CHAPTER 06

CLOSTRIDIAL DISEASES HEALTH PERSPECTIVE IN FARM ANIMALS

Salma A Shoulah[1], Abdelfattah Selim[1], Ehab El-Sayed Mohamed[2] and Mohamed MS Gaballa[3]*

[1]Department of Animal Medicine (Infectious Diseases), Faculty of Veterinary Medicine, Benha University, Toukh. 13736, Egypt
[2]Foot and Mouth Disease Research Department (FMDRD), Agricultural Research Center (ARC), Veterinary Serum and Vaccine Research Institute (VSVRI), Abassia, Cairo, Egypt
[3]Department of Pathology, Faculty of Veterinary Medicine, Benha University, Toukh. 13736, Egypt
*Corresponding author: mohamed.gaballah@fvtm.bu.edu.eg

INTRODUCTION

In nature, Clostridium (C.) is a genus of widely distributed commensal or soil-borne bacteria as well as often being a part of the enteric flora of many animals and humans. We tend to pay more attention to pathogenic clostridium species as *C. botulinum, C. chauvoei, C. haemolyticum, C. novyi, C. perfringens, C. septicum*, and *C. tetani*, rather than the commensal members of this genus. Clostridium species are distinguished from other bacteria by their anaerobicity and the presence of heat-resistant endospores. Predisposing conditions are always needed for clostridial infections to occur, i.e., a deep wound or a traumatic injury that compromises the skin or intestinal barrier, or the alteration of the gastrointestinal microbiota because of a change in feed or the treatment of antimicrobial agents. When these conditions are met, the bacteria usually produce toxins which are primarily responsible for the pathogenesis of the diseases caused by the bacteria. Because the bacteria's toxins act quickly, treating diseases caused by them is very challenging. We can classify clostridial diseases and infections into three major categories: neurotoxic diseases, histotoxic diseases, and enteric diseases. Animals that are afflicted with clostridial diseases typically die suddenly, with no apparent symptoms. Bacteria present in animal' intestine can multiply, causing the bacteria to spread throughout the carcass. In addition to putrefaction of carcasses caused by growth of clostridial organisms' postmortem, other pathologies can be affected. So, carcasses must be examined early to make a definitive diagnosis and tested specifically for bacteria and their toxins, with histopathology also crucial. As well as morphological and biochemical characteristics, antigenic specificity of toxins and surface antigens are used to distinguish between the various pathogenic and related species. In order to prevent these diseases, vaccination is the best method for protection.

The current chapter reviews a number of histotoxic and neurotoxic clostridial diseases as well as enterotoxaemia's that have been reported in farm animals. Moreover, it includes information concerning their etiology, epidemiology, and the mechanisms underlying their pathology. Furthermore, it covers treatment, control, and prevention strategies.

Clostridial Histotoxic Infections

Blackleg

Overview

'Blackleg,' or clostridial myositis is a highly fatal and febrile bacterial disease that affects cattle as well as sheep, goats, and infrequently horses. Blackleg breaks out in either the shoulder or the hindquarter then triggers black, edematous, emphysematous, or crepitating swelling of the heavy body muscles, and severe toxaemia and myonecrosis of the skeletal and/or cardiac muscles (Abreu et al. 2018).

Etiology and Epidemiology

An infection occurs when a rod-shaped Gram-positive, spore-forming bacterium called *C. chauvoei* gets into the body. Despite environmental variations and disinfectants, the spores remain viable for many years**.** According to current information, the predominant route of infection in sheep is believed to be penetrating skin or mucosal injuries, but the primary entrance point in cattle is unknown. However, ingestion of contaminated feed or eruption of teeth might allow entry through the alimentary mucosa (Radostits 2007). There is a positive correlation between annual rainfall and incidence of blackleg during warm, wet months. In spite of the lack of understanding of this correlation, it is hypothesized that rain may aid in spores' allocation. Moreover, water saturation supports anaerobiosis coupled with enhanced pasture growth, thus encouraging pastured cattle to consume more feed (Useh et al. 2006). While sheep of any age can potentially be infected, fast-growing cattle under two years of age that are on a great amount of nourishment and are rapidly growing in particular are most susceptible (Radostits 2007; Snider and Stern 2011; Cooper et al. 2016).

