Clostridium spp. **Dr. Sahar Mahdi**

Phylum: firmicutes

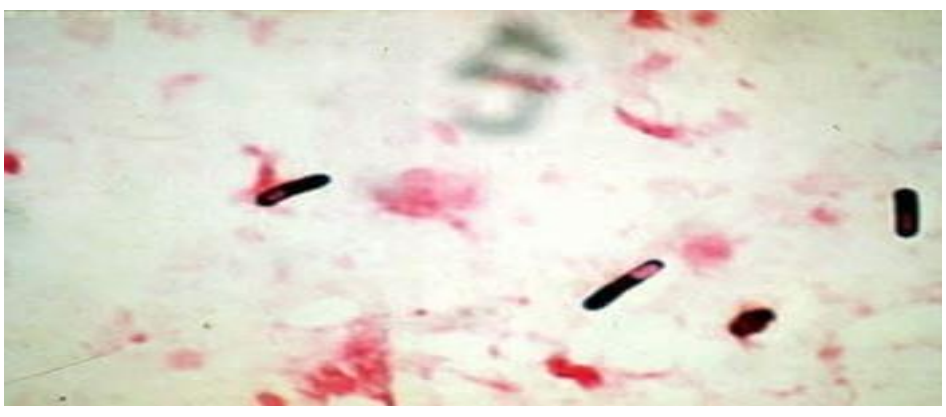
Class: clostridia

Order :clostridiales

Family: clostridiaceae

Genus ; Clostridium

Clostridia are prokaryotic bacteria of the phylum Firmicutes, which are large, anaerobic, spore-forming, rod-shaped, gram-positive organisms. They can be living cells (vegetative forms) or dormant spores. Their natural habitats are soils and intestinal tracts of animals, including humans. Dormant spores of several clostridial species have been found in healthy muscular tissue of horses and cows. The endospores are oval, sometimes spherical, and are located centrally, subterminally, or terminally. The vegetative forms of clostridia in tissue fluids of infected animals occur singly, in pairs, or rarely in chains. Differentiation of the various pathogenic and related species is based on morphological characteristics in culture including spore shape and position, biochemical testing, and the antigenic specificity of toxins or surface antigens. The genomes of many clostridial spp have been sequenced and published. Pathogenic strains or their toxins may be acquired by susceptible animals via either wound contamination or ingestion. Diseases thus produced are a constant threat to successful food animal production worldwide.



Clostridial diseases can be divided into two categories:

1. those in which the organisms actively invade or when locally dormant spores are activated and reproduce in tissues of the host, with the production of toxins that enhance the spread of infection (the gas-gangrene group, the clostridial cellulitides group)

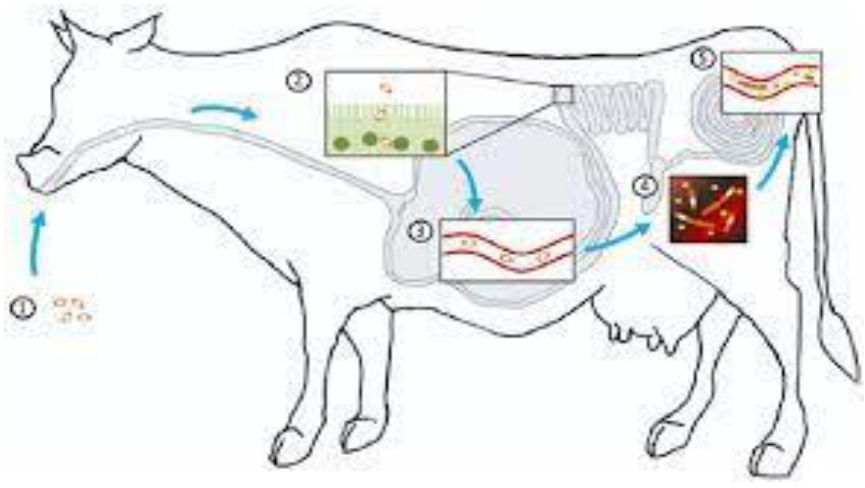
2. those characterized by toxemia resulting from the absorption of toxins produced by organisms within the digestive system (the enterotoxemia), in devitalized tissue (tetanus), or in food or carrion outside the body (botulism)

