

STREPTOCOCCUS

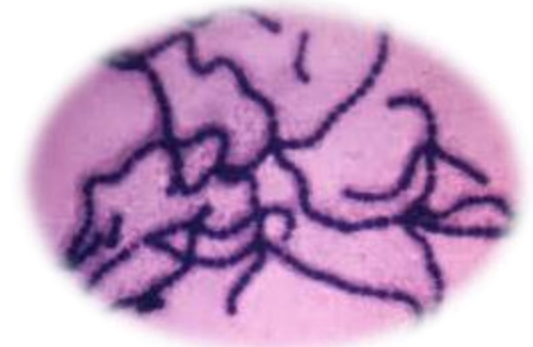
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MORPHOLOGY

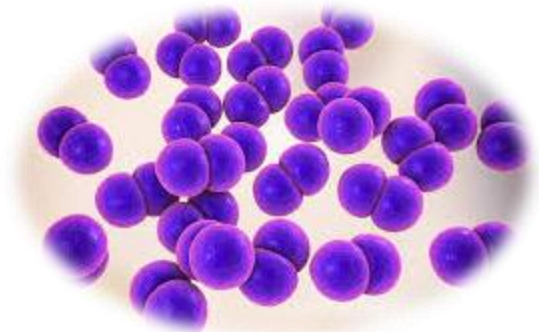
- Gram positive , non motile except ***Lactococcus lactis*** non spore forming cocci occurs singly, in pairs or in chains.
- Long chains are observed in ***Streptococcus equi***
- ***Streptococcus pyogenes*** round to ovoid and occur in pairs or chains of varying length
- ***Streptococcus pneumoniae*** occur as diplococcus and are lancet shaped. Also known as pneumococcus
- In older cultures they will lose Gram positive character.
- Strict anaerobe/ facultative anaerobe (catalase and oxidase negative)



Lactococcus lactis



Streptococcus equi

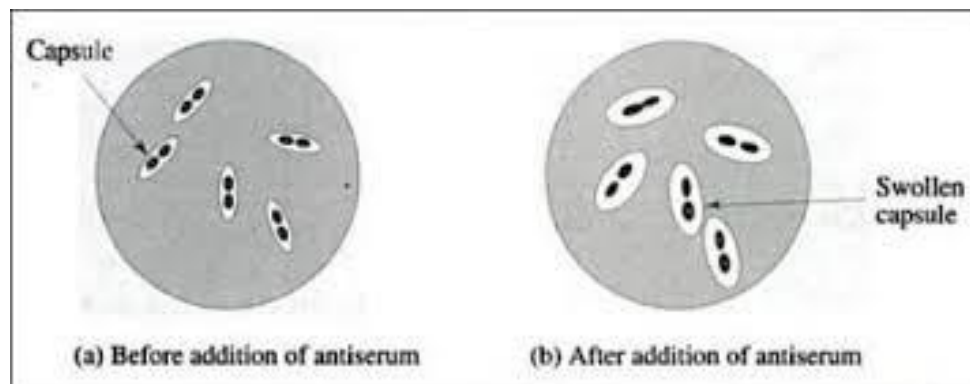


Streptococcus pneumoniae



CAPSULE

- *Streptococcus pyogenes* have hyaluronic acid capsule
- *Streptococcus pneumoniae* have polysaccharide capsule
- *Streptococcus pneumoniae* give **Quellung reaction**, it is a test for capsule demonstration
- In quellung reaction colonies are mixed with hyper immune sera against capsule , there will be swelling of capsule



CLASSIFICATION OF STREPTOCOCCI

- Streptococci can be classified on the basis of
- Growth characteristics
- Haemolysis,
- Serologic specificity



GROWTH CHARACTERISTICS

- Pyogenic streptococci: *Streptococcus pyogenes*
- Oral streptococci: *Streptococcus salivarius*
- Enterococci: *Enterococcus faecalis*
- Lactic streptococci: *Lactococcus lactis*
- Anaerobic streptococci
- Other streptococci