Pathogenesis

As previously stated, *C. chauvoei* spores are prevalent in soils, and the disease usually manifests in injured sheep, whereas in cattle, the spores multiply after ingestion before crossing the intestinal mucosa. Spore-intake causes spores to multiply and enter the general circulation, where they are distributed in a variety of tissues, including skeletal muscles (Quinn et al. 2011; Frey and Falquet 2015; Cooper and Valentine 2016).

How to cite this chapter: Shoulah SA, Selim A, Mohamed EES and Gaballa MMS, 2022. Clostridial diseases health perspective in farm animals. In: Abbas RZ, Khan A, Liu P and Saleemi MK (eds), Animal Health Perspectives, Unique Scientific Publishers, Faisalabad, Pakistan, Vol. 2, pp: 44-53. https://doi.org/10.47278/book.ahp/2022.41

Blackleg has been categorized as an "endogenous" clostridial infection since the pathogen seems to be set up in tissue in a dormant state prior to disease happens (Cooper and Valentine 2016).

An anaerobic environment for germination and multiplication is created by local muscle damage ahead of low oxygen tension, which triggers the dormant spores to germinate. As soon as *C. chauvoei* reverts to its vegetative form, it generates a number of toxins, including oxygen-stable and oxygen-labile hemolysins, DNase, hyaluronidase, and neuramidase, causing substantial necrotizing myositis locally and a fatal toxemia systemically (Abreu et al. 2017; Pires et al. 2017).

Clinical Findings

The majority of blackleg cases are acute or sub-acute, though cases can also occur chronically. When acute cases are found, animals are dead without clinical signs, but for some of the cases where clinical signs are evident, such as depression, lethargy, anorexia, and refusal to move, clinical signs are followed by circulatory collapse and death (Groseth et al. 2011; Snider and Stern 2011). Observations of the animal before death usually reveal severe lameness, as well as swelling of the upper part of the leg. At first, the swelling is painless and hot to the touch with discolored skin covering, then quickly it come to be cold and painless, with edema and emphysema being felt and the skin appears dry and cracked (Irisk 2007). When blackleg lesions are present in the limb musculature of sheep, there is a stiff gait, and the sheep experiences lameness in one limb or more along with is reluctant to move. Subcutaneous edema, however, is scarce and gaseous crepitation cannot be felt before death (Radostits 2007). The skin may appear discoloured above and beyond, although there is no skin necrosis or gangrene (Coetzer et al. 1994). Clinically, the syndrome in horses is not well characterized, yet it has been associated with edema of pectoral muscle, stiff gait, and incoordination (Radostits 2007).

Gross and Microscopic Lesions

The carcass of a blackleg-dead animal is often found lying sideways with the affected hind limb sticking out. In a few hours, bloating, putrefaction, and blood-stained froth is exuded from the nostrils and anus (Songer 2004). Acute hemorrhagic inflammation is evident in internal organs such as the lungs, heart, stomach, and intestines. Moreover, local lymph nodes are emphysematous and swollen as a result of acute lymphadenitis. Besides, there is also blood-tinged fluid in the serous cavities and the internal organs display degenerative changes. Swelling is often observed externally in superficial skeletal muscles, as well as stretched and dark skin overlying the affected muscles. Under the skin of affected areas, crepitation can be palpated since the sero-sanguineous fluid, which is accompanied by gas bubbles, usually expands the subcutaneous tissues and fasciae in affected areas (Hogarth 2000). On cutting sections of the affected muscles, dark red to black discolorations are observed, a sweet odor, similar to rancid butter can be smelled along with edema at the periphery of the lesions can be noticed. The center of the lesions is usually dry and friable, with numerous tiny cavities filled with gas bubbles.

Microscopically, there are swollen, hypereosinophilic, vacuolated, fragmented muscle fibers; they have lost cross striations; gas bubbles separate the fascia from the muscular fibers, and hypercontraction bands are often seen. Infiltrated neutrophils may infiltrate fragmented myofibers; however, macrophages, plasma cells, and lymphocytes are gradually replacing the neutrophils as the lesions progress. likewise, Gram-positive bacteria occur singly or in small irregular clumps could be observed. Hemorrhage and proteinaceous edema expand the interstititium, whereas the interstitial vessels contain fibrin thrombi besides arterioles and arteries show fibrinoid necrosis, with fibrin thrombi in the interstitial vessels and fibrinous necrosis in arteries and arterioles along with intramural neutrophil infiltration (Abreu et al. 2018).