Clostridial diseases are not spread from animal to animal or from animals to humans. They have been classified into three forms:

a-histotoxic diseases

b-neurotoxic diseases

c-enteric diseases



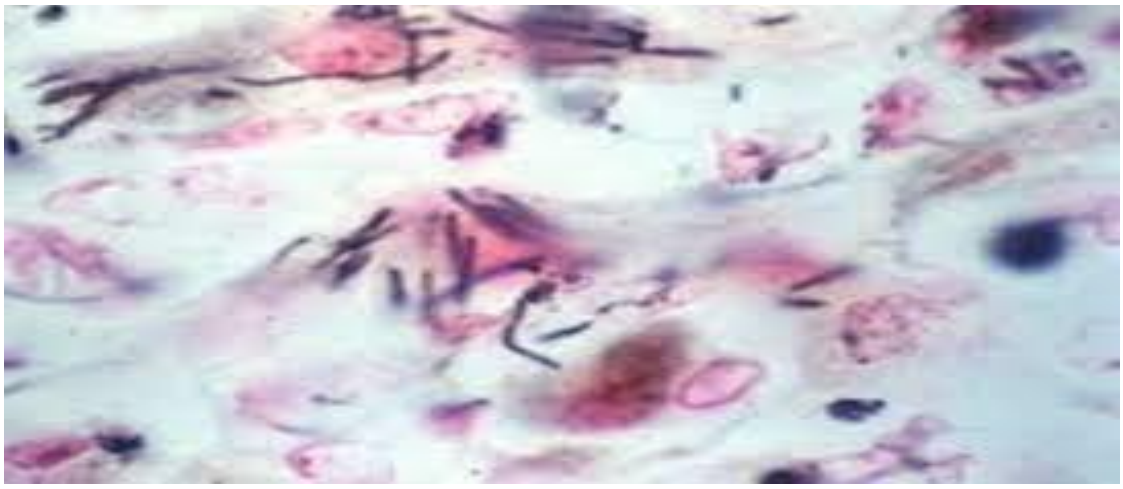
1-Clostridium piliforme

Tyzzer disease is caused by *Clostridium piliforme*. The disease affects a variety of animals, including mammals and birds. It is characterized by a triad of lesions that include **colitis, hepatitis, and myocarditis.** Tyzzer disease is affects a wide range of animals in many regions of the world. It was first described in

mice in 1917. Several years later, it was reported in laboratory rabbits and then in other small laboratory mammals, including guinea pigs, hamsters, gerbils, and rats. It is a highly fatal disease of **young foals**, but it is rare in other domestic animals, including dogs, cats, and calves. It has been reported, albeit rarely, in a variety of wildlife, including muskrats, cottontail rabbit, coyote, gray fox, lesser panda, snow leopard, raccoon, marsupials, white-tailed deer, and a few avian species.

The disease primarily affects young, well-nourished animals, especially those **fed high-protein diets**, during periods of stress. Some species appear resistant unless stressed or immunosuppressed, whereas others appear to be susceptible without immunosuppression. Dietary factors, including excessive nitrogenous diets fed to laboratory animals or to nursing mares, may cause immunosuppression and may predispose susceptible animals to the disease. In all animal species, Tyzzer disease is characterized clinically primarily by liver failure and icterus, a consequence of hepatic necrosis. Less frequently, diarrhea is observed. A triad of lesions consisting of hepatitis, colitis, and myocarditis is considered classic for the disease. However, although hepatitis seems to be constant in all species, colitis and myocarditis occur sporadically in some species. For instance, colitis and/or myocarditis rarely occurs in horses.

Liver, cut sections: The pale yellow necrotic areas contrast to the congested dark red areas. Within the dark red areas are multifocal areas of necrotizing, hemorrhagic hepatitis.



***Clostridium piliforme*, liver**

Diagnosis

Clostridium piliforme, a gram-positive, motile, spore-forming, rod-shaped, flagellated, obligate anaerobes, intracellular bacterium. can be cultured in the yolk sac of chick embryos or tissue culture cells. The vegetative phase is very labile, but spores may survive in soiled bedding at room temperature for >1 year and are resistant to heating up to 60°C for 30 minutes.

C piliforme appears to be common in the environment, but because it is a difficult organism to culture, little is known about the epidemiology, pathogenesis, and immunity of this infection The feces samples of sick or carrier animals are the primary source of spores that contaminate the environment.

Once the disease is present on a farm, it may be seen sporadically every year. The mode of transmission is believed to be fecal-oral, by ingestion of spores, after which most immunocompetent animals clear the infection within a few weeks. In susceptible individuals, however, *C piliforme* replicates in the intestinal mucosa, likely the ileum, colon, and cecum, where it results in enterocyte death and, in some cases, associated inflammation. From there, the microorganism is absorbed into the portal circulation, from where it is distributed to the liver and other organs. The bacterium has an affinity for the epithelial and smooth muscle cells of the intestines, hepatocytes, and cardiac myocytes. Stress factors such as capture, overcrowding, shipping, and poor sanitation appear to be predisposing.

