ENTEROTOXIGENIC CLOSTRIDIA

Learning objectives

To know in detail about,

- Classificaiton of *Cl.perfringens*
- Diseases caused by *Clostridium perfringens* in domestic animals and poultry
- Morphology cultural and biochemical characteristics of *Clostridium perfringens*
- Stormy fermentation and Nagler's reaction
- Enterotoxaemias, struck, pulpy kidney disease and necrotic enteritis
- General approaches used to demonstrate and identify toxins of enterotoxigenic clostridia

HISTORY

- Clostridial enterotoxaemias are acute, highly fatal intoxications that affect sheep, lambs, calves, piglets and occasionally foals.
- The diseases are caused by the major exotoxins (enterotoxins) of *Clostridium perfringens* types B, C, D and occasionally types A and E.
- The bacillus was originally cultivated by Achalme (1891), but it was first described in detail by Welch and Nuttal (1892)-who isolated it from the blood and organs of cadaver.

HABITAT

- Based on toxin production *Clostridium perfringens* organisms are classified into 5 types (A to E).
- *Clostridium perfringens* Type A occurs in the intestinal tract of human and animals and in most soils.
- Type B to E are more adapted to survival in the intestines but in outbreaks of disease they survive long enough in soil to infect other animals

MORPHOLOGY

- Gram +ve, bacillus, straight, parallel sides, rounded or truncated ends, occur either singly or in chains or small bundles.

- It is highly pleomorphic, filamentous and involution forms are common. It is capsulated and non-motile .

- The spores are oval, sub-terminal and bulge the mother cell.They are rarely produced and their absence is one of the characteristic morphological features of *C.welchii*.

CULTURAL AND BIOCHEMICAL CHARACTERISTICS

- It is an anaerobe, but can also grow under micro aerophilic conditions.
- Oxygen is not actively toxic to the bacillus and cultures do not die on exposure to air.
- Though, this bacillus is grown at 37°C, pH 5.5-8.0, the temperature of 45°C is optimal for many strains.
- The generation time at this temperature is ten minutes only.
- This property can be utilized for obtaining pure cultures of *C.welchii*.
- Robertson's cooked meat broth inoculated with mixtures of *C.welchii* and other bacteria and incubated at 45°C for 4-6hrs serves as enrichment.
- Subcultures from this onto blood agar plates yield pure or predominant growth of *C.welchii*.Good growth occurs in Robertson‘s cooked meat medium.

- The meat is turned pink but it is not digested.
- In litmus milk, fermentation of lactose leads to formation of acid, which is indicated by the change in the color of litmus from blue to red.
- The acid coagulates the casein (acid clot) and the clotted milk is disturbed due to the vigorous gas production.
- The paraffin plug is pushed up and shreds of clot are seen sticking to the sides of the tube. This is known as stormy fermentation. (Click here for visual)
- After overnight incubation on rabbit or sheep blood agar, colonies of most strains show a target haemolysis resulting from a narrow zone of complete haemolysis due to theta toxin and a much wider zone of incomplete haemolysis due to alpha toxin. This double zone haemolysis pattern is characteristic for *C.welchii*.

- All the five types of *C.welchii* (A –E) produce alpha toxin.
- This is a lecithinase C, which, in the presence of calcium and magnesium ions splits lecithin into phosphotidyl choline and diglyceride.
- This specific lecithinase effect can be demonstrated by Nagler‘s reaction.
- Type A antitoxin is spread over half of an egg yolk agar plate and allowed to dry.
- The suspect *C.perfringens* is streaked across both sides of the plate. Click here for visual

- All the types of *C.perfringens* produce the alpha toxin, that is a lecithinase.
- On the half of the plate without the antitoxin, the lecithin in the medium is attacked causing opalescence around the streak.
- The lecithinase reaction is neutralized on the other half of the plate with the antitoxin but the growth of *C.perfringens* is unaffected.
- *C.welchii* ferments several sugars (glucose, maltose, lactose and sucrose) and produces acid and gas. Indole –ve, MR +ve, VP-ve, H_2S +ve.