Treatment, Prevention and Control

Unless the animal is morbid, penicillin and surgical debridement of the lesion, including fasciotomy, are recommended. Unfortunately, the extensive nature of the lesions, however, limits the recovery rate. In the presence of high fever and a toxemic condition, antiallergic and antipyretic as well as large doses of crystalline penicillin (44,000 IU/Kg BW) should be given intravenously, followed by a longer-acting preparation. Blackleg antiserum, unless very high doses are given, is unlikely to be of much benefit in treatment (Radostits et al. 1994; Constable et al. 2017). In order to prevent the spread of infection, the corpse of an infected animal must be buried deeply.

In order to control blackleg, Bacterins vaccines prepared from *C. chauvoei* cultures that were formalin-inactivated are generally used for vaccination against blackleg. Most of the vaccines can be found in a polyvalent formulation with *C. novyi, C. septicum*, and *C. sordellii*. A vaccination schedule of two vaccinations given four weeks apart followed by a booster vaccination at the end of the first year is generally recommended on farms where the disease is enzootic (Butler 1998; Tolera et al. 2019).

Malignant Edema

Overview

Known also as gas gangrene, malignant edema is a type of necrotizing clostridial infection of horses, sheep, and cattle that is characterized by a short duration, fever, and the presence of hot, painful swelling in the vicinity of the site of infection (Cebra and Cebra 2012; Silva et al. 2016).

Etiology and Epidemiology

Several Clostridium species have been isolated from injuries associated with malignant edema in animals, involving *C. septicum*, *C. chauvoei*, *C. perfringens* and *C. sordellii* (Peek et al. 2003; Silva et al. 2016). Oftentimes, mixed infections occur. Additionally, *C. sordellii* has been linked to malignant edema in cattle, as well as swollen heads in sheep. Infection is caused by gram-positive bacteria that enter the body through skin wounds or mucosal wounds. Like *C. chauvoie*, these organisms generate spores that are resistant to heat, alcohol and acid pH leading to endurance of the infection for extended times in a local area but germinate when subjected to an anoxic environment with the proper nutrients and temperatures. Gas gangrene's clostridial agents, including their spores, are ubiquitous. Animals' intestinal contents, organic matter, and soil containing high levels of humidity are the major sources of

these clostridial agents. Thus, seasonally flooded soils are more likely than dry soils to contain these microorganisms**.** The disease can be sporadic and harm animals of all ages and species in response to injections, while outbreaks in sheep can occur following some management practices such as vaccinations, umbilical cord contamination in newborns, shearing and docking or after lambing. When it comes to cattle, following parturition, lacerations of the vulva are often associated with the precipitating cause, whereas in equine, intramuscular inoculation of medications, usually associated with colic therapy, is the most common cause (Constable et al. 2017).

Pathogenesis

The disease set off when wounds are contaminated with the spores or with the vegetative forms of one or more clostridial species, germination, vegetation, and production of toxins then occur; hemolytic and necrotizing toxins, in particular (alpha toxins), are most threatening. The histotoxic clostridia produce toxins that act locally, causing tissue necrosis, which offers an ideal condition for the continued multiplication of these microorganisms and higher concentrations of toxins, which are ultimately released into the body's circulation, causing toxaemia, shock, and death (Morris et al. 2002; Costa et al. 2007; Popoff and Bouvet 2009; Aronoff 2013; Silva et al. 2016; Sunagawa and Sugitani 2017).

Clinical Findings

Most animals show similar clinical signs, including depression, tachycardia, muscle tremors, and hyperthermia within about six to 48 hours after infection. Usually, when a limb is affected, the animal lame, reluctant to move, and eventually falls to its death within 48 hours. A reddish-brown fluid is released from the vulva within 2-3 days when an infection occurs at parturition. In some cases, swelling extends to the pelvis and perineum. Among young male rams, fighting injuries can cause the development of a clinical condition referred to as "big head." This is a specific type of gas gangrene, distinguished by an edematous swelling of the head, face, and neck that is not gaseous or hemorrhagic (Choi et al. 2003; Costa et al. 2007; Odani et al. 2009; Farias et al. 2014; Silva et al. 2016)