HAEMOLYTIC PATTERN

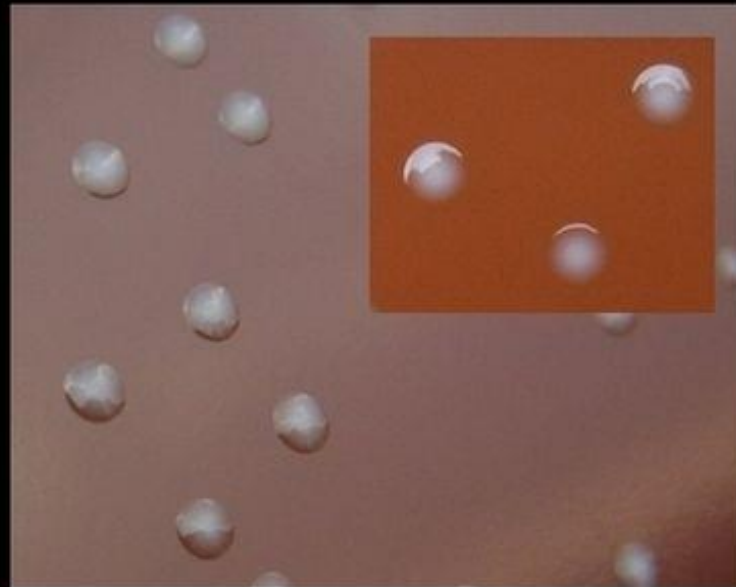
- **α haemolysis:** Partial haemolysis with a zone of green coloration around the colonies.
- **β haemolysis:** complete clear zone of haemolysis around the colonies.
- **γ haemolysis:** No detectable haemolysis
- **α' haemolysis:** a small zone of partial haemolysis followed by a zone of complete haemolysis