RESISTANCE

- Spores are usually destroyed within 5 minutes by boiling, but those of the food poisoning strains of type A and type C resist boiling for 1-3 hrs.
- Autoclaving temp is lethal.

TOXINS

Classification

- *C.welchii* is one of the most prolific of toxin producing bacteria forming at least 12 different toxins , besides many other enzymes and biological active substances.

C. perfringens	**Major toxins**			
Type	Alpha	Beta	Epsilon	Iota
A	+	-	-	-
B	+	+	+	-
C	+	+	-	-
D	+	-	+	-
E	+	-	-	+

- Alpha toxin is produced by all types. Mostly by type A strains. It is lethal, dermonecrotic and haemolytic.
- This is a lecithinase C (phospholipase) that attacks cell membranes causing cell death and destruction and also responsible for Nagler's reaction .
- It is haemolytic for the red cells of most species except horse and goat. This toxin gives a zone of partial haemolysis on blood agar.
- The haemolysis is of hot-cold variety being best seen after incubation at 37°C followed by chilling at 4°C.
- Beta toxin is lethal and necrotising. It is sensitive to trypsin and this explains the predilection of types B and C for neonates as colostrum has anti trypsin activity.
- It is a labile toxin and may be destroyed if there is a delay in small intestinal contents, containing the toxin, reaching the laboratory.
- Epsilon toxin is secreted as a protoxin (proto toxin) and is activated in the intestines by proteases such as trypsin.
- Pulpy kidney disease is not usually seen in neonatal lambs as colostrum contains an antitrypsin factor that can prevent the epsilon toxin being activated.
- The toxin itself increases gut permeability, assuring absorption of the toxin into the blood stream.
- It damages vascular endothelium (including blood vessels in the brain) leading to fluid loss and edema.
- This epsilon toxin can be regarded as an enterotoxin and neurotoxin.
- Iota toxin is also produced as a protoxin and is not unique to *C.perfringens* type E as it is also formed by *C.spiroforme* and *C.difficile*
- Besides several minor toxins are produced such as the theta (haemolysin),

Kappa (collagenase), lambda (Proteinase), Mu(hyaluronidase) and Nu(DNase) – all these may contribute to tissue damage.

- Based on the type of toxin productions, *Clostridium perfringens* are classified into 5 types

C.perfringens types	Major toxins	Host	Disease
A	Enterotoxins	Human	Food poisoning
	Alpha	Lambs Broiler chickens	Enterotoxaemic jaundice Necrotic enteritis
B	Beta and alpha	Lambs under 3 weeks old	Lamb dysentry
C	Beta and alpha	Piglets 1-3days old	Haemorrhagic enteritis (Clostridial enteritis)
		Broiler chickens (2 weeks old)	Necrotic enteritis
		Adult sheep and goats	Struck
D	Epsilon	Sheep all ages (except neonates)	Pulpy kidney disease (over eating disease)
E	Iota and alpha	Calves and lambs	Enterotoxaemia (Haemorrhagic enteritis)

PATHOGENESIS

- The enterotoxaemias are often precipitated by certain husbandry and environmental factors such as abrupt changes in feeding, usually to a richer diet and overeating and voracity on high protein and energy rich feeds.
- This leads to slowing of peristalsis with retention of bacteria in the intestines, absorption of toxins, inadequately digested carbohydrate and the provision of a rich medium for the proliferation of *C.perfringens*
- The bacterium inhabits the large intestine in normal animals, but if overgrowth occurs *C.perfringens* can spill over into the small intestine with the production of a large amount of toxin and enterotoxaemia.