Gross and Microscopic Lesions

In most animals, gas gangrene results in comparable gross changes, with diffuse hemorrhagic and gelatinous subcutaneous edema and emphysema, irrespective of the clostridial species. A soft, doughy swelling is produced at the site of infection accompanied by local erythema, severe pain on palpation, then the swelling becomes more edematous, but less painful. A diffuse edema involving the perineum and perivaginal area of cattle often occurs following postpartum gas gangrene, which, in some cases, extends to nearby muscles. Among sheep and cows, an externally visible line of demarcation dividing affected and unaffected tissue along with severe edema and bleeding of the subcutaneous tissue distinguishes gangrenous myositis. In an edematous or hemorrhagic area, gram-positive bacilli with central, subterminal, or sometimes terminal spores are usually observed microscopically. There may also be infiltrated inflammatory exudate, mainly neutrophil-based, as well as vasculitis, and thrombosis, which may extend to the fasciae and muscle tissue, in addition to necrosis of adjacent skeletal muscle similar to that found in cases of blackleg. An unpleasant, putrid smell is frequently present with infections caused by *C. perfringens* and *C. sordellii*. Edema fluid is generally blood-stained and contains gas bubbles, apart from in *C. novyi* infections where it is gelatinous, clear, and without gas bubbles. It is not uncommon for subcutaneous hemorrhages and serosanguineous fluid to accumulate in body cavities. A ram's "swelled head" can involve the entire neck and head, as well as the pleura, the chest, and the lungs (Cooper and Valentine 2016; Constable et al. 2017).

Treatment, Prevention and Control

Locally, penicillin inoculation immediately into and across the edge of the lesions are often suggested with irrigation with hydrogen peroxide and iodine solution. In order to control the toxemia on a systemic level, high dosages of procaine penicillin G, repeated at 4-6 hours intervals along with antitoxin drugs, fluid therapy, and anti-inflammatory drugs should be given as soon as possible in the course of the illness (Constable et al. 2017). Vaccination of the animals, strict hygienic measures at lambing, shearing, castration, docking, navel treatment with antiseptics (acridine dyes, betadine lotion, etc.), as well as avoidance of soil or fecal contamination of wounds are all part of a proven approach to disease prevention. When gas gangrene is endemic in an area, animals aged 4 to 6 months or younger should be vaccinated with bacterin toxoid twice, with a gap of four weeks between each vaccination (Boyd et al. 1972; Barnes et al. 1975; Lewis 2011; Parish et al. 2019; Oliveria Jounior et al. 2020).

Bovine Bacillary Hemoglobinuria

Overview

Bacillary hemoglobinuria (BHU; red water disease) is an acute, toxemic, and often lethal disease of cattle, less frequently noticed in sheep, and it has been notified rarely in horses and pigs. It is marked by intravascular hemolysis, sudden onset of hemoglobinuria, hepatic infarction, and death within one to two days (Oliver and Staempfli 1999; Radostits 2000; Shinozuka 2011).

Etiology and Epidemiology

The strict anaerobic *C. haemolyticum* (*C. novyi* type D) is a Gram-positive, motile, and sporulated rod (Navarro and Uzal 2016) causes BHU, and its primary virulence factor is beta toxin, a phospholipase C (Oliver and Staempfli 1999; Radostits 2000; Shinozuka 2011). Under anaerobic conditions, it produces a necrotic and hemolytic beta toxin causing damage to the hepatocytes as well as capillaries endothelium.

Bacillary hemoglobinuria is usually sporadic, but it can be endemic in areas with a high prevalence of fascioliasis. Flooding, drainage, polluted hay from infected areas, or carrier animals spread the disease from infected areas to noninfected ones. Conditions of dry weather may affect animal movement patterns, which may result in animals converging on areas of pastures that have liver fluke infestations, such as those located around small ponds, drains, dams, and swamps, thus being more prone to exposure to metacercariae. The disease is more common in animals over the age of one year, in good nutritional condition and are often introduced to infected areas recently.