Tyzzer disease is relatively common in horses, which are susceptible to at least two distinct strains, although very little information is available about specific features of disease caused by each of these strains. Some isolates of *C piliforme* produce toxins; however, others do not..

The disease in foals occurs most often between 1 and 6 weeks of age, with most cases occurring between 1 and 2 weeks, and at weaning in rabbits. In some species, Tyzzer disease has been identified concurrently with other diseases, such as feline infectious peritonitis and feline herpesvirus infection in cats, distemper and mycotic pneumonia in dogs, and cryptosporidial and coronaviral enteritis in calves.

The disease in foals is more common during spring, when nursing mares are exposed to lush, **high-protein pastures**. The increase in the availability of nutrients from pasture forages and supplemental diets may encourage the overgrowth of *C piliforme* in the gut of nursing mares. This process predisposes neonatal foals to the disease when they are exposed to massive numbers of the bacterium by ingesting the feces of their dams soon after birth as a mechanism to establish their normal intestinal flora.

In foals, Tyzzer disease affects primarily the liver, where it induces widespread multifocal necrosis, and foals usually die by acute liver failure. Only young foals up to 6 weeks old develop lesions. all foals with Tyzzer disease had liver lesions, while fewer of those animals had enteric or myocardial lesions. Older foals become more resistant to the disease as the gut matures. The disease is not recognized in adult horses, but they carry the bacterium in their gut.

In horses, immunologic factors seem to affect the likelihood of disease, because many adults have antibodies against *C piliforme* and do not develop clinical signs. Tyzzer disease is more common in foals when young, nursing mares are introduced to a farm where the disease is endemic, and it is less common in suckling foals with older mares; this suggests that older mares are immune to the disease and may transfer *C piliforme* antibodies to their foals in colostrum.

Clinical Finding of Tyzzer Disease in animals

Tyzzer disease often affects apparently healthy, fast-growing foals. The incubation period in experimentally infected foals is **4–7 days after oral exposure to bacterial spores.** Most foals are found in a coma or dead. Clinical signs, if seen, are of short duration, from a few hours to 2 days. Clinical signs are variable but may include depression, anorexia, pyrexia, jaundice, diarrhea, and recumbency. Near death, convulsions and coma occur.

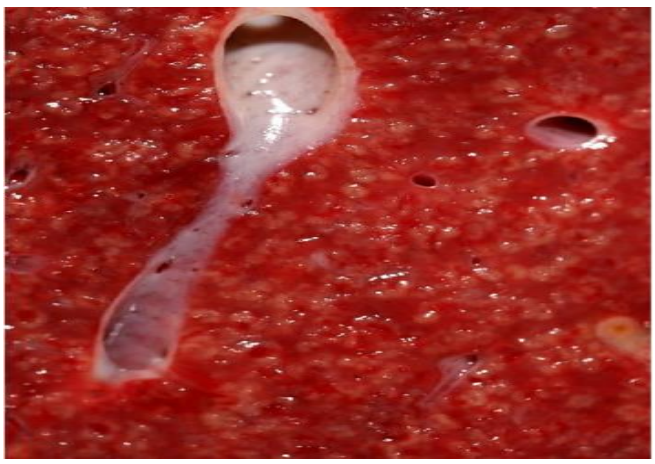
Laboratory animals, including rabbits, may be found dead at the start of an outbreak. As the disease progresses in the colony, animals may show depression, a ruffled coat, and varying extents of watery diarrhea. Clinical signs in other animal species are highly variable. In foals, the serum enzymes sorbitol dehydrogenase, aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase, and gamma-glutamyltransferase are increased. Hyperbilirubinemia, leukopenia, hemoconcentration, and terminally profound hypoglycemia may also develop. Clinicopathologic tests are of minimal value in laboratory animals because of quick death.

Lesions

Characteristic gross lesions of Tyzzer disease are seen in the liver, and less frequently in the myocardium and/or intestinal tract. Rarely, lesions in other organs are seen. Throughout the liver of most animal species, many white, gray, or yellowish foci of necrosis, ~2 mm in diameter, may be seen. In addition, hepatomegaly is marked, and the hepatic lymph nodes are edematous and hyperplastic.

Foals with Tyzzer disease always have liver lesions; however, lesions occur much less frequently in the colon and heart. In rabbits, in addition to liver lesions, severe lesions develop in the intestine and heart

Microscopically, in all animal species, numerous multifocal areas of lytic hepatic necrosis can be seen**. In foals, the hepatic lesions are more pronounced than in other animals.** The causative filamentous bacilli are found in a crisscross pattern in the cytoplasm of hepatocytes .



