Genus: Clostridia

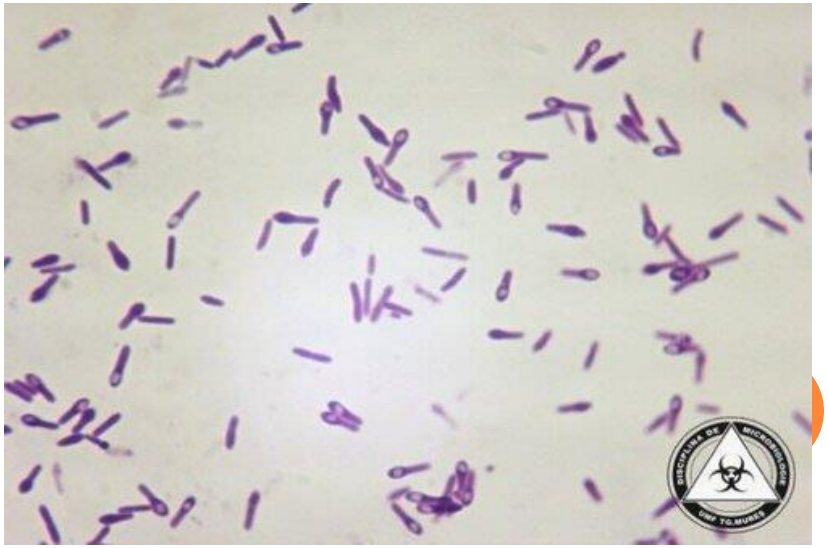
Dr. Bincy Joseph

Assistant professor

PGIVER, Jaipur

Clostridium

- Kingdom -Prokaryotes
- Division -Fermicutes
- Class -Clostridia
- Order -Clostridiales
- Family -Clostridiaceae
- Genus -*Clostridium*



HISTORY:

- Tetanus has been known from very early times, having been described by Hippocrates.
- But the knowledge of the disease was achieved only in 1884.
- *Rosenbach –1886* - demonstrated a slender bacillus with round terminal spores in a case of tetanus.
- *Kitasato –1889* – isolated *C. tetani* in pure culture and reproduced the disease in animals by inoculation of pure culture.
- *C. botulinum* was first isolated by Van Ermengam (1896) from a piece of ham that caused an outbreak of botulism.
- The Greek term "tetanus" which means 'contracture' has been taken from the Latin medicine "rigor".

Introduction

- The clostridia are large Gram-positive bacteria which are fermentative, catalase-negative and oxidase-negative, and require enriched media for growth.
- They are straight or slightly curved rods and the majority arc motile by flagella which are peritrichous (except *C. perfringens*).
- *Clostridium* species produce endospores which usually cause bulging of mother cells
- The size, shape and location of endospores can be used for species differentiation.
- Clostridia are anaerobic. *C. odematiens* (*C. novyi*) are strict anaerobes and die on exposure to oxygen.
- *C. histolyticum* and *C. welchii* are aerotolerent and may even grow aerobically.