Pathogenesis

C. haemolyticum spores are soil-borne, they can withstand to exposure to environmental conditions and remain viable for several years (Jasmin 1947). Usually, the disease is caused by ingesting contaminated materials and after multiplying in the intestinal tract, bacteria enter the bloodstream, phagocytized by Kupffer cells, and then remain latent in the liver until hepatic damage renders the environment anaerobic (Van Kampen and Kennedy 1969). In addition to causing liver necrosis, migrating flukes also cause anaerobic conditions in the liver, which allow it to germinate, multiply, and produce toxins such as phospholipase C (hemolytic beta toxin), causing hepatocellular necrosis and intravascular hemolysis (Cullen and Stalker 2016; Navarro and Uzal 2016). As a result of beta toxin action, the arachidonic acid cascade is activated, thromboxane and leukotrienes are produced, platelets aggregate, and capillary permeability increases. Infarcts with large anemic area are typical of the disease both because of hepatocyte necrosis and thrombus formation due to endothelial disruption. Among the main clinical features of hemolysis are hemoglobinuria and jaundice, as well as severe hypoxia that leads to hemoglobinuric nephrosis (Navarro and Uzal 2016).

Clinical Findings

Clinically, it may present as either an acute form lasting 10 to 12 hours, or a subacute form lasting 3 to 4 days. Both forms are characterized by hemoglobinuria, jaundice, and high fever (40 to 41°C) that decreases rapidly at the time of death (Oliver and Staempfli 1999). A marked reduction in hemoglobin concentration, hematocrit, and red blood cell count is associated with hematological changes, followed by leukocytosis. Furthermore, symptoms include decreased rumination, lactation, defecation of dark brown feces, an increased heartbeat, severe dyspnea, and edema of the brisket (Van Kampen et al. 1969; Navarro 2017).

Gross and Microscopic Lesions

Macroscopically, it is characterized by rapid rigor mortis, soiled perineum with bloodstained urine and feces, subcutaneous gelatinous edema, and severe petechial or diffuse hemorrhages are common features. However, pathognomonic features of BHU are a large, usually single area of necrosis delimited by a hyperemic rim, typically seen at the liver's diaphragmatic surface. Microscopic examination of hepatic lesion reveals focally extensive coagulative necrosis with a rim of inflammatory cells mainly neutrophils, and fewer lymphocytes and plasma cells surround the necrotic tissue with large numbers of Gram-positive rods, mostly along the inner margin of the leukocytic rim in sinusoids of necrotic areas. A homogeneous or globular acidophilic substance occupies the glomerular spaces and tubular lumen, giving the kidneys a mottled appearance (speckled red or brown by hemoglobin). Furthermore, the tubule lumens contain varying levels of eosinophilic granules and protein casts as a result of epithelial degeneration and necrosis. Therefore, the urine in the bladder appears deep red colored (Ahourai et al. 1990; Hussein et al. 2013; Navarro 2017).

Treatment, Prevention and Control

If available, special treatment is immediate administration of antitoxic serum with Procaine penicillin G, plus supportive treatment such as blood transfusions, parenteral fluids, and mineral supplements containing iron, copper, and cobalt. Treatment must be in the early stages, unfortunately, there is not enough time to initiate treatment, and even when it is attempted, the success rate is low (Crowe et al. 1989; Oliver and Staempfli 1999; Shinozuka 2011). During an outbreak, it may be more useful in preventing the disease in animals that haven't been vaccinated. Considering the disease's association with liver flukes, reducing parasitic burdens and restricting access to swampy and poorly drained pastures have been very effective in reducing BHU incidences (Kahn and line 2005). Burning or deep burial is also suggested for disposing of animals who have died of BHU.

Black Disease

Overview

Infectious necrotic hepatitis (INH) is a bacterial infection, more commonly known as black disease in livestock, that occurs most often in sheep, infrequently in cattle, and is caused by a cytotoxin of *C. novyi* type B. It produces focal areas of coagulative necrosis in the liver (Cullen and Stalker 2016).