Hepatic necrosis, Tyzzer disease, foal, gross specimen

2- *Clostridium tetani*

A- Causes tetanus in many human and animals , with the spores being distributed everywhere.

B- Organisms are slender rods, motile (most strains). Gram+ve, and the form large and terminal spores with characteristic tennis racket appearance

C- Many are strict anaerobes growing at 14 to 44 °C.

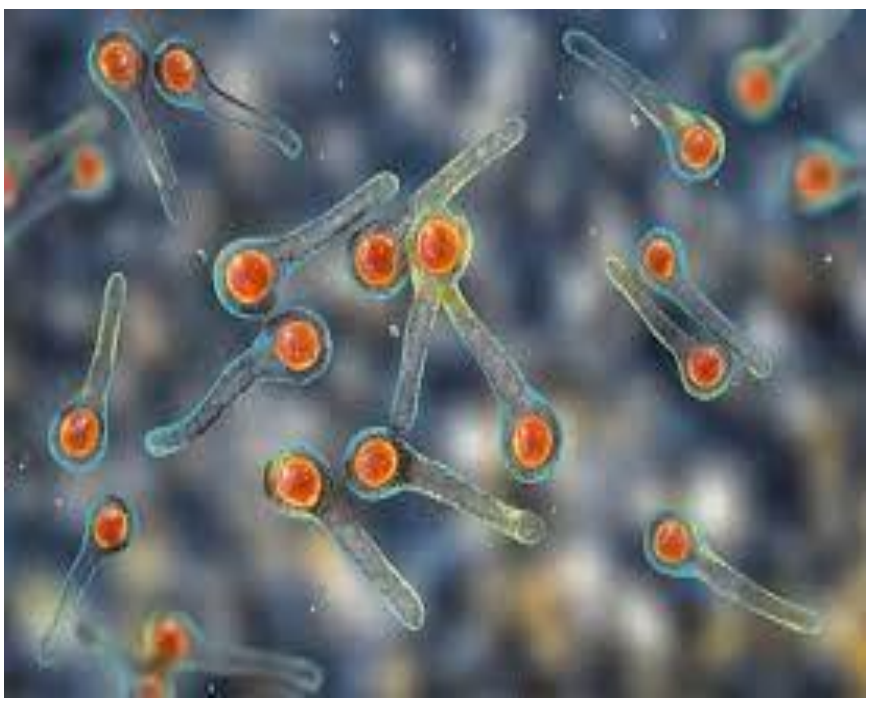
D- Toxigenic strains may produce tetanolysin and tetanospasmin which may cause a condition characterized by rigidity of jaw, neck, limbs and trunk. This condition depends entirely on germination of spores into bacilli and the elaboration of toxins

C- used the fildes technique for cultured

Clostridium tetani grows on ordinary media.

Growth can be improved by blood and serum.

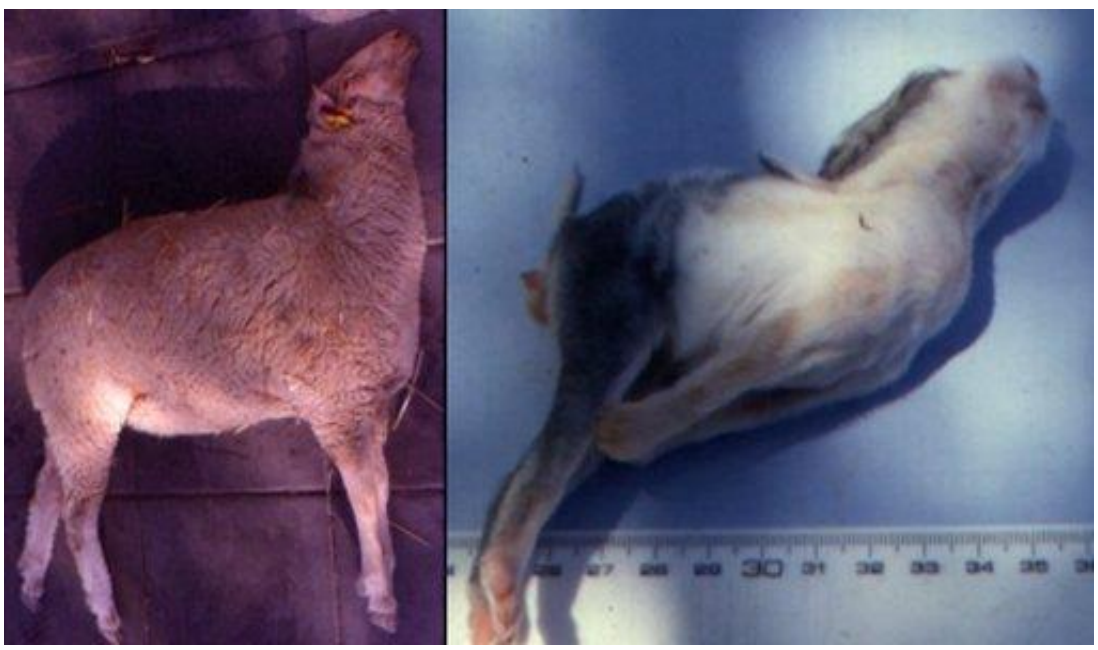
Because of marked tendency to **swarm** over the surface of the agar, surface colonies are difficult to obtain.