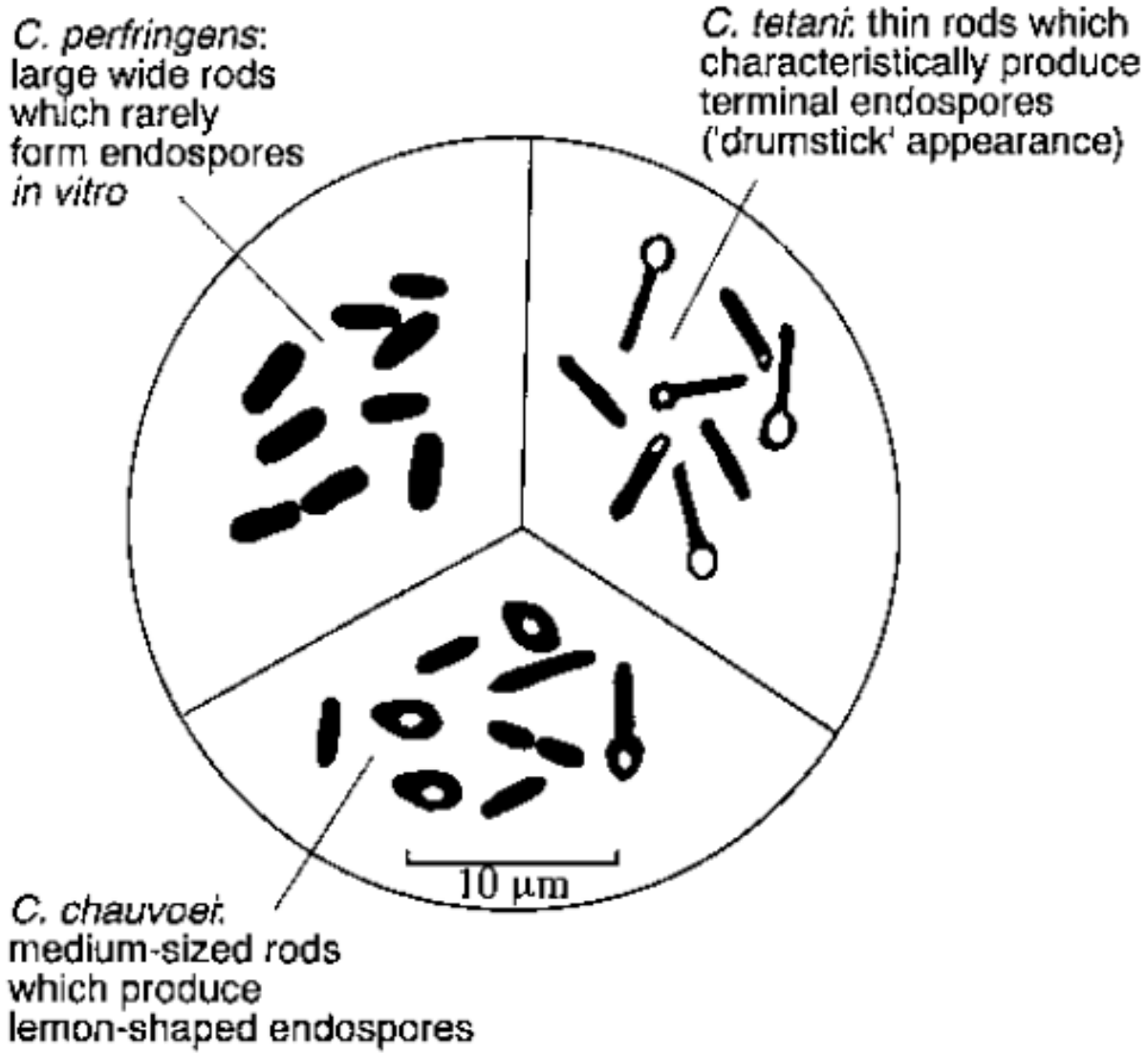


Figure 16.1 Characteristic morphology of some clostridial species.

Natural Habitat

- Clostridia are saprophytes which are found in soil, fresh- water or marine sediments with suitably low redox potentials.
- They constitute part of the normal intestinal flora and some may be sequestered as endospores in muscle or liver. Sequestered endospores, if activated, may produce disease.

Table 16.1 Nomenclature changes of some *Clostridium* species.

Present name	Former name
Clostridium perfringens	*Clostridium welchii*
Clostridium argentiense	*Clostridium botulinum* type G
Clostridium haemolyticum	*Clostridium novyi* type D
Clostridium novyi	*Clostridium oedematiens*
Clostridium piliforme	*Bacillus piliformis*

Classification

- These can be grouped in **four categories**, three based on toxin activity and tissues affected and the fourth containing pathogens of lesser importance.
- ***Clostridium tetani*** and *C.* ***botulinum,*** the **neurotoxic clostridia**, affect neuro- muscular function without inducing observable tissue damage.
- In contrast, **histotoxic clostridia** produce relatively localized lesions in tissues such as muscle and liver, and may subsequent1y cause toxaemia.
- ***Clostridium perfringens*** types A to E, important members of the third category, produce inflammatory lesions in the gastrointestinal tract along with enterotoxaemia.
- Clostridia in the fourth category are associated with sporadic diseases, usually affecting individual animals.

