by *Leptospira interrogans* serovar *icterohemorrhagiae.*<br>➢ Infected dogs sporadically shed *Leptospira* in their urine and serve as sources of *Leptospira interrogans* serovar *canicola* and *Leptospira interrogans* serovar *icterohemorrhagiae* infections. Dogs may shed *Leptospira* in their urine for 2 to 6 months and a risk to humans close to them.<br>➢ Four principal forms are recognized: 1. The hemorrhagic form 2. The icteric form 3. The uremic or subacute form 4. The inapparent form. The first two forms are primarily caused by serovar *icterohemorrhagiae* whereas the latter two forms are usually caused by serovar *canicola*. Acute Leptospirosis commencing with vomiting and red eyes progresses to nephritis (Arched back, immobility, blood stained urine), dehydration and frequently death within four days. Rapid death within hours characterizes a peracute form of *icterohaemorrhagiae i*nfection. |

| | |
|---|---|
| Equine Leptospirosis | ➢ Congenital infection, stillbirth, abortion. Clinical picture is usually the same as in cattle. A form of chronic recurrent eye infection (uveitis) may develop 2 to 8 months later in survivors. |
| Swine Leptospirosis | ➢ Abortions and stillbirth, congenital infections. |

**Diagnosis**

**Specimens**

1. Urine, portions of kidney and liver. Because of the fragility of *Leptospira* and the time and effort involved, culture is not usually practicable.
2. Portions of liver and kidney, both fresh and fixed.
3. Fresh urine or urine containing formalin. Add 1.5 ml of 10% formalin to 20 ml of urine. Formalin will keep *Leptospira* intact for days to weeks.
4. Paired serum samples for serological test.

**Procedure**

1. **Microscopic examination:** Demonstration of *Leptospira* in fresh urine or formalized urine by Dark field examination of thick smears stained by Giemsa's technique. Thin, bright, coiled bacteria will be seen. Occasionally show *Leptospira* in fresh blood from early infection. Microscopic examination can also be done by silver impregnation technique described by Fontana.
2. **Culture:** Whole fresh blood or urine cultured in Fletcher's semisolid medium. Growth is slow and culture should be kept for several weeks (4 weeks) at 25°C to 30°C.
3. **Animal inoculation:** Guinea pig is inoculated intraperitonially with fresh plasma or urine. Within a few days, spirochetes become demonstrable in the peritoneal cavity on the death of the animal, haemorrhagic lesions with spirochetes are found in many organs.
4. **Serological Test**
   a. Microscopic Agglutination test (MAT)
   b. Fluorescent Antibody Technique (FAT)
   c. ELISA
5. PCR (Polymerase chain reaction)

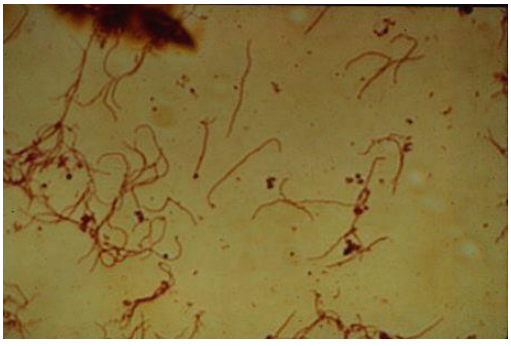


**Fig: *Leptospira***

**Control**

- Rodent control
- Treatment of carrier animals

- Protective clothings and occupational hygiene
- Active protection: In dog, cat and pigs – Vaccines are available containing suspensions of killed serovars. The vaccines are given s/c or i/m in two initial doses, one month apart followed by boosters.

**Public Health Significance**

- Human beings acquire infections from infected domestic animals, rodents and contaminated water. The organisms may also enter through mucous membranes or breaks in the skin.
- The disease is referred to several names – Weil's disease, Fort Bragg fever and Swineherd's disease.
- Various serovars of *Leptospira*, including *canicola, icterohemorrhagiae* and *pomona* can infect humans.
- Veterinarians, slaughterhouse workers and farmers are at particular risk. The acute form of the human disease is characterized by febrile jaundice and nephritis.

# Borrelia

**Species:** *Borrelia anserina*

**Synonym:** *Borrelia gallinerum*

Causes fowl spirochetosis (Avian Borreliosis): A disease of chicken, ducks, turkeys and geese characterized by an acute septicemia accompanying with fever, diarrhoea, drowsiness and emaciation. It is transmitted by the bites of ticks, e.g. *Argas persicus* and others. The organism may be passed in eggs to the next generation of ticks.

**Diagnosis**

- Direct examination – Giemsa stained blood film
- Dark field microscopy
- Can be cultivated in chicken embryo.

**Relapsing Fever**

A disease is caused by a number of *Borrelia spp*., e.g. *Borrelia recurrentis*, occurs sporadically in U.S. *Borrelia persica* – Asiatic-African tick borne relapsing fever. It is characterized by recurrent febrile attacks during which the organism can be recovered from the blood.

*Borrelia vincentii*, *Borrelia buccale*: Opportunistic pathogen; cause ulcerative disease involving the gum and oral cavity. Vincent angina (Acute sorethroat), ulcerative tonsillitis in dogs and other mammals.

*Borrelia burgdorferi*: Lyme disease in man.

# Treponema

**Species**

*Treponema suis*: Preputial infections in pigs.

*Treponema hyodysenteriae*: Swine dysentery

*Treponema cuniculi*: Rabbit syphilis or Vent disease. It is a true venereal disease in which lesions consisting of vesicles and scabs are seen mainly involving the prepuce, vagina and perineal region. Thick scaly crusts persist in the female for months.

**Diagnosis**

Dark field microscopy.

*Treponema pallidum*: Causes exclusively syphilis in human.

Three forms of the disease – Primary, Secondary, Tertiary lesion.

Primary lesions occur by sexual contact and subsequent appearance of syndrome characterized by papule which undergoes lysis and form hard chancre. After 3 to 5 weeks secondary lesions appear, characterized by red maculopapular lesion and moist papule appears the anogenital region. After 3 to 5 years tertiary stage begins characterized by the development of granulomatous lesions in skin, bones and liver.

**Distinguishing features of *Borrelia, Treponema and Leptospira:***

| **Characteristics** | ***Borrelia*** | ***Treponema*** | ***Leptospira*** |
|---|---|---|---|
| Length | 8-16 µm | 5-18µm | 6-20µm |
| Width | 0.25-0.3µm | 0.09-0.5µm | 0.1-0.2µm |
| Spiral number | 4-8, loose | 6-14, regular | Many, fine, tight |
| Ends | Taper terminally to fine filaments | Pointed, may have terminal filaments | One or both ends have a semilunar hook |
| Staining: | | | |
| Gram | Yes | No | No |
| Giemsa | Yes | Poor | Poor |
| Silver impregnation | Not necessary | Yes | Yes |