Clostridium tetani

A B

Robertson cooked meat broth showing proteolytic activity. (B) Blood agar showing swarming.

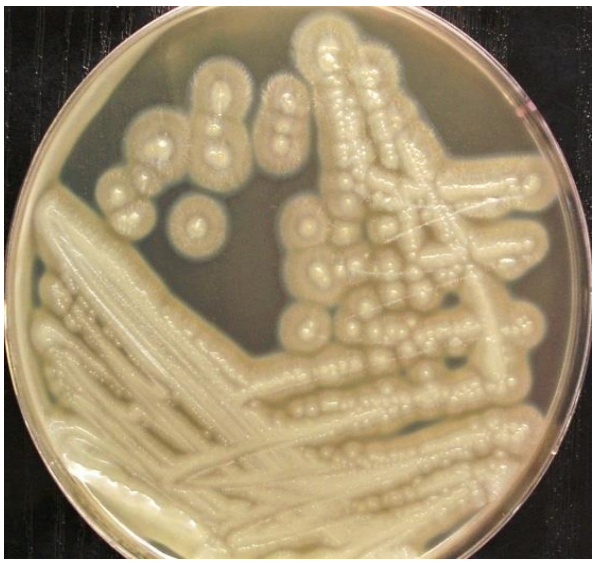


rigidity of jaw, neck, limbs and trunk

3- *C. botulinum*

A- This organism and its spores are well distributed in soil, vegetables. Fruits and pastures.

B- Strict anaerobe, may grow within 20-35°C and does not ferment lactose.



C- It may cause food poisoning in man and animals after ingestion of performed toxin which is excreted in the foodstuff under suitable conditions. These toxins are extremely lethal, especially type A toxin

Poisoning signs may involve paralysis of the hind limbs, muscles of the mouth, pharynx and neck, ending in coma and death.

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4- Clostridium difficile:

Is gram-positive, a spore-forming, toxin-producing, anaerobic bacterium that causes antibiotic-associated colitis. It colonizes the human intestinal tract after the normal gut flora has been altered by antibiotic therapy. C. difficile infection is one of the most common healthcare-associated infections and a significant cause of morbidity and mortality among older adult hospitalized patients.

Man cause pseudomambranous colititis in human and ileocolitis in laboratory animals.

It is of minor importance in veterinary public health.

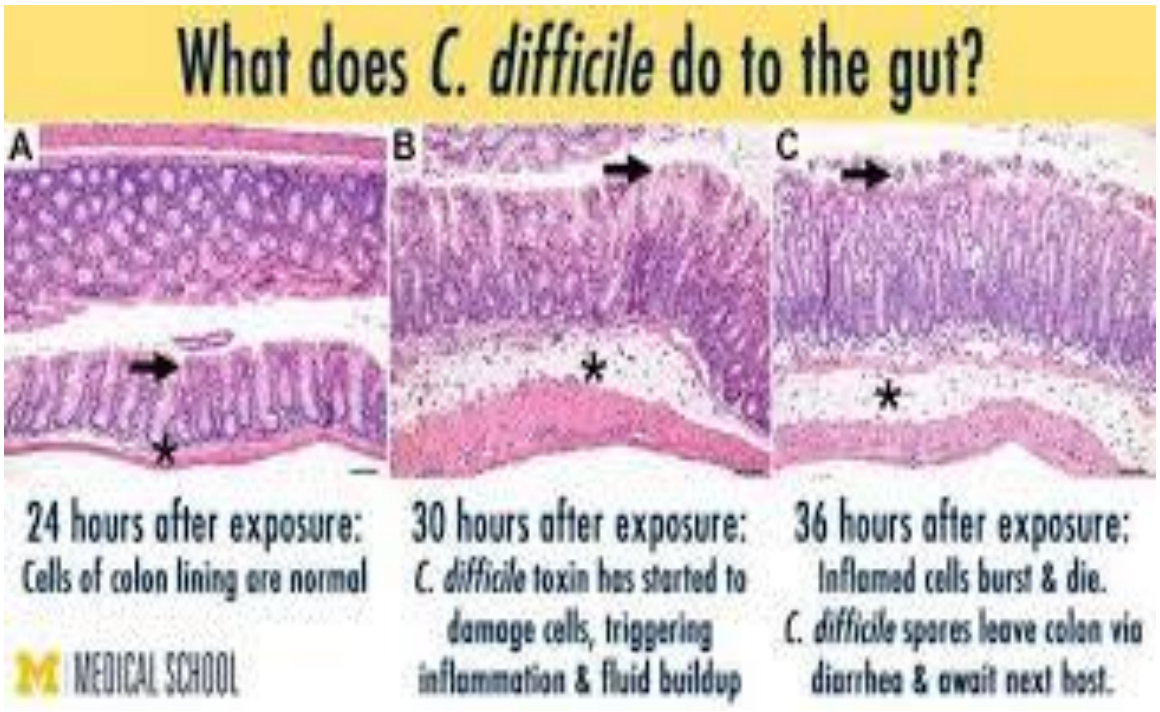
This organism may be a normal constituent of the fecal flora of health infants; however , it is rare and transient in the intestine of the healthy adult.