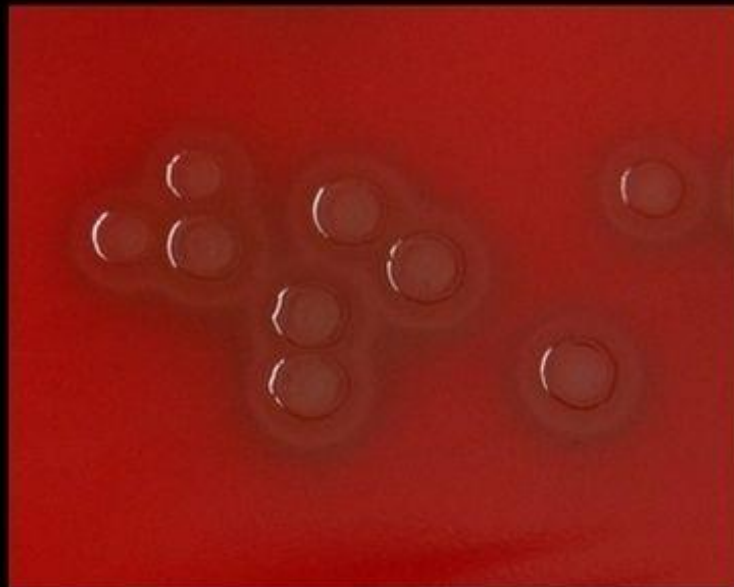
Klebsiella pneumoniae

gamma hemolysis



Enterococcus faecalis

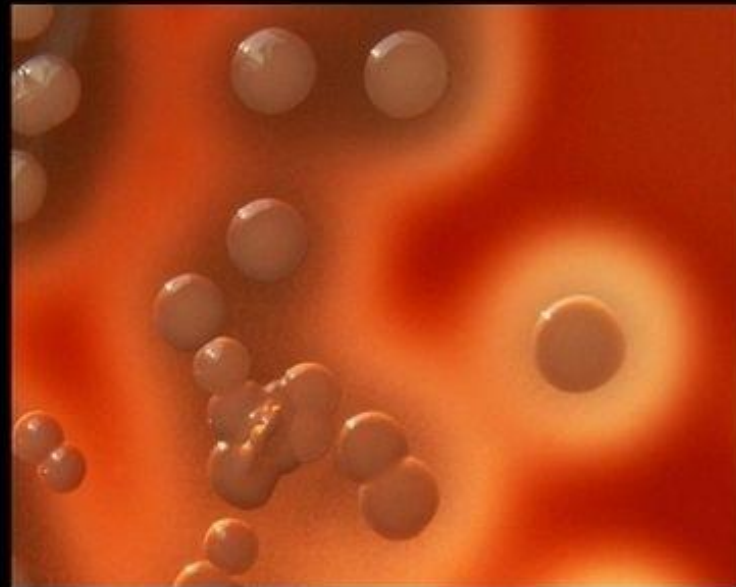
gamma hemolysis



FN

Streptococcus pneumoniae

alpha hemolysis



Staphylococcus aureus

beta hemolysis



LANCEFELD CLASSIFICATION

- by Rebecca Lance field in 1926
- This classification of streptococci is based on the serologic difference in a carbohydrate substance called '**C**' **substance** found on the cell wall of streptococci.
- Here streptococci are divided into different groups and each group is further divided into different types.
- Precipitation test is used to differentiate each group.
- Letters A,B,C used to designate different groups and Arabian numerals used to designate different types
- Typing within the group is based on the serological difference in M protein.



LANCEFIELD CLASSIFICATION

Classification

- Group A - *Streptococcus pyogenes*
- Group B - *Streptococcus agalactiae*
- Group C - *Streptococcus equisimilis*, *Streptococcus equi*, *Streptococcus zooepidemicus*, *Streptococcus dysgalactiae*
- Group D - *Enterococci*, *Streptococcus bovis*
- Group E - *Streptococcus milleri* and *mutans*
- Group F - *Streptococcus anginosus*
- Group G - *Streptococcus canis* and *Streptococcus dysgalactiae*
- Group H - *Streptococcus sanguis*
- Group L - *Streptococcus dysgalactiae*
- Group N - *Lactococcus lactis*
- Group R&S - *Streptococcus suis*
- other *Streptococcus* species are classified as 'non-Lancefield Streptococci'



| Lancefield group | Species | Haemolysis | Host(s) | Disease | Natural habitat (if known) |
|------------------|--|---------------------------------|------------------------------|---|-------------------------------|
| A | <i>S. pyogenes</i> | β | Humans | Scarlet fever, septic sore throat, puerperal fever, erysipelas, abscesses and rheumatic fever | Human upper respiratory tract |
| | | | Cattle | Mastitis (rare) | |
| | | | Foals | Lymphangitis | |
| B | <i>S. agalactiae</i> | β (α , γ) | Cattle, sheep and goats | Chronic mastitis | Milk ducts |
| | | | Humans and dogs | Neonatal septicaemia | Maternal vagina |
| | | | Cats | Kidney and uterine infections | |
| C | <i>S. dysgalactiae</i> | α (β , γ) | Cattle | Acute mastitis | Buccal cavity and genitalia |
| | | | Lambs | Polyarthritis | |
| | <i>S. dysgalactiae</i> subsp. <i>equisimilis</i> | β | Horses | Abscesses, endometritis and mastitis | Skin and vagina |
| | | | Pigs, cattle, dogs and birds | Various suppurative conditions | |
| | <i>S. equi</i> subsp. <i>equi</i> | β | Horses | Strangles, genital and suppurative conditions, mastitis and purpura haemorrhagica | Equine tonsils |
| | <i>S. equi</i> subsp. <i>zooepidemicus</i> | β | Horses | Mastitis, abortion, secondary pneumonia and navel infections | Vagina and skin |
| | | | Cattle | Metritis and mastitis | |