PATHOGENECITY

Disease	Clinical and postmortem signs
Food poisoning	Sudden onset, diarrhoea, abdominal pain and nausea. But vomiting is uncommon. Short course and rarely fatal
Enterotoxaemic jaundice	Depression, anaemia, icterus, haemoglobinuria and lambs die within 6-12hrs

	of first signs known as the yellows or yellow lamb disease
Lamb dysentry	A haemorrhagic and rapidly fatal enterotoxaemia. Lambs are often found dead
Haemorrhagic enterotoxaemia (Clostridial enteritis)	Dysentery, collapse and death. Small intestine is dark red and has gas bubbles in mucosa
Necrotic enteritis in broilers	Depression, diarrhoea, death in a few hrs. Mortality 2-50%. Mucosa of small intestine has a brown psuedomembrane. Most common in deep litter units.
Struck	Sudden death due to an enterotoxaemia
Pulpy kidney disease (Over eating disease)	Odema of brain, glycosuria, sudden death. Excess fluid in body cavity, focal symmetrical encephalomalacia occurs in well-grown lambs.

DIAGNOSIS

- Gram stained smears can be made from the mucosa of the small intestine of a recently dead animal.
- Large numbers of Gram-positive rods are suggestive of *C.welchii*.
- Saccharolytic in Robertson's cooked meat media, opalescence in egg yolk agar, haemolysis on blood agar, stormy clot fermentation and sugar fermentation tests are helpful for identification.
- Histopathology on brain sections helps to demonstrate focal symmetrical encephalomalacia in pulpy kidney disease.
- Rapid kidney autolysis, pulpy cortical softening and Glucosuria are suggestive of pulpy kidney disease.

Demonstration of toxin in the small intestine

- Collect 20-30ml of ileal contents from a recently dead animal and send it to the laboratory as soon as possible.
- The ileal contents are centrifuged and the clear supernatant is tested for toxin.
- In ileal contents, the epsilon and iota toxins are usually in the active form.
- To demonstrate the toxin 0.4ml of the clarified ileal content can be inoculated i/v ly into mice.
- If mouse dies within 5 mts this is probably due to shock. Death from toxin usually occurs within 10hrs.
- Identification of toxin in the clarified ileal content is carried out by a neutralization test by using suitable antitoxin.

CONTROL AND PREVENTION

- Before the lambing season the ewes are vaccinated with formalised whole culture or alum precipitated vaccine.

- The resulting passive immunity, with unweaned lambs, derived from colostrum protect lambs for first 3 weeks of life.
- Similarly alum precipitated trypsin –treated toxoid is also satisfactory.
- Lambs can also be vaccinated by giving the first dose within 72 hrs of birth and repeated at 4 weeks of age.

Immunity may not last more than 6-12 months unless booster dose is given.

HISTOTOXIC CLOSTRIDIA

Learning objectives

To know in detail about,

- Diseases caused by histotoxic clostridia in domestic animals
- Morphology, cultural and biochemical characteristics of *Cl.novyi*, *Clostridium septicum* and *Clostridium chauvoei*.
- Pathogenesis of Histotoxic Clostridia
- Describe antigens and toxins of *Cl.novyi*.
- B raxy, black quarter and gas gangrene
- Difference diagnostic methods for histo toxic clostridia

HABITAT

- World wide in distribution. The principal habitat of *C.novyi* (also called as *Clostridium oedematiens*) is soil and the intestine of animals.
- Based on toxin production *Clostridium novyi* is classified into four types (A-D).
- The type A is commonly found in soil. Type B is rarely found in soil, and it is common in the normal intestinal tract of herbivores.
- Strains of type A and B are recovered from the livers of normal animal. Type D (*C. haemolyticum*)- is found in the ruminant digestive tract, liver and in the soi