Non- hemolytic and non –proteolytic.

Diarrhea with colitis

Watery diarrhea is the cardinal symptom of

C. difficile–associated diarrhea (CDAD) with colitis Other manifestations include lower abdominal pain and cramping, low-grade fever, nausea, anorexia, and leukocytosis . Diarrhea may be associated with mucus or occult blood.



5- *Clostridium perfringens (or welchii)*

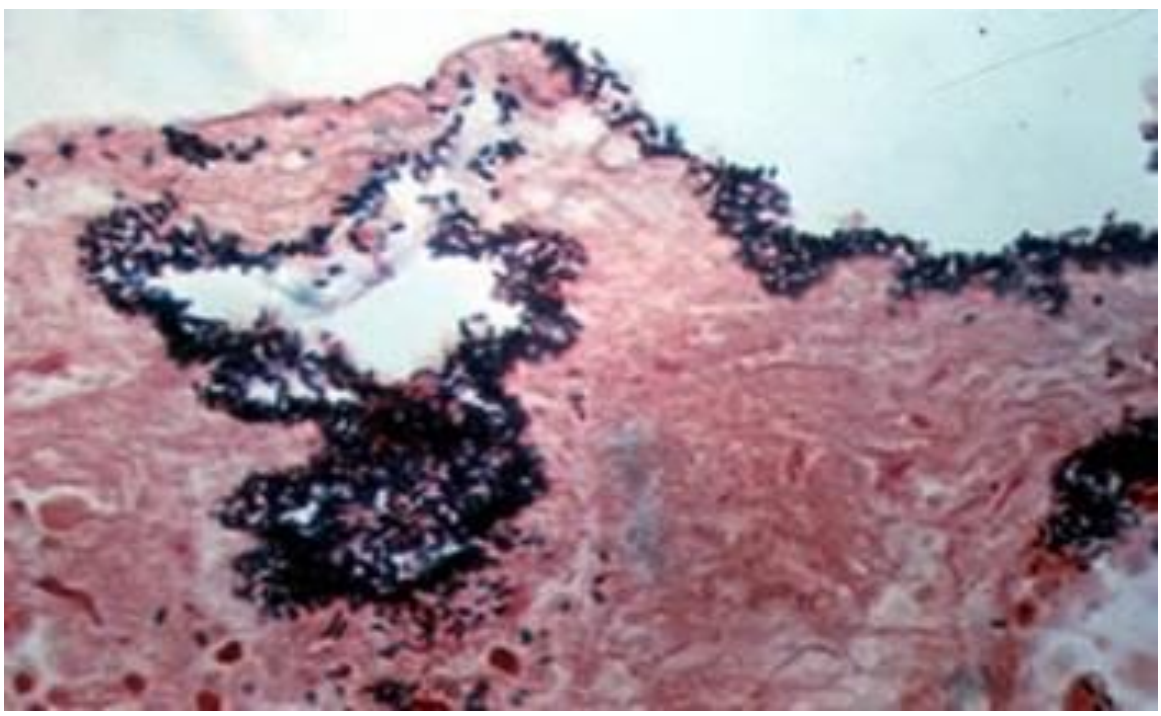
A- The cause of gas gangrene in human and animals plus other important Enterotoxaemic infections in sheep and other animals.

B- different strains produce several toxins which can be differentiated by neutralization test . On this basis *C. perfringens* is classified into type A, B, C, D, E, each of which causes a certain disease.

C- the organism is found in soil and intestines of healthy animals and human , but under certain encouraging conditions may reverse to become pathogenic and cause disease.