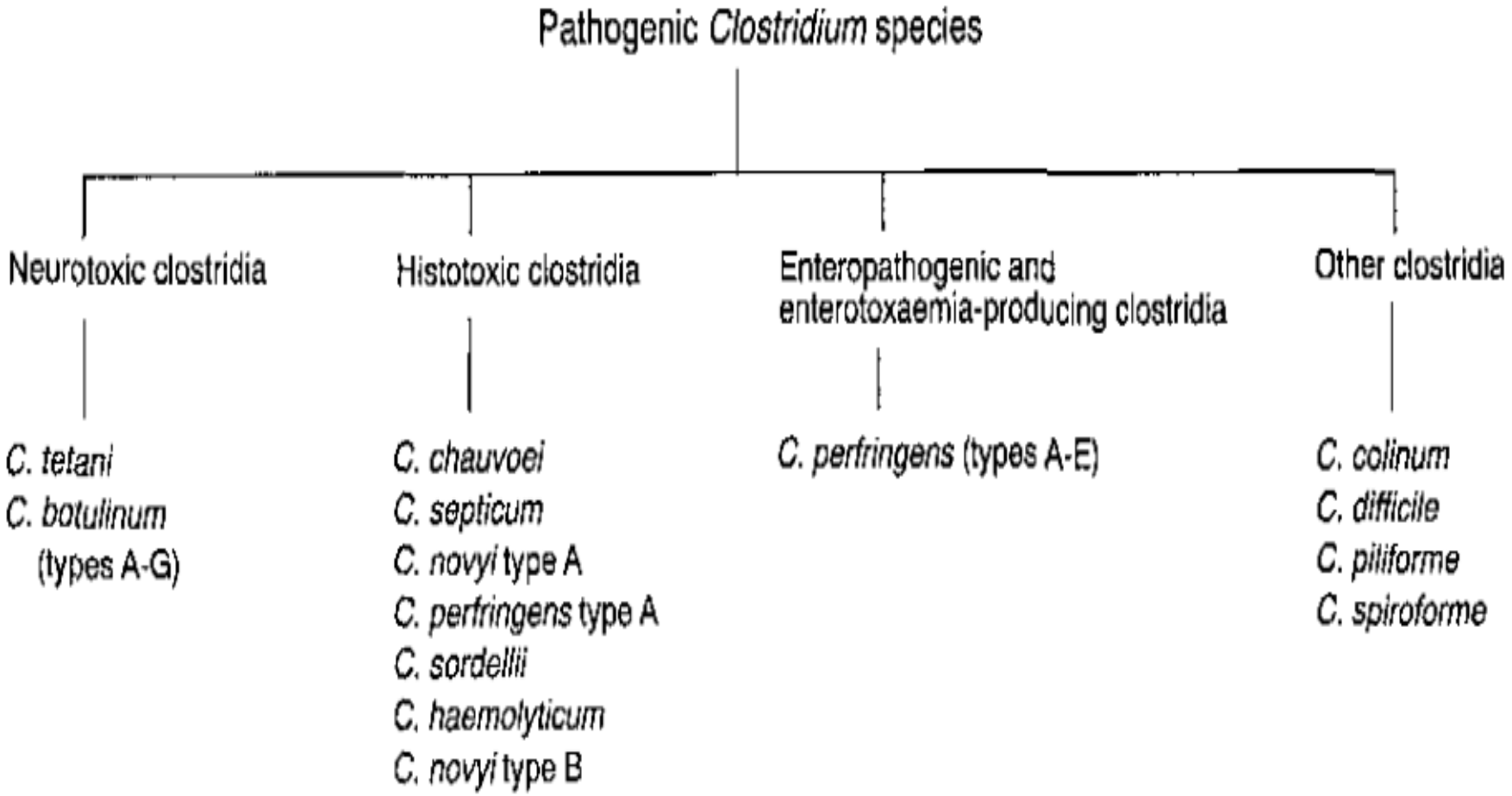


Figure 16.2 Pathogenic *Clostridium* species of veterinary importance.

Clinical Conditions by neurotoxic clostridia

- The neurotoxic clostridia, *C. tetani* and *C. botulinum* produce their effects by elaborating potent neurotoxins.

Feature of neuro-toxin	*Clostridium tetani*	*Clostridium botulinum*
Site of production	In wounds	In carcases, decaying vegetation, canned foods. Occasionally in wounds or in intestine (toxico-infections)
Genes which regulate production	In plasmids	Usually in genome (in bacteriophages for types C and D)
Antigenic type	One antigenic type (tetanospas-min)	Eight antigenically distinct toxins, types A to G
Mode of action	Synaptic inhibition	Inhibition of neuro-muscular transmission
Clinical effect	Muscular spasms	Flaccid paralysis

Tetanus

- Tetanus is an acute potentially fatal intoxication which affects many species including humans.
- However, Horses and Man are highly susceptible, ruminants and pigs moderately so, and carnivores are comparatively resistant.
- Poultry are not susceptible to tetanus.
- ***Clostridium tetani,*** the aetiological agent, is a straight, slender anaerobic Gram-positive rod.
- Spherical endospores, which are terminal and bulge mother cells, impart a characteristic 'drumstick' appearance to sporulated organisms

TETANUS

- The endospores are resistant to chemicals and boiling but are killed by autoclaving at 121'C for 15 minutes.
- ***Clostridium tetani*** has a swarming growth and is haemolytic on blood agar due to the production of tetanolysin.
- Ten serological types of *C.* ***tetani*** can be distinguished by their flagellar antigens.
- The neurotoxin, tetanospasmin, is antigenically uniform irrespective of serotype, and antibodies induced by the neurotoxin of any one of the serotypes neutralize the neurotoxins produced by the others.
- Infection occurs when endospores are introduced into traumatized tissue from soil or faeces.

Tetanus

- Common sites of infection include deep penetrating wounds in the horse, castration and docking wounds in sheep, abrasions associated with dystocia in cows and ewes, and the umbilical tissues in all young animals.
- The presence of necrotic tissue, foreign bodies and contaminating facultative anaerobes in wounds may create the anaerobic conditions in which ***C. tetani*** spores can germinate.
- The clostridial organisms may replicate more readily in the tissues when the haomolytic toxin (tetanolysin), is released.
- Vegetative bacteria multiplying in necrotic tissues produce the potent tetanospasmin which is responsible for the clinical signs of tetanus.

Pathogenesis

- Structurally, tetanus toxin consists of two chains joined by a disulphide bridge.
- The light chain is the toxic moiety and the heavy chain is responsible for receptor binding and internalization of the toxin.
- The neurotoxin binds irreversibly to ganglioside receptors on motor neuron terminals and is transported to the nerve cell body and its dendritic processes in the central nervous system in toxin- containing vesicles, by retrograde intra-axonal flow.
- Toxin is transferred trans-synaptically to its site of action in the terminals of inhibitory neurons, where it blocks pre- synaptic transmission of inhibitory signals.