MORPHOLOGY, CULTURAL AND BIOCHEMICAL CHARACTERS

- Large, pleomorphic, Gram+ve rods with oval to cylindrical subterminal spores are characteristic**.**
- There is little or no swelling of the mother cell, non-capsulated, motile by peritrichous flagella.
- *Clostridium novyi* type B and *C.haemolyticum* are very demanding in both their anaerobic and nutritional requirements.
- Very strict anaerobic procedures are necessary and media containing cysteine should be used. These clostridia can die within 15mts of being exposed to atmospheric O_2.
- These organisms are difficult to grow on primary culture and the growth isenhanced by agar enriched with glucose or freshly prepared blood or fresh brain infusions.
- On blood agar *C.novyi* produces characteristic large, irregular colonies with a rhizoid edge and a large zone of clear haemolysis.
- On moist surface of solid media after 3-4 days of incubation colonial motility develops which is characterized by the movement of daughter colonies moving away from parent colonies in spirals or arc and few return and fuse to the parent colony.
- In horse blood agar, the colonies are haemolytic, small and usually rhizoidal in nature.
- Areas of hemolysis develop beneath the colonies and develop into wider zone after 48-72hrs incubation.

- In Sheep blood agar very slight haemolysis develop. In Robertson's cooked meat medium *C.novyi* type D is very strongly proteolytic.
- Type A, B and C are saccharolytic. The lecithinase activity of beta toxin of type B and D, and Gamma toxin of type A produces quite distinct opacity changes on egg-yolk agar.
- *C.novyi* type A exhibits lipase activity on egg-yolk agar.
- It will produce characteristic iridescent pearly layer on the surface of the colonies, extending on to the surface of the medium immediately surrounding them.
- *C.novyi* type A is the only species among clostridia that produces both a lecithinase and a lipase.
- Saccharolytic type ferment glucose and maltose but not lactose.

RESISTANCE

- Spores of most strains survive heating to 950C for 15mts. But are killed at autoclaving.
- Spores are resistant to 5% phenol, 10% formalin or 0.1% merthiolate.
- They are killed rapidly by exposure to hypochlorite.
- Spores remain viable for years in soil.

TOXINS

- *C.novyi* possess several somatic and flagellar antigens, which are not of muchimportance
- Based on toxin production *C.novyi* is classified into 4 types.
- *C.novyi* synthesizes five major toxins, α, β, γ, δ and ε.

	Alpha	Beta
C. novyi type A	+	-
Type B	+	+
Type C	-	-
Type D *C. haemolyticum*	-	+++

- Type A produces all toxins except beta. Type C isolates are non toxigenic.
- In addition to this type B also produce zeta, eta and theta toxin.
    - Alpha toxin produced by type A and B is lethal, necrotizing, causes increased capillary permeability, and is toxic to several tissues including muscle, heart and liver.
    - Beta toxin is a lecithinase and produced by type B and by *C.haemolyticum* in greater amounts.
    - This may account for the haemolytic crisis and death in bacillary haemoglobinuria.
    - Gamma toxin is a necrotising phospholipase D.

- Delta toxin is an oxygen labile haemolysin.
- Epsilon toxin is a lipolytic enzyme.
- Zeta toxin is partly haemolytic.
- Theta toxin is a lipase.
- Eta toxin is a tropomyosinase, which degrades tropomyosin and myosin and may play a role in destruction of infected muscles.

PATHOGENESIS

- In black disease and bacillary haemoglobinuria, the spores, normally present in the intestine, may reach the liver and remain dormant in the kupffer cells.
- Any destruction of liver tissue could be the initiating factor.
- The tissue damage is usually due to migration of immature liver fluke (*Fasciola hepatica*), and anaerobic conditions permits germination of spores, growth of vegetative cells and subsequent production of toxin.
- Alpha toxin produced in the local area of necrosis and in the liver is adsorbed into the circulation and results in systemic effects.
- In case of bacillary haemoglobinuria the dominant toxin is beta toxin.
- Big head in rams develop when sub cutaneous tissues traumatized during fights are subsequently invaded by *C.novyi* type A.
- The oedema is the result of vascular damage inflicted by the alpha toxin.