Morphology and cultural characteristics of clostridia:

1-rod-shaped, occurs singly or in pairs , forms capsules especially in smears from infected tissues, non-motile, gram+ve, and forms oval, central or subterminal spores.

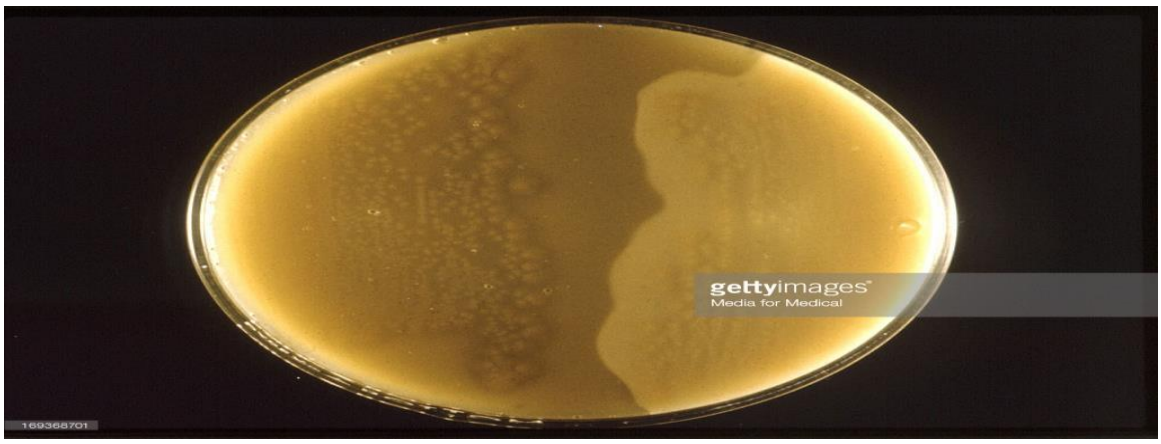


***C. perfringens* in tissue**

2- grow well on ordinary media at 37-47 °C, and growth is enhanced by the presence of glucose or blood.

3- on blood agar, the round colonies of smooth strains are surrounded by a narrow zone of β- haemolysis, and a winder zone of ά – heamolysis. This feature will become very distinct when the plates are stored at a lower temperature.

4- On media containing human serum of filtered egg-yolk, the organisms produce a marked opalescence caused by ά toxin (lecithinase) and this phenomenon is called : Nagler or lecithinase reaction .



culture of the anaerobic clostridium bacterium on egg-yolk medium

5- Enterotoxaemic infections are characterized by the presence of very large numbers of *C. perfringens* in the intestines , clearly demonstrate by gram‘s stain smear prepared (immediately after death of the animal from the intestinal contents or lesions in the intestinal wall.

Main important toxins of C. perfringens

Type of *C. perfringens*	major lethal toxins produced
A	alpha
B	alpha, beta and epsilon
C	alpha and beta
D	alpha and epsilon
E	alpha and iota

A- alpha toxin is a major lethal toxin

B- beta is a major lethal and necrotizing toxin

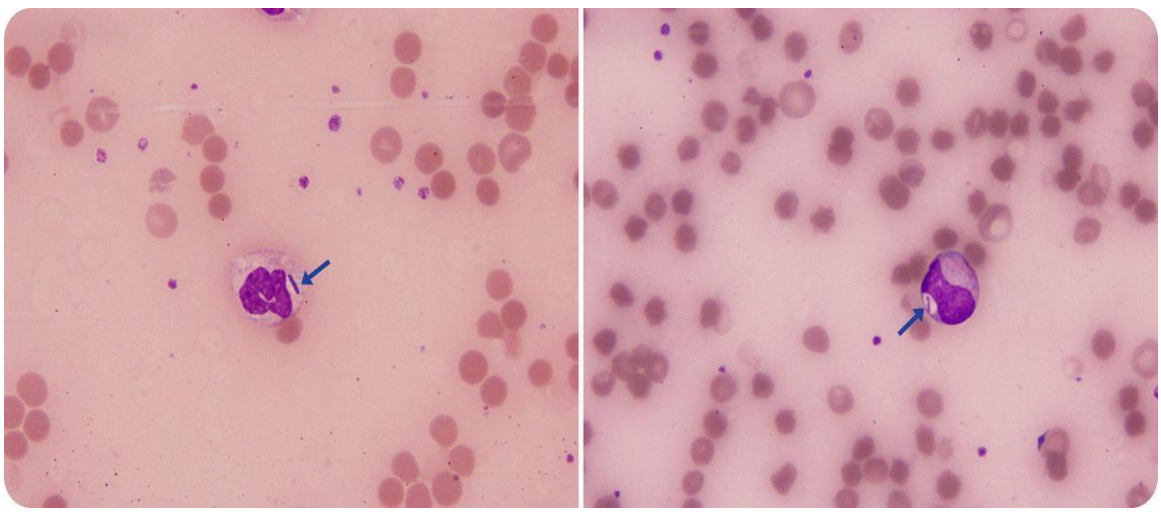
C- Epsilon toxin is a major lethal and necrotizing toxin

D- iota toxin is a major lethal and necrotizing prototoxin.