Etiology and Epidemiology

C. novyi type B, the etiologic agent of infectious necrotic hepatitis, a soilborne organism found in the intestinal microbiota and in herbivores' livers. It is also capable of infecting wounds by resting on the surface of the skin or lying dormant in muscle. Infection occurs via fecal-oral route and spread from farm to farm by transport of contaminated soil during flooding or by infected wild animals and birds. Adult animals are more susceptible to INH, while young animals are not affected as often. In many countries, outbreaks take place during the summer or autumn months and are correlated with liver damage caused by fascioliasis, and typically end within a few weeks after a frost since the encysted metacercaria is destroyed by the freezing

Pathogenesis

During infection, *C. novyi* passes through intestinal walls, settles in the liver, and remains dormant until local anaerobic conditions, such as those created by migrating flukes, which then promote *C. novyi* to multiply and produce alpha-toxin, which is necrotic and causes further liver necrosis and widespread damage to the blood vessels. In some cases, nervous signs can occur as a result of this general vascular disturbance or from the effect of specific neurotoxin.

Clinical Findings

Clinical signs are usually non-specific, such as weakness, restlessness, drowsiness, anorexia, hyperthermia, tachypnea, tachycardia, and recumbency are among the common symptoms. Affected sheep can die within a few hours of disease onset without any clinical signs being present. Cows show the same signs as sheep, but the course is more prolonged, the

feces are semifluid, feces are semi-fluid sometimes mixed with blood.
Peritonitis, severe progressive toxemia, depression, reluctance to walk, pain on palpation of the abdomen, frequent straining, and recumbency are the common symptoms displayed by horses with INH. They may also have variable degrees of jaundice, tachypnea, hematuria, and, rarely, neurological signs such as ataxia and head tilt. Jaundice is unexpectedly seen in horses but not in ruminants. This may be because horses are more susceptible to the action of C. novyi beta toxin (Smith 2015; Whitfield et al. 2015; Navarro and Uzal 2016).

Gross and Microscopic Lesions

A characteristic feature of this disease is hemorrhagic subcutaneous edema and congestion in ventral regions of the carcass that result in a very dark appearance, earning the name "black disease". A large amount of straw-colored fluid or blood-tinged fluid has been observed to accumulate in the pericardial, pleural, and peritoneal cavities as well as petechial hemorrhages on the epicardium and endocardium. The liver is swollen, gray-brown, and shows characteristic yellow necrotic areas approximately one to two cm in diameter that are bordered by red areas of hyperemia. On microscopic examination, the liver lesion appears as an eosinophilic inflamed fluke tract encircled by coagulation necrosis, dilated and congested vessels, and neutrophil infiltration. At the margin of the lesion, large, gram-positive bacteria are found just inside a zone of neutrophil infiltration (Nyaoke et al. 2017).

Treatment, Prevention and Control

Besides supportive care, procaine penicillin is given in high doses. The produced toxin may, however, make antibiotics ineffective (Cebra and Cebra 2002; Smith 2015). Vaccination is repeated every four to six weeks on affected farms, followed by annual vaccination. In outbreaks, vaccination with alum-precipitated toxoids is highly effective. By controlling the liver fluke and destroying the snails in streams and marshes by using molluscicides, this disease can be controlled (Hjerpe 1990).

Clostridial Neurotoxic Infections

Tetanus

Introduction

Tetanus, otherwise known as Lock Jaw, gets its name from the Greek word 'tetanos,' meaning 'contract'. Rather than a transmissible disease, it is a neurologic condition that has a worldwide distribution in humans and animals and results from the intoxication of the nervous system with the exotoxin of *C. tetani* and is characterized by persistent spasmodic contractions of the entire body musculature without impairment of consciousness (Bleck 1991; Hassel 2013).

Etiology and Epidemiology

As a result of wound contamination with soil-borne, sporulating, anaerobic bacterium infection by *C. tetani*, clinical disease often arises from tetanus neurotoxin (Bleck 1991; Hassel 2013; Popoff 2020). All farm animals are susceptible to tetanus throughout the world. The susceptibility of animal species to tetanus, however, varies considerably between them. The most susceptible species are horses, guinea pigs, monkeys, sheep, mice, goats, and humans but less sensitive species are cats and dogs, and birds are relatively invulnerable (Aslani et al. 1998; Wernery et al. 2004; Driemeier et al. 2007; Popoff 2020). A deep puncture wound, including those in the hoof, is commonly employed as the portal of entry in horses. Further, after castration in young pigs, as well after shearing, docking, vaccinations, or injection of pharmaceuticals, particularly anthelmintic drugs, among lambs. Additionally, insanitary conditions at parturition can cause tetanus to develop in the newborn (Linnenbrink and Macmichael 2006; Smith and Sherman 2009; Kumar Das et al. 2011; Pugh and Baird 2012).