| Lancefield group | Species | Haemolysis | Host(s) | Disease | Natural habitat (if known) |
|------------------|---|---------------------------------|----------------------------|---|--|
| D | <i>Enterococcus faecalis</i> <i>E. faecium</i> <i>E. durans</i> | α (β , γ) | Many species | Opportunistic infections such as septicaemia in chickens, bovine mastitis, endocarditis in cattle and lambs, and urinary-tract infections in dogs | Intestinal tract of many animals |
| | <i>S. equinus</i> <i>S. bovis</i> | α | Many species | Opportunistic infections | Intestinal tract of many animals |
| E (P, U, V) | <i>S. porcinus</i> | β | Pigs | Jowl abscesses and lymphadenitis | Mucous membranes |
| G | <i>S. canis</i> | β | Carnivores | Neonatal septicaemia. Genital, skin and wound infections | Genital tract and anal mucosa |
| | | | Cattle | Occasional mastitis | |
| N | <i>Lactococcus lactis</i> | α | Cattle | Unknown pathogenicity | Milk, plants and tonsils of pigs fed on whey |
| Q | <i>Enterococcus avium</i> | α , γ | Many species | Unknown pathogenicity | Faeces of birds and mammals |
| R(D) | <i>S. suis</i> type 2 | α | Pigs (weaning to 6 months) | Meningitis and arthritis | Tonsils and nasal cavity |
| | | | Humans | Meningitis and septicaemia | Pigs |
| S(D) | <i>S. suis</i> type 1 | α (β) | Pigs (2–4 weeks old) | Meningitis, arthritis, pneumonia and septicaemia | Tonsils and nasal cavity |
| Unclassifiable | <i>S. uberis</i> | α (γ) | Cattle | Mastitis | Skin, vagina and |



CULTURAL CHARACTERISTICS

- The bacteria grow well in ordinary laboratory medium enriched with blood or serum.
- *Enterococcus faecalis* grow at high temperature of 45°C.
- Primary isolation can be done in sheep or ox blood agar
- Blood agar and Edwards medium are the most preferred medium for streptococci
- Small round smooth glistening dew drop like colonies are produced
- Virulent streptococci produce matt colonies and less virulent organism produce glossy colonies



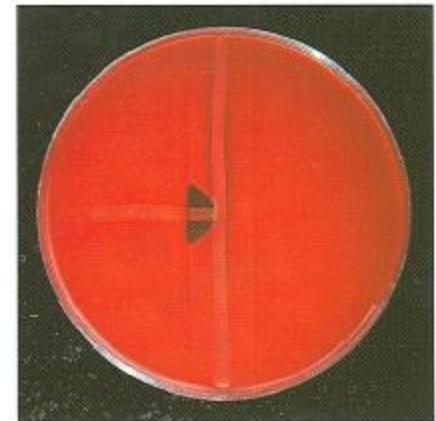
CONTD..

- Produce α , β , γ , and α' haemolysis
- CAMP test is used for identification of *Streptococcus agalactiae*
- ***Streptococcus bovis*, *Streptococcus uberis*** and *Enterococcus faecalis* will grow on Mc Conkey agar.
- In broth organism grow and produce faint growth with uniform turbidity.
- *Streptococcus agalactiae* produce long chains of deposits on the sides of the tube and supernatant will be clear.



CAMP TEST

- A culture of *Staphylococcus aureus* with wide zone of partial haemolysis is streaked across the centre of a sheep or ox blood agar plates
- A streak of suspect group B *Streptococcus* is made at right angles to and taken to within 1 to 1.5 mm of the *Staphylococcal* streak
- The plate is incubated at 37°C for 18-24 hours
- A positive CAMP test is indicated by an arrow head of complete haemolysis
- The group B streptococci produce a diffusible metabolite that complete the lysis of red cells, only partially lysed by beta haemolysis of the *Staphylococcus*



zone of hemolysis of
S.aureus

an augmentation of the effect of *S.aureus*
 β -hemolysin on erythrocytes
(arrowhead configuration)



Staphylococcus aureus

Streptococcus agalactiae

CAMP reaction

BIOCHEMICAL CHARACTERISTICS

- They are **catalase** and **oxidase negative** and fermentative.
- Hydrolyses aesculin and sodium hippurate
- Reduces methylene blue milk
- Able to grow in the presence of 6.5% NaCl
- **Catalase negative** test differentiate Streptococcus from Staphylococcus
- *Streptococcus agalactiae* positive for hippurate hydrolysis
- *Streptococcus uberis* & *Enterococcus faecalis* positive for aesculin hydrolysis



PATHOGENESIS

- The infection can be either endogenous or exogenous
- Exogenous infection is through inhalation, ingestion or direct contact with fomites.
- The streptococcal infection is characterized by production of pus (pyogenic infection).
- When pyogenic bacteria invade the system, they stimulate inflammatory response that is characterized by vascular dialation and exudation of plasma and neutrophils.
- Neutrophils phagocytose the bacteria and kill the cells.
- But some bacteria can grow in neutrophils resulting in production of toxins which kills phagocytic cells.
- The enzymes liberated by dead phagocytic cells liquefy dead tissues and phagocytic cells.
- The liquefied mass is yellow and thick consistency of pus due to deoxyribonucleoprotein.
- Streptococcal infection is generally localized and rarely it become septicaemic or bacteraemic.