6- *C.chauvoei*

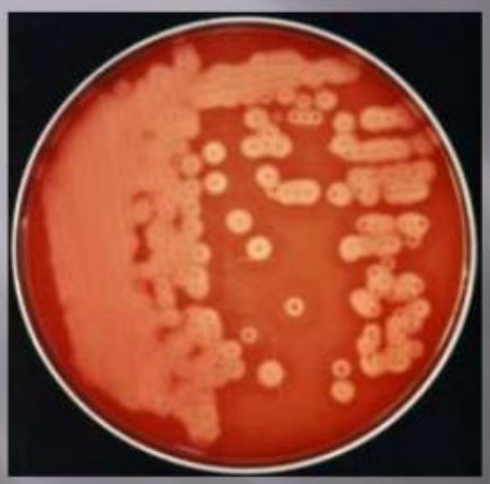
A- The cause of black or **black quarter** in sheep and cattle .

B- cigar-shaped rod , non –encapsulated, forms spores which are oval, wide and central or subterminal. Black leg in cattle

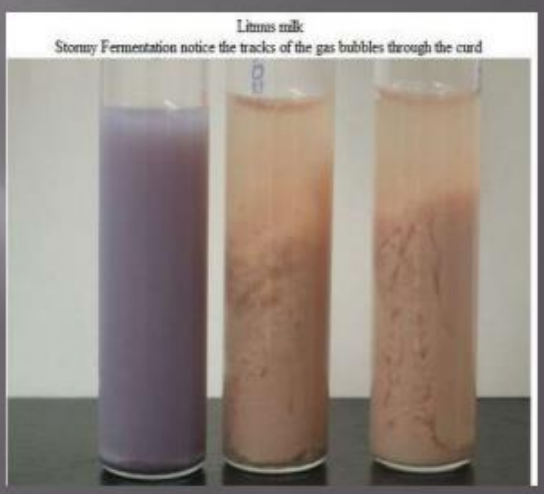


Clostridium. perfringens

Culture & identification



B – Haemolysis
On blood agar media



1= Control, 2 and 3= "Stormy clot fermentation"
Clostridium perfringens will produce acid and gases

Media used for Cultivation

- Liquid medium for cultivation cooked meat broth
- Thiglyclolate broth
- CMB contain unsaturated fatty acids which take up oxygen
- Proteolytic medium turns the medium black and Saccharolytic medium turn the meat pink

1- fresh samples from an infected animal are cultured onto blood agar, incubated anaerobically for 24-48 hr. Another alternative is to inoculate the sample into Cooked meat broth (freed of oxygen by preheating at

100°C in a water bath for few minutes then cooled and cultured and then subcultured onto blood agar.

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Diagnosis of clostridia infections

A- direct examination : making smear from the intestinal contents and staining them with grams stain to check for large numbers of G+ve rods.

B- isolation , either by direct cultivation into media like thioglycollate or cooked meat broths or by streaking onto agar media, like blood or SPS(sulphite-polymyxin-sulphadiazine) agar.

C- identification: by different techniques, like fluorescent antibody and gas chromatography.

D- laboratory animal inoculation.

E- biochemical identification: using the following tests:

- Heamolysis on blood agar
- Shape and location of spore
- Gelatin liquification
- Nitret reduction
- Motility
- Litmus milk
- Indole
- H_2S productions
- Urease
- Starch hydrolysis
- Sluggish double zone of heamolysis (β and ά)

Antigenic Structure:

• Flagella (H), somatic (O), and spore antigens.

Pathogenicity Determinants:

• play a role in local infection only in conjunction with other bacteria that create suitable environment for their invasion

• systemic-acting, **plasmid-mediated A-B neurotoxin (tetanospasmin)** produced intracellularly

• Mode of Action --- one of most poisonous substances

• binds gangliosides in synaptic membranes (synapses of neuronal cells) and **blocks release of inhibitory neurotransmitters,** continuous stimulation by excitatory transmitters• muscle spasms (spastic aralysis) (trismus **(lockjaw)**, risus sardonicus, opisthotonus), cardiac arrhythmias, fluctuations in blood pressure