PATHOGENECITY

Symptoms

- Big head
  - Odematous swelling occurs in head, face and neck.
  - It will be followed by collapse and death of animals.
  - The mortality rate may be more than 90%.
- Black disease
  - Acute toxaemia leads to sudden death.
  - The signs include rapidly decreasing ability to move, unsteady gait and collapse.
- Bacillary haemoglobinuria
  - Common in summer months, affected animals suffer from fever, abdominal pain, port-wine coloured urine , diarrhoea and haemoglobinuria. The mortality rate is 90%.

Lesions

- Black disease
  - Number of clearly defined gray-yellow foci (necrotic areas) in the liver.
  - The lesion consists of a central core of necrosis surrounded by a zone of leucocytes in which there will be masses of *C.novyi.* Excess fluid in body cavities.
  - Straw-coloured exudates will be present in pericardial and peritoneal cavities.
  - Extensive subcutaneous and bloodstained odema can be noticed in the carcass.
  - Venous congestion occurs that darkens the skin(Black disease).
- Bacillary haemoglobinuria:
  - Number of typical anaemic infarcts in the liver.
  - Pale and raised areas surrounded by a blue-red zone.

- There will be blood stained intestinal contents, dark colored urine in the bladder, marked icterus of the carcass, widespread odema and haemorrhages in the myocardium

DIAGNOSIS, CONTROL AND PREVENTION

- Based on history
- Direct Gram stained smears
  - Presence of characteristic liver lesions together with large number of Gram +ve rods in liver impression smears from a recently dead animal is suggestive of the disease.
- Flourescence Antibody Test is useful for the identification of *C.novyi* type B and *C.haemolyticum* in acetone fixed liver impression smears.
- Isolation of organism from affected tissue (as like other clostridial infections) and by characteristic cultural characters.
- Animal inoculation
  - Toxin in the liver can be demonstrated by intra muscular injection of homogenates into guinea pigs.
  - The pathogenicity is enhanced if the homogenate is added to an equal amount of 5% $CaCl_2$ solution before inoculation.
  - The guinea pigs die in 1-2 days with very extensive subcutaneous edema. Specific antitoxin is not readily available for neutralization tests.

Control and prevention

- Elimination of liver flukes through destruction of snail.
- Aluminum hydroxide adsorbed formalized whole culture vaccines are available.
- Outbreaks of the disease may be controlled by the prompt injection of hyper immune sera.

Clostridum septicum

HISTORY AND HABITAT

- *Clostridum septicum* is very closely related with *Clostridum chauvoei*, hence it is called as *Clostridum chauvoei* type A.
- The bacillus was first described by Pasteur and Jourbert (1887) and named it as Vibrion septique .
- *Clostridum septicum* causes
  - Malignant odema in Cattle, sheep and pigs
  - Braxy (Bradsot) in Sheep
  - Necrotic dermatitis in Chickens
- It is found in soil and the intestine of animals.

MORPHOLOGY, CULTURAL AND BIOCHEMICAL CHARACTERS

- Gram +ve, highly pleomorphic, chracteristic long filamentous forms are seen in stained smears of affected muscle.
- Cigar shaped rods and citron forms are more common. Spores are oval, central or subterminal. Non-capsulated, motile by peritrichous flagella.
- Strict anaerobe, growth at an opt.temp of 37°C, growth is promoted by glucose.
- On ordinary media, the colonies are irregular and transparent initially, turning opaque (large, grayish white on continued incubation).
- The colonies are swarming and spreading over the entire surface.

- On stiff agar, the colonies are irregular with a rhizoid edge. Some strains produce smooth, round colonies.
- In cooked meat medium meat turns pink with rancid odour, and produces abundant gas (because, it is saccharolytic).
- Like, *C.perfringens,* the *C.septicum* inoculated into litmus milk produces the classical stormy clot or stormy fermentation reaction.
- Ferment glucose, lactose, maltose and salicin but not sucrose. Acid and gas are not produced.