VIRULENCE FACTORS OF STREPTOCOCCI

- Major virulence factor of Streptococci are surface M protein and hyaluronic acid
- Haemolysin : Streptolysin O (oxygen labile haemolysin) and Streptolysin S (Oxygen stable haemolysin) which are toxic for neutrophils and macrophages.
- Streptokinase: Also known as Fibrinolysin. It activates plasminogen to plasmin which prevents the formation of fibrin clots. So it is used in cardiac arrest for dissolving the clots.
- DNases A,B,C and D: Also known as streptodornase. Assist in the production of substance required for growth.
- Hyaluronidase A: A major virulence factor which promotes the spread of organism in tissues.
- Erythrogenic toxins A,B,C: responsible for rashes in Scarlet fever
- NADases: kills the phagocytic cells
- Proteinase, lipoproteinase, Amylase, and Esterase



DIAGNOSIS

- Materials for diagnosis: Pus, joint fluid, milk , organs, blood swab, meningeal swab
- Examination of culture smear by Gram staining method and also milk smear by Newman's staining method.
- Biochemical reaction
 - a) haemolytic pattern
 - b) Differentiation in fermentation of sugars like trehalose, sorbitol, mannose, salicin, lactose, raffinose, inulin, esculin *etc.*
 - c)Hydrolysis of esculin and sodium hippurate
 - d)Reduction of methylene blue milk and preference of 6.5% sodium chloride for growth.

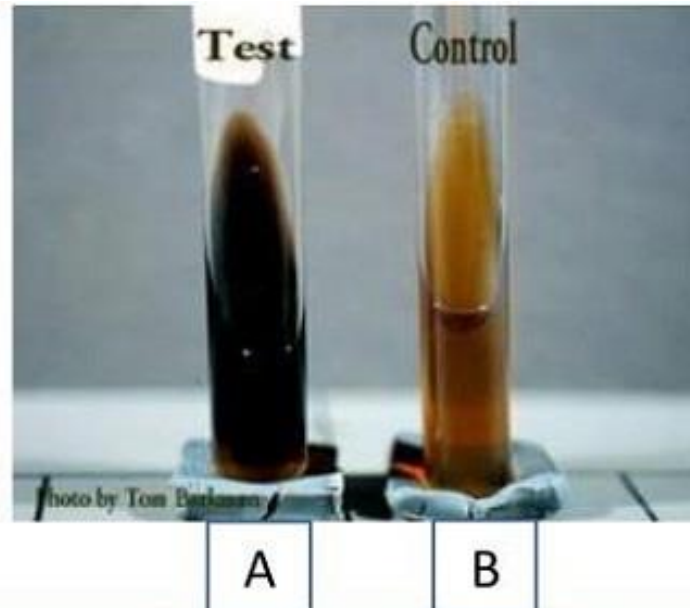


CULTIVATION

- The medium commonly used are nutrient agar, blood agar or **Edward's medium**.
- The Edwards medium contains crystal violet and thallium acetate. Is the selective medium for streptococcus
- Most pyogenic bacteria produce **haemolysis**
- **CAMP test** (Christie, Atkinson, Munch, Peterson): It is a presumptive test for diagnosis of ***Streptococcus dysagalactiae***. The test is based on the ability of streptococcal organism to complete partial haemolysis produced by *Staphylococcus aureus*.
- **Hydrolysis of esculin:** Esculin agar selective or differential media that is used primarily to distinguish faecal streptococci (Enterococcus species) from other streptococcal organism.
- Enterococcus is the only organism that can hydrolyse esculin
- The hydrolysed esculin complexed with iron to form a dark black brown colour in the tube.