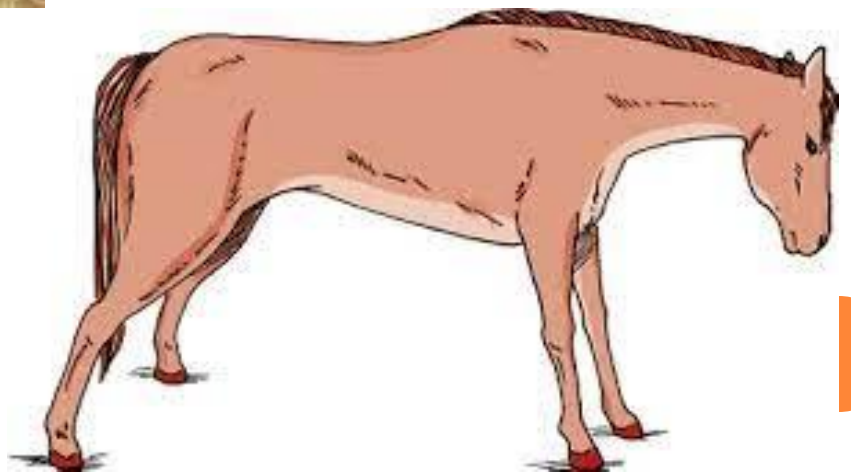
Pathogenesis

- It does this by hydrolysis of synaptobrevins, protein components of vesicles containing neuro- transmitters.
- Because release of inhibitory neurotransmitters is prevented, spastic paralysis results.
- Toxin can also be blood-borne, especially when produced in large amounts and can then bind to motor terminals throughout the body prior to transfer to the central nervous system.
- Bound toxin is not neutralized by antitoxin.

Clinical sign

- The incubation period of tetanus is usually between 5 and 10 days but may extend to three weeks.
- The clinical effects of the neurotoxin are similar in all domestic animals.
- The nature and severity of the clinical signs are dependent on the anatomical site of the replicating bacteria, the amount of toxin produced and species susceptibility.
- Clinical signs include stiffness, localized spasms, altered heart and respiratory rates, dysphagia and altered facial expression.
- Spasm of mastigatory muscles may lead to 'lockjaw'. Generalized muscle stiffness can result in a 'saw-horse' stance especially in horses.

Saw-horse Projection



Diagnostic procedures

- The diagnosis of tetanus is usually presumptive and is based on the clinical signs and a history of recent trauma in unvaccinated animals.
- Gram stained smears prepared from material from lesions may reveal the characteristic 'drumstick' forms of *C. tetani*.
- Anaerobic culture of C. *tetani* from necrotic wound tissue may be attempted but is often unsuccessful.
- Serum from affected animals may be used to demonstrate circulating neurotoxin, using mouse inoculation.

Botulism

- Botulism is a serious, potentially fatal intoxication usually acquired by ingestion of preformed toxin.
- *C. botulinum*, the aetiological agent, is an anaerobic Gram- positive rod which produces oval, subterminal endospores.
- The endospores of *C. botulinum* are distributed in soils and aquatic environments worldwide.
- Eight types of *C. botulinum* are recognized on the basis of the toxins (A, B, C_{α}, C_{β}, D, E, F and *G)* which they produce.
- These neurotoxins, which are inactivated by boiling for up to 20 minutes, induce similar clinical signs but differ in their antigenicity and potency.

BOTULISM

Table 16.3 Toxins of *Clostridium botulinum*.

Toxin	Source	Susceptible species
Type A	Meat, canned products	Humans
	Toxico-infection	Infants
	Meat, carcases	Mink, dogs, pigs
Type B	Meat, canned products	Humans
	Toxico-infection	Infants
	Toxico-infection	Foals (up to two months of age)
Type C	Dead invertebrates, maggots, rotting vegetation and carcases of poultry	Waterfowl, poultry
	Ensiled poultry litter, baled silage (poor quality), hay or silage contaminated with rodent carcases	Cattle, sheep, horses
	Meat, especially chicken carcases	Dogs, mink, lions, monkeys
Type D	Carcases, bones	Cattle, sheep
	Feed contaminated with carcases	Horses
Type E	Dead invertebrates, sludge in earth-bottomed ponds	Farmed fish
	Fish	Fish-eating birds, humans
Type F	Meat, fish	Humans
Type G	Soil-contaminated food	Humans (in Argentina)

Botulism

- Some *C. botulinum* types are confined to particular geographical regions. Germination of endospores, with growth of vegetative cells and toxin production, occurs in anaerobic locations such as rotting carcases, decaying vegetation and contaminated canned foods.
- Toxico-infectious botulism, an uncommon form of the disease, occurs when spores germinate in wounds or in the intestinal tract.
- Intestinal toxico-infectious botulism has been recorded in foals (shaker-foal syndrome), pups, broiler chickens and turkey poults.

Botulism

- *C. botulinum* types C and D cause most out- breaks of botulism in domestic animals.
- Outbreaks of disease occur most commonly in waterfowl, cattle, horses, sheep, mink, poultry and farmed fish.
- Botulism in cattle has been associated with ingestion of poultry carcases present in ensiled poultry litter used as bedding or spread on pasture
- Poor quality baled silage and silage or hay containing rodent carcases have been linked to outbreaks of botulism in horses and ruminants.