ANTIGENS AND TOXINS

- Six groups have been recognized, based on somatic and flagellar antigens.
- *Clostridum septicum* produces at least four distinct toxins and fibrinolysin.
    - The α toxin is oxygen stable haemolysin, dermonecrotic and lethal.
    - The β toxin is leucotoxic and DNAse.
    - The γ toxin is a hyaluronidase.
    - The δ toxin is an oxygen labile haemolysin.
- α toxin has direct effect on cardiac muscle and is capable of causing capillary damage.
- Iron is required both for growth of bacteria and for production of α toxin.

PATHOGENESIS

Braxy in sheep

- The disease is more common in winter months.
- When animals ingest large volume of frozen grass, the spores that are present in the soil are ingested with feed.
- The mucosa of the abomasum is damaged due to cold conditions from an adjacent rumen.
- Any *C. septicum* spores present can germinate and replication of the bacterium leads to toxin production, toxaemia and rapid death.

Malignant odema (Anaerobic cellulites)

- In this exogenous gas gangrene infection, spores are introduced into wounds where they may germinate in the anaerobic necrotic material and toxin is produced by the vegetative cells.

PATHOGENECITY

Symptoms

- Braxy usually occurs in well-nourished one-year-old sheep, ailing animals show signs of abdominal pain and diarrhoea. Death occurs within few hours.
- In malignat odema, infected wound, become gas gangrenous.
- Fever, soft swelling around wound spreading to muscles.
- Swelling odematous and wet with much exudates and gas. Muscles appear dark red to black color.

Lesions

- In braxy, the lesion may be confined to the abomasum; there will be the characteristic area of haemorrhagic inflammation in the wall of the abomasum.
- There will be an extensive quantity of blood stained fluid in the peritoneal cavity.

DIAGNOSIS, CONTROL AND PREVENTION

- Based on history, symptoms and characteristic lesions strongly suggestive of this disease.
- FAT is to be employed for differentiate it from *C.chauvoei* .
- Identification of specific clostridial toxin by mixing 1.2ml of culture fluid with 0.3ml of specific antitoxin, allowing the mixture to stand for 30 minutes at 37°C and inoculate two guinea pigs intra peritoneally. If specific toxin present it will be neutralized.
- Penicillin alone or with hyper immune serum can be used to treat infections.
- Sheep can be effectively immunized against the disease and multi component vaccine is used.