BILE ESCULIN TEST



Name of the test: Bile Esculin test

Example A: Positive - Group D streptococcus (Enterococcus species)

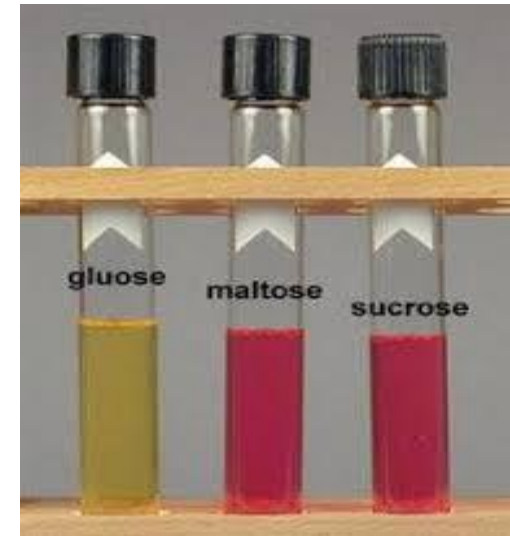
Example B: Negative - Group B streptococcus

Principle: The selective agent **bile**, inhibits most gram positive bacteria.

Esculin in the medium is hydrolyzed to esculetin and dextrose.

The esculetin reacts with ferric chloride in the media to form a black-brown color.

- **Cystine tryptic agar (CTA) sugar fermentation test:** used to identify Streptococci by fermentation reaction.
- The fermentation of carbohydrates in the media produces acids which turns the pH indicator to yellow.
- Colonies obtained from a blood agar culture is stabbed into the media in a CTA tube.
- After incubation a positive fermentation test will give yellow colour.



HIPPURATE HYDROLYSIS TEST

- Hydrolysis of hippurate: distinguishes Group B streptococci from other streptococci

BIOCHEMICAL TESTS

7. **Hippurate hydrolysis test**: Detect hippuricase enzyme production, used to differentiate beta-hemolytic streptococci (*S. pyogenes* & *S. agalactiae*).



➤ **Positive test**

- Deep purple colour
- *S. agalactiae*

➤ **Negative test**

- No change in colour



PRODUCTION OF CAROTENOID PIGMENT

- On media such as GBS agar about 97% of group B streptococci produce orange to red pigment when incubated anaerobically



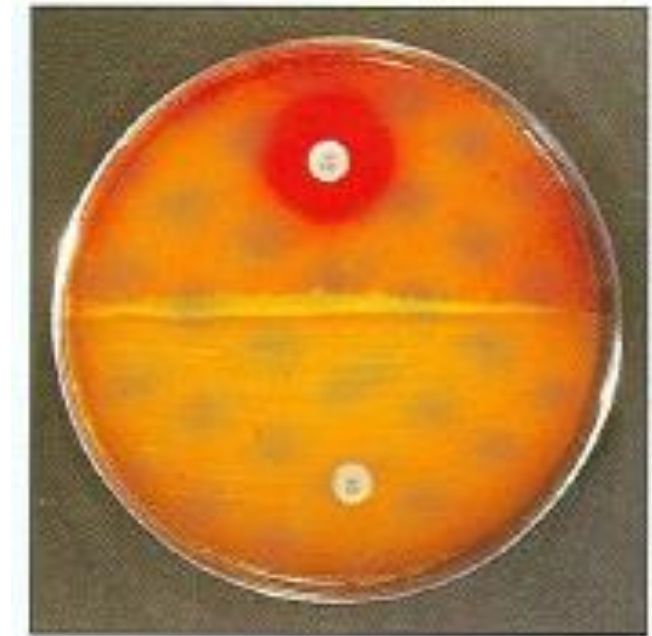
BACITRACIN SUSCEPTIBILITY TEST

- Distinguishes between Group A and Group B streptococci
- Group A Streptococci (*Streptococcus pyogenes*) are susceptible to bacitracin (0.04 U) and group B are resistant