Botulism

- Pica, arising from starvation or phosphorus deficiency in herbivores may induce affected animals to chew bones or carcases containing botulinum toxin.
- The resultant botulism is known as lamsiekte in South Africa, bulbar paralysis in Australia and loin disease in the USA.
- Contaminated raw meat and carcases are often sources of toxin for carnivores.
- Waterfowl and other birds can acquire toxin from dead invertebrates, decaying vegetation or from the consumption of maggots containing toxin.

Pathogenesis

- The neurotoxins of C. *botulinum* are the most potent biological toxins known.
- Preformed toxin in food, absorbed from the gastrointestinal tract, circulates in the bloodstream and acts at the neuromuscular junctions of cholinergic nerves and at peripheral autonomic synapses.
- Its structure is similar to that of tetanus toxin and it binds to receptors on nerve endings and enters cells during acetylcholine release.
- As with tetanus toxin, hydrolysis of synaptobrevins causes irreversible interference with the release of the transmitter, acetylcholine in this instance, resulting in flaccid paralysis.
- Death results from paralysis of respiratory muscles.

PATHOGENESIS

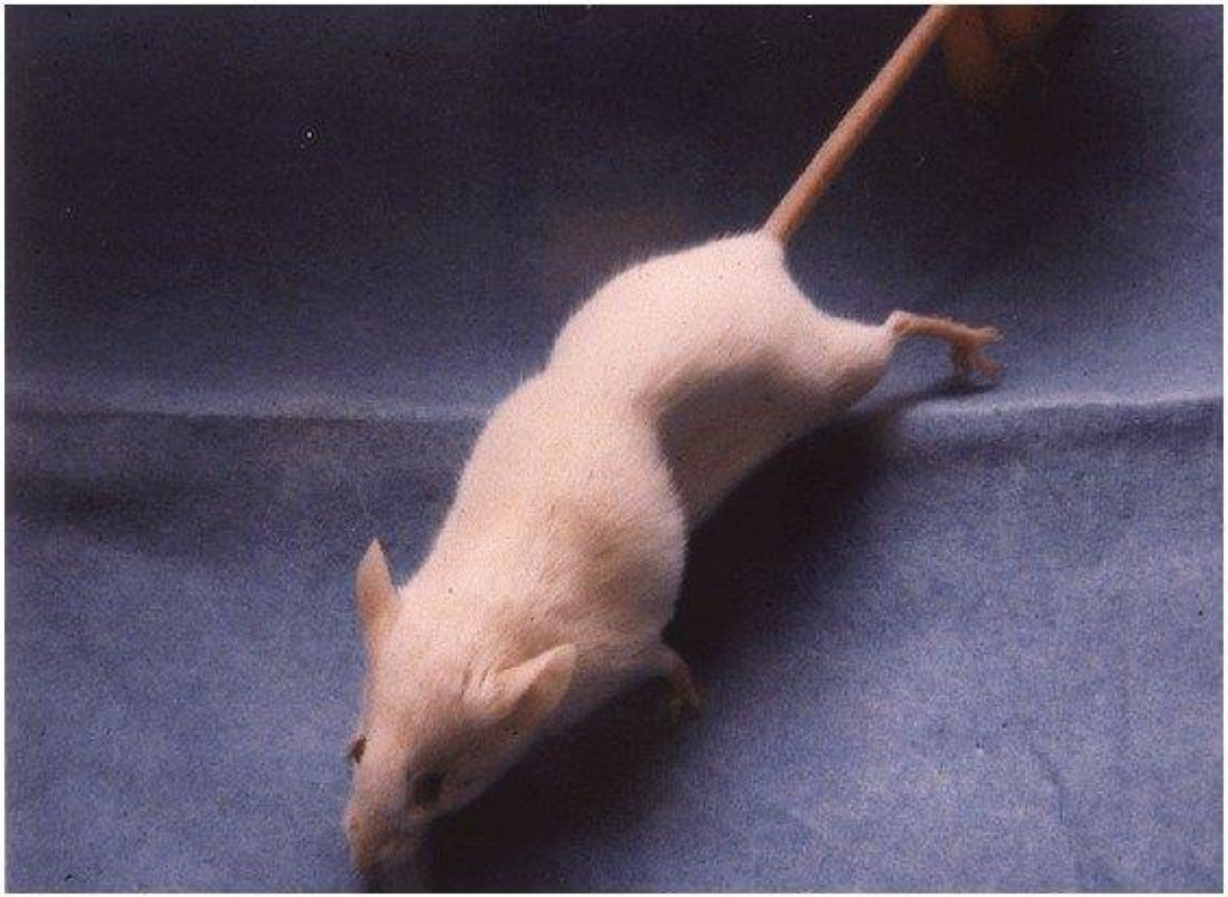
- The difference between the effects of tetanus and botulinum toxins is due to their different sites of action.
- Tetanus toxin travels up the nerve axon to the ventral horn whereas botulinum toxin remains at the neuromuscular junction.
- Ingested spores of *C. botulinum* arc normally excreted in the faeces.
- In toxico-infectious botulism, however, germination of spores in the intestine, results in toxin production by the vegetative organisms.
- The factors which predispose to toxico-infectious botulism are not known.
- The shaker-foal syndrome, a form of toxico- infectious botulism in foals up to two months of age, has been attributed to the impact of stress on the dam leading to increased corticosteroid levels in the milk.