Pathogenesis

When the oxygen tension in the local tissue decreases, the tetanus bacilli proliferate and produce tetanolysin and tetanospasmin. Whenever tetanolysin triggers local necrosis, surrounding tissues are invaded and tetanospasmin is brought into the bloodstream, bound to motor endplates, transported retrogradely by intra-axonal transport, inhibiting the release of neurotransmitters such as glycine and g-amino butyric acid, thereby hindering the inhibitory spinal interneuron activity. Inhibitory neurotransmitters act to inhibit the actions of excitatory nerve impulses from upper motor neurons. If normal inhibitory mechanisms cannot inhibit these impulses, generalized muscle spasms, and as a result, death by asphyxiation occurs (Wernery et al. 2004; Lotfollahzadeh et al. 2018).

Clinical Findings

An infection usually incubated for 3-4 weeks but may persist for several months afterward. At the beginning, muscle stiffness is accompanied by muscle tremor and the affected animal may continue to eat and drink, but tetany soon prevents mastication. Muscular tetany increases with the progression of the disease, which causes the animal to assume a "saw horse" posture. An unsteady, straddling gait caused by stiff hind limbs could also be noticed.
In all except sheep, quick movement of the third eyelid across the cornea before slow retraction is one of the initial and most reliable indications. Among young cattle, bloat is an initial sign but not always harsh and usually associated with frequent powerful rumen contractions. Infected horses initially display signs of colic and muscle stiffness in the lips, nostrils, ears, jaw (lockjaw), and tail. Death occurs as the entire musculature is affected. Horses and cattle usually die from a fatal illness within 5-10 days, but sheep usually die at about the third or fourth day.

Gross and Microscopic Lesions

The entry wound is the only visible change in animals dying of tetanus. Microscopically, there is tygrolysis of the C.N.S. neurons and a tabby-cat appearance caused by fatty changes in the myocardium

Treatment, Prevention and Control

For treating infections, penicillin G or metronidazole are the

best antibiotics, but they will not affect an existing disease. Although tetanus does not have a specific treatment, Anti-tetanus neurotoxin antibodies block free tetanus neurotoxin from entering neurons, but anti-toxin antibodies can't control toxin that has been taken up into neuronal cells. Once the infection site is found, a wound must be aggressively cleaned and debrided, but only after antitoxin is administered, as debridement, irrigation with hydrogen peroxide, and the use of local penicillin may facilitate toxin absorption. To avoid injury if convulsions occur, keep the affected animals calm and provide them with a calm, dark room with non-slip flooring. The injection of 1500 IU tetanus antitoxin can be effective for short-term prophylaxis. Furthermore, inactivated toxoid requires two doses 3-6 weeks apart. A protective antibody titer can be obtained within 14 days of the second injection and lasts for at least a year and up to 5 years (Acke et al. 2004; Sprott 2008).

Botulism

Overview

Botulism is a neuromuscular disorder that causes profound generalized flaccid paralysis in most of animals, and is often regarded as a zoonotic disease since foodborne botulism can end up infecting humans.

Etiology and Epidemiology

The disease is caused by one of seven neuroparalytic toxin subtypes (A to G) produced by *C. botulinum* (Galey et al. 2000; Ettinger and Feldman 2004; Bohnel and Gessler 2010), spore-forming anaerobe. Mammals, birds, and fish are typically affected; horses are mainly vulnerable to type B toxins; cattle and sheep are typically affected by types C and D. Under environmental circumstance, spores can survive for over 30 years, nonetheless if moist and warm conditions occur, the spores will germinate and revive vegetative cells that release a stable highly lethal toxin that blocks the release of acetylcholine at the neuromuscular junction, causing flaccid paralysis. Throughout drought times when feed is scant, phosphorus intake is minimal, and carrion is ample, silage-associated botulism outbreaks are most likely to occur. The majority of botulism cases are caused by ingestion of preformed botulinum toxins; additionally, toxins in feed can be caused by direct growth of *C. botulinum* in feeds (forage botulism) or contamination of feeds with toxin-containing carrion (carrion-associated botulism), while other less common ways to get botulism are through a wound or toxins produced by the growth and infection of the alimentary tract (toxicoinfectious botulism) (Rings 2004; Braun et al. 2005; Radostits 2007).