OPTOCHIN SENSITIVITY TEST

- Distinguishes *S. pneumoniae* from other alpha haemolytic bacteria
- It is a disc diffusion test
- The zone of inhibition when using 6 mm disc should be equal to or greater than 14 mm



BILE SOLUBILITY TEST

- Test is conducted by adding suspension of equal amount of *S. pneumoniae* broth culture and 10% solution of Sodium taurocholate
- Within 10-15 minutes pneumococcus will autolyse and the solution become clear



AMYLASE REACTION AND RAPID VP TEST FOR *S. SUI*

- *Streptococcus suis* produce an amylase reaction on Columbia agar with starch solution
- *S. suis* is negative for rapid VP (acetoin) test





STRANGLES

STRANGLES

- Highly contagious infection of horses and other equids caused by the bacterium ***Streptococcus equi***.
- The disease is characterized by severe inflammation of the mucosa of head and throat with extensive swelling and rupture of the lymph nodes, which produce large amounts of thick creamy pus.
- **Etiology:** ***Streptococcus equi subspecies equi***
- Organism isolated from nose and lymph nodes of affected animals and can be readily identified by **simple sugar tests**.



SYMPTOMS AND LESIONS

- Susceptible horses produce strangles within 3-14 days of exposure.
- Animal shows depression, inappetence, and fever of 39°C-39.5°C.
- Most typically the horses develop nasal discharge (initially mucoid, rapidly thickening and purulent), a soft cough and slight and painful swelling of submandibular lymph node.
- Horses usually keeps their head low and extended so as to relieve the head and throat pain.
- With the progression of the disease abscess develop in the submandibular and retropharyngeal lymph nodes.
- The lymph nodes hard very painful and obstruct breathing (Strangles).
- The lymph node abscess will burst in 7-14 days releasing thick pus contaminated with *S. equi*.
- The horses will rapidly recover once abscess have ruptured.



Strangles in Horses

Symptoms:

Nasal discharge
Decreased appetite
Increase in Temperature

Swelling of lymph nodes
-Can obstruct the airway
Puss secreted from abscess
-Abscess ruptures in 7
days and 4 weeks



CONTD..

- The fatal complications of strangles are **bastard strangles** and **purpura haemorrhagica**.
- Bastard strangles which describes the dissemination of infection to unusual sites other than lymph nodes of throat like abdominal or lung lymph nodes.
- A brain abscess may rupture causing sudden death.
- Retropharyngeal abscess may burst in the throat and pus may be inhaled into the lung.



CONTD

- **Purpura haemorrhagica** is an immune mediated acute inflammation of peripheral blood vessels that occurs within 4 weeks of strangles.
- It results from the formation of immune complex between the horse antibody and bacterial components. These immune complex become trapped in capillaries where they cause inflammation visible in the mucus membrane as pin point haemorrhages.
- These haemorrhages leads to wide spread severe edema of the head, limbs and other parts of the body.



- **Diagnosis**

- By culturing pus from nose , from abscessated lymph nodes and from the throat of clinically affected horses

- Sugar tests are done for *Streptococcus equi*

- **Treatment**

- Drug of choice : Penicillin G



MASTITIS

- Mastitis by *Streptococcus agalactiae* , *Streptococcus dysagalactiae*, *S. bovis*, *E. faecalis* and *S. uberis*.
- *S. agalactiae* causes chronic mastitis in cattle, sheep and goat.
- *Streptococcus dysagalactiae* causes acute mastitis and polyarthrititis in lambs.
- **Hotis test.** For the identification of mastitis by *Streptococcus agalactiae*.
- 9.5 ml of milk taken in a test tube and add 0.5 ml of bromocresol purple. Incubate at 37°C for 24 hour. If organism present canary yellow colonies on the side of the test tube.



STREPTOCOCCUS PYOGENES

- Streptococcus pyogenes causes scarlet fever, septic sore throat, rheumatoid fever, and erysipelas.
- Dick test is used for *Streptococcus pyogenes*



A photograph of three paper boats floating on a body of water. The boat on the left is purple, the one in the center is red, and the one on the right is red with white polka dots. The water is dark blue with white ripples. The background is a blurred shoreline with green foliage.

THANK YOU