Clinical sign

- The clinical signs may develop in 3 to 17 days after ingestion of toxin, are similar in all species.
- Dilated pupils, decreased salivation, tongue flaccidity and dysphagia are in farm animals.
- Incoordination and knuckling of the fetlocks is followed by flaccid paralysis and recumbency.
- Paralysis of respiratory muscles leads to abdominal breathing.
- Body temperature remains normal and affected animals are alert.
- Death may occur within days of the emergence of clinical signs.
- In birds, there is progressive flaccid paralysis which initially affects legs and wings. Paralysis of muscles of the neck (limberneck) is evident only in long-necked species.

Diagnostic procedures

- Clinical signs and a history of access to contaminated food may suggest botulism
- Confirmation requires the demonstration of toxin in the serum of affected animals. Serum collected from dead animals is unsuitable for mouse inoculation
- The traditional method for demonstrating toxin is by mouse inoculation. Injected mice develop a characteristic 'wasp-waist' appearance, a consequence of abdominal breathing following paralysis of respiratory muscles.
- The PCR and nucleic acid probe- based methods have been used for the detection of *C. botulinum* toxin genes.
- Immunological methods using ELISA or chemiluminescent assays are sensitive and specific procedures for toxin detection.
- Toxin neutralization tests in mice, using monovalent antitoxins, can be employed to identify the specific toxin involved if required.



Mouse with classical “wasp waist” sign after intoxication with botulinum toxin

Clinical conditions by histotoxic clostridia

- Endospores of histotoxic clostridia are widely distributed in the environment and can persist for long periods in soil.
- The endospores of particular clostridial species are often found in certain localities and in well-defined geographical regions.

Pathogenesis

- It is probable that the majority of ingested endospores are excreted in the faeces but some may leave the intestine and become distributed in the tissues where they remain dormant.
- The sequence of events which lead to endospore distribution in tissues is unclear.
- Spores originating in the intestinal lumen may be transported to the tissues in phagocytes.
- Tissue injury leading to reduced oxygen tension is required for spore germination and replication of vegetative bacteria.
- Local necrosis produced by the exotoxins of the replicating bacteria allows further proliferation of the organisms in the tissues with extension of the necrotizing process.

Pathogenesis

- Endogenous infections which include blackleg, infectious necrotic hepatitis and bacillary haemoglobinuria result from activation of dormant spores in muscle or liver.
- The anaerobic environment in necrotic tissue is conducive to replication of the clostridia, which are often present together with facultative anaerobes in mixed infections.
- Extension of local tissue destruction results from exotoxin production.

Clinical conditions by histotoxic clostridia

Clostridium species	Disease	Toxin	
		Name	Biological activity
C. chauvoei	Blackleg in cattle and sheep	α	Lethal, haemolytic, necrotizing
		β	Deoxyribonuclease
		γ	Hyaluronidase
		δ	Oxygen-labile haemolysin
C. septicum	Malignant oedema in cattle, pigs and sheep Abomasitis in sheep (braxy) and occasionally in calves	α	Lethal, haemolytic, necrotizing
		β	Deoxyribonuclease
		γ	Hyaluronidase
		δ	Oxygen-labile haemolysin
C. novyi type A	'Big head' in young rams Wound infections	α	Necrotizing, lethal
C. perfringens type A	Necrotic enteritis in chickens Necrotizing enterocolitis in pigs Gas gangrene	α	Haemolytic, necrotizing, lethal, lecithinase
C. sordellii	Myositis in cattle, sheep and horses Abomasitis in lambs	α	Lecithinase
		β	Oedema-producing lethal factor
C. novyi type B	Infectious necrotic hepatitis (black disease) in sheep and occasionally in cattle	α	Necrotizing, lethal
		β	Necrotizing, haemolytic, lethal, lecithinase
C. haemolyticum	Bacillary haemoglobinuria in cattle and occasionally in sheep	β	Necrotizing, haemolytic, lethal, lecithinase

Black leg

- Blackleg, an acute disease of cattle and sheep caused by *C. chauvoei*, occurs worldwide.
- In cattle, the disease is most often encountered in young thriving animals from 3 months to 2 years of age and infection is usually endogenous, the latent spores in muscle becoming activated through traumatic injury.
- The disease may affect sheep of any age and, 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.

Malignant oedema and gas gangrene

- Malignant oedema and gas gangrene are exogenous, necrotizing, soft tissue infections.
- The bacteria most commonly implicated are *C. septicum* in malignant oedema and *C. perfringens* type A in gas gangrene.
- Malignant oedema manifests as cellulitis with minimal gangrene and gas formation. Tissue swelling due to oedema, and coldness and discolouration of the overlying skin are obvious clinical features.
- Gas gangrene is characterized by extensive bacterial invasion of damaged muscle tissue. Gas production is detectable clinically as subcutaneous crepitation. The clinical features of toxaemia in gas gangrene are similar to those encountered in malignant oedema.

Braxy

- Braxy, an abomasitis of sheep, is caused by the exotoxins of *C. septicum*.
- The disease, which occurs in winter during periods of heavy frost or snow, has been recorded in parts of northern Europe and occasionally elsewhere in the world.
- It has been suggested that ingestion of frozen herbage may cause local devitalization of abomasal tissue at its point of contact with the rumen, allowing invasion by *C. septicum*.
- The course of the disease is rapid and most animals die without premonitory signs. Anorexia, depression and fever may be evident immediately before death.

Infectious necrotic hepatitis (black disease)

- It is an acute disease affecting sheep and occasionally cattle.
- Rare cases have been described in horses and pigs.
- The hepatic necrosis is caused by exotoxins of *C. novyi* type B replicating in liver tissue which has been damaged by immature *Fasciola hepatica* or other migrating parasites.
- Death is rapid with no premonitory signs and the disease requires differentiation from acute fascioliasis.
- The term 'black disease' relates to the dark discolouration of the skin caused by the marked subcutaneous venous congestion observed at postmortem examination.

Bacillary haemoglobinuria

- It occurs primarily in cattle and occasionally in sheep. In this endogenous infection with *C. haemolyticum*, the clostridial endospores are dormant in the liver, probably in Kupffer cells.
- As in infectious necrotic hepatitis, the main factor which facilitates spore germination and clostridial replication is fluke migration.
- The β toxin, a lecithinase, produced by vegetative cells, causes intravascular haemolysis in addition to hepatic necrosis.
- Haemoglobinuria, a major clinical feature of the disease, is a consequence of extensive red cell destruction.

Diagnostic procedures

- Histotoxic clostridia contributing to these conditions can be identified by fluorescent antibody techniques.
- *C. perfringens* is cultured anaerobically on blood agar at 37°C for 48 hours. Colonies of *C. perfrigens* type A are up to 5 mm in diameter, circular, flat, greyish and surrounded by a zone of double haemolysis.

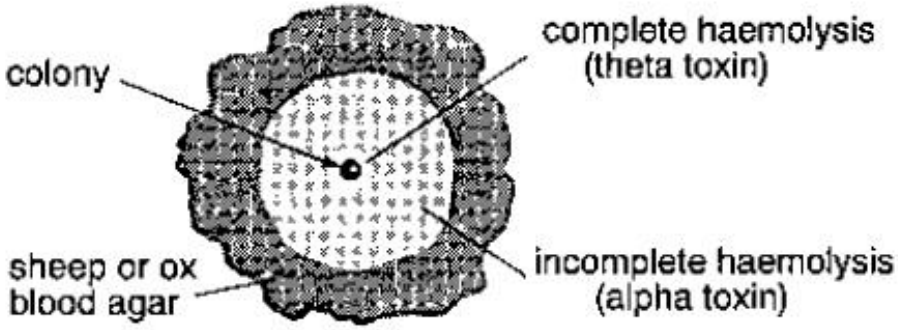


Figure 16.3 Double haemolysis on blood agar around a colony of *Clostridium perfringens*.

- A positive CAMP test occurs with *Streptococcus agalactiae*. A diffusible factor produced by *S. agalactiae* enhances the partial haemolysis of the alpha toxin of *C. perfringens*.
- The pattern of haemolysis is similar to that observed in the *S. agalactiae* reaction with the beta haemolysin of *Staphylococcus aureus*.
- A PCR-based method for the identification of *Clostridium* spp.

- The Nagler reaction, a plate neutralization test, identifies the alpha toxin of *C. perfringens*, which has lecithinase activity

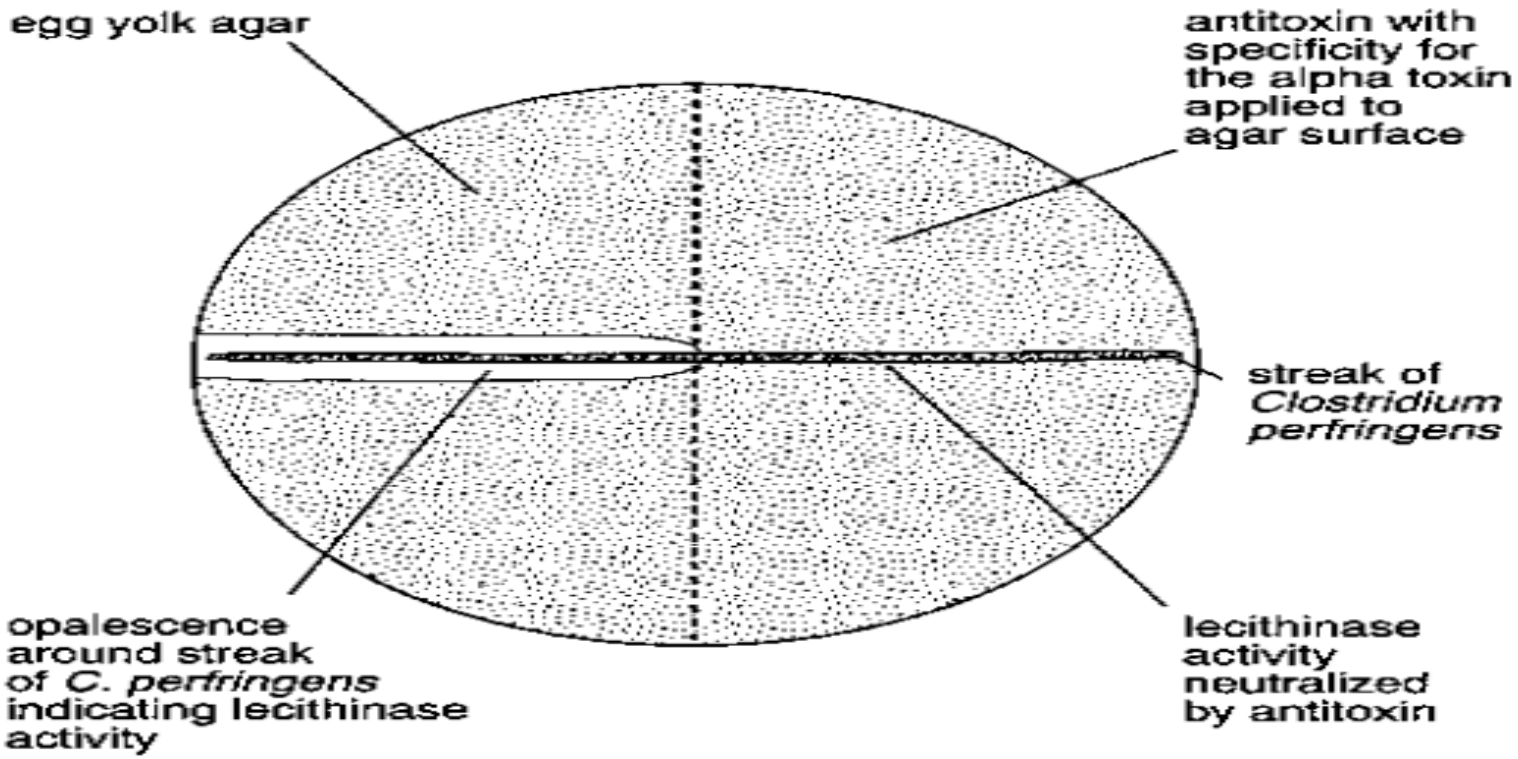


Figure 16.4 Nagler reaction produced by *Clostridium perfringens* growing on egg yolk agar. Antitoxin with specificity for the alpha toxin is applied to the surface of one half of an egg yolk agar plate and allowed to dry. *Clostridium perfringens* is streaked across the plate which is incubated anaerobically 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.

Enteropathogenic and enterotoxaemia-clostridia

- *Clostridium perfringens* types B, C and D are of particular significance in domestic animals.

Usual habitat

- *Clostridium perfringens* is found in soil, in faeces, and in the intestinal tracts of animals and man.
- *C. perfringens* types B, C and D may survive in soil as spores for several months.
- *C. perfringens* type A, which constitutes part of the normal intestinal flora, is widely distributed in soil.

PATHOGENESIS AND PATHOGENICITY

- *C. perfringens* types A to E produce a number of potent, immunologically distinct exotoxins which cause the local and systemic effects encountered in enterotoxaemias.
- A range of minor toxins, some of which may enhance virulence, is also recognized. These include two haemolysins (δ and θ), a collagenase (K) and a hyaluronidase (μ)

Table 16.5 Types of *Clostridium perfringens* and their major toxins.

Clostridium perfringens	Disease	Toxin	
		Name	Biological activity
Type A	Necrotic enteritis in chickens	α (significant toxin)	Lecithinase
	Necrotizing enterocolitis in pigs, Canine haemorrhagic gastroenteritis	Enterotoxin	Cytotoxic
Type B	Lamb dysentery	α	Lecithinase
	Haemorrhagic enteritis in calves and foals	β (significant toxin)	Lethal, necrotizing
		ε (exists as a prototoxin and requires activation by proteolytic enzymes)	Increases intestinal and capillary permeability, lethal
Type C	'Struck' in adult sheep	α	Lecithinase
	Sudden death in goats and feedlot cattle	β (significant toxin)	Lethal, necrotizing
	Necrotic enteritis in chickens	Enterotoxin	Cytotoxic
	Haemorrhagic enteritis in neonatal piglets		
Type D	Pulpy kidney in sheep	α	Lecithinase
	Enterotoxaemia in calves, adult goats and kids	ε (significant toxin, exists as a prototoxin and requires activation by proteolytic enzymes)	Increases intestinal and capillary permeability, lethal
Type E	Haemorrhagic enteritis in calves	α	Lecithinase
	Enteritis in rabbits	ι (significant toxin)	Lethal

Pulpy kidney disease

- This disease, caused by *C. perfringens* type D, occurs in sheep worldwide.
- The condition is also described as 'over-eating disease' because gorging on a high grain diet or on succulent pasture predisposes to its development.
- Clinical signs include dullness, opisthotonos, convulsions and terminal coma.
- Bloating may be evident in the later stages of illness.
- Hyperglycaemia and glycosuria arc constant features of the disease.
- Affected adult sheep, which have survived for several days, may exhibit diarrhoea and staggering.

Diagnostic procedures

- 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.
- Glycosuria is a constant finding in pulpy kidney disease.
- Toxin neutralization tests using mouse and guinea-pig inoculation can definitively identify the toxins of *C. perfringens* present in the intestinal contents of recently-dead animals

Further Readings

- Clinical Veterinary Microbiology 2nd Edition 2013 By Bryan Markey
- Veterinary Microbiology and Microbial Disease