Pathogenesis

Soil and animals' gastrointestinal tracts can harbor *C. botulinum* spores. Under anaerobic or alkaline conditions, intestinal or wound-borne botulinum toxins are absorbed by the circulatory system and reach peripheral cholinergic terminals, and peripheral ganglia, causing functional paralysis without causing any pathological changes (Aoki et al. 2010). A toxin's heavy chain binds to receptors and translocates inside cells; the toxin's light chain blocks the release of acetylcholine at the neuromuscular junction as a result. The animal dies of respiratory failure after developing flaccid paralysis of the diaphragm (Lobato et al. 2013).

Clinical Findings

It can take from 18 hours to 17 days for botulism to develop. Acute cases can result in sudden death, whereas chronic ones aren't. When a cow becomes botulism-infected, it has difficulty moving, digestive problems, weakness in the hind limbs and sternal recumbency; while in horses, there is paralysis of muscles in the limbs, mandibles, larynx, pharynx, eyelids, tongues, and tails, usually end also with sternal recumbency. As the affected animals lie in sternal recumbency, they rest their heads on the ground or on their flanks, similar to a cow with parturient paresis while sheep hold their heads tipped to one side or move up and down while walking (limber neck). Furthermore, dysphagia and tongue weakness can also be seen in these sheep. Sometimes, the tongue will become paralyzed and hang from the mouth, incapable to grind or swallow, and will drool saliva (ACMSF 2006; Sherein 2013; Alemu and Ayele 2018).

Gross and Microscopic Lesions

The occurrence of suspicious feedstuffs in the stomach or forestomach can be a signal even if no specific changes are observed during necropsy. Jejunal hemorrhages can occur infrequently, especially in cattle. Horses with botulism types A and C have been reported to have edema of the nuchal ligament, which may result from weak neck muscles making it difficult for the horses to keep their heads up (Chao et al. 2004; Alemu and Ayele 2018).

Treatment, Prevention and Control

Advanced stages of botulism usually do not respond to treatment, and euthanasia is often recommended. In affected mammalian species, antitoxins can be given, but this approach must be applied before the toxins interact with the neuromuscular junction. Specific or polyvalent antiserums can be highly effective when given early in the course along with high quality fluid physiotherapy, enteral or parenteral nourishment, and mechanically ventilated if needed with inhaled oxygen nasally. It is important to dispose of carcasses hygienically in order to avoid further contamination of pastures. When herds are infested, vaccination can be effective (Beran 1994; Fitzpatrick and Katherine 2006; Nusair et al. 2009; Desta et al. 2016).

Clostridial Infections of Gastrointestinal System

Braxy

Overview

"Braxy" is another name for hemorrhagic abomasitis (gastritis) that occurs in sheep as well as other ruminants, which often results in rapid mortality with no or few symptoms (Cebra and Cebra 2012).

Etiology and Epidemiology

A gram-positive soil-borne bacillus called *C. septicum* causes

this infection, which is occasionally linked to other gastrointestinal infections as well as wound infection. During winter when grasses are of poorer quality, sheep may change their grazing behavior, resulting in increased consumption of woody forages and soil that holds *C. septicum* spores. As a result, this may damage the abomasal mucosa, thereby providing an entry point for *C. septicum* that may result in fetal toxemia in yearlings and weaner sheep.

Clinical Findings

After onset of disease, animals suffer from fever reaching 42°C followed by recumbency and death within 12–36 hours. Also, there may be sudden onset of symptoms, such as complete anorexia, depression, and bloating and distension of the abdomen. When an animal is comatose, there may be a bloody discharge from its nose

Gross and Microscopic Lesions

Macroscopically, abomasal wall is markedly edematous, thickened, congested, and hemorrhagic with blood-tinged fluid within the abdomen and abomasum (Maria et al. 2009). Upon microscopic examination, there is extensive necrosis, ulceration, edema, and congestion of the mucosa and submucosa. Besides, in the lamina propria and submucosa, there is heavy neutrophilic infiltration. Similarly, the small intestine is hemorrhagic, swollen, edematous, and necrotic.

Treatment, Prevention and Control

In most cases, antibiotics such as penicillin G cannot cure this disease because