MORPHOLOGY, CULTURAL AND BIOCHEMICAL CHARACTERS

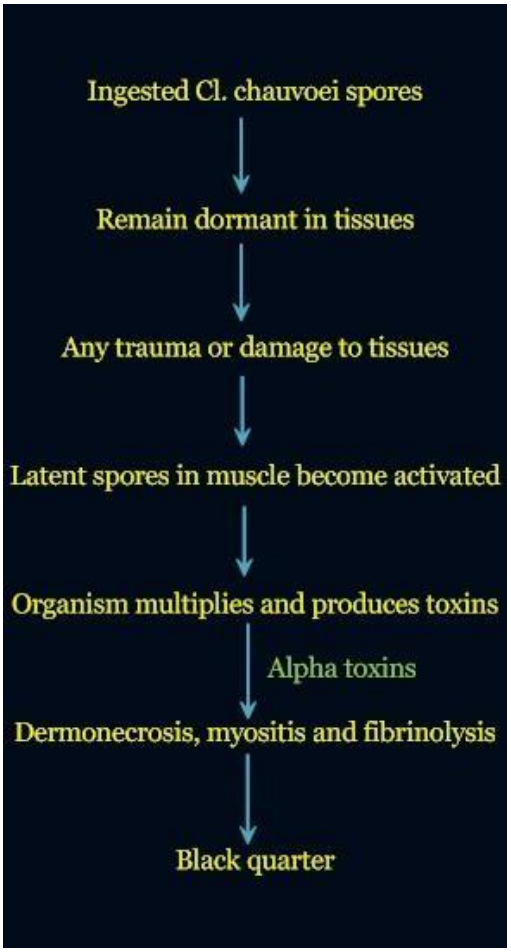
- Worldwide distribution in soil and pastures. *C. chauvoei* (also called as C. *chauvoei* type B, C. *feseri*) causes black quarter or black leg in Cattle & Sheep.
- Gram positive, rod shaped with rounded ends 3-8mm in length & 5mm in width.
- Sometimes pleomorphic, large cigar shaped rods or citron forms occur.
- Non-capsulated and motile by peritrichous flagella. Non-motile variants do occur.
- Spores are oval and located centrally or subterminally. Old cultures stain Gram negative.
- Strict anaerobe. Growth occurs at an optimum temperature of 370C. Growthenhancedby the addition of liver extract or glucose.
- In blood agar whitish grey colonies with irregular edges develop, surrounded by a zone of haemolysis.
- In cooked meat medium growth is slow and meat is turned pink with sour odour
- *C. chauvoei* ferments glucose, lactose, sucrose, maltose with acid & gas, but not salicin.

ANTIGENS AND TOXINS

Classification

- *C. chauvoei* has somatic and flagellar antigens and produces 4 toxins
  - Alpha toxin - oxygen stable haemolysin, necrotoxin that causes dermonecrosis and fibrinolysis
  - Beta toxin – DNA ase
  - Gamma toxin – hyaluronidase
  - Delta toxin – Oxygen – labile haemolysin.
  - This toxin is lethal for mice & guinea pigs when given I/v.

PATHOGENESIS



PATHOGENECITY

Symptoms

- The disease usually occurs in young cattle of 6 months to about 2-3 years of age.
- The most obvious sign is crepitating swelling particularly in the hind or fore quarter which rackels when rubbed with the fingers as a result of gas production.

- The affected animal will become lame and the affected muscles shows trembling with violent twitching. Death usually occurs within 24 hours.
- In sheep an acute febrile condition develops within 1-2 days following aninjuryand a typical black quarter lesion can be observed at the site. Death occurs suddenly

Lesion

- In the central part of the lesion there is usually a well-defined area of muscle, which is dark red in colour, dry, necrotic and filled with small gas bubbles, which give a swollen appearance to the muscles.
- The lesion has a characteristic rancid odour. Surrounded this area of muscle there will be yellowish or blood stained oedematous fluid.
- In ewes there will be necrosis of the vaginal mucosa and skin with extensive oedema involving the hind limbs and thigh muscles.

DIAGNOSIS, CONTROL AND PREVENTION

- Based on History
- Based on Symptoms - The most obvious sign is crepitate swelling particularly in the hind or fore quarter which rackels when rubbed with the fingers as a result of gas production
- Smears prepared from the lesions and oedmatous fluid reveal Gram positve rod.
- Isolation can be done from the center of the lesion, oedematous fluid and from heart blood & spleen.
- FAT used to differentiate from *C. septicum*
- Broth cultures or oedematous fluid from the lesions can be tested for toxicity and specific neutralization by antitoxin in mice or guinea pigs.
    - The most reliable results are obtained from using a formalized alum precipitated whole culture that confers immunity against the bacteria as well as the toxin.
    - For economic reasons a multi-component vaccine containing *C. chauvoei, C. septicum, C.welchii type D and C. tetani* is used.
    - A stronger immunity is stimulated by two doses of vaccine at a time interval of at least 2-3 weeks

Control and prevention

- Hyper immune serum (HIS) is used to control explosive outbreaks. Penicillin along with HIS is used to treat the disease.
- Oxytetracycline & Chlortetracycline can also be employed effectively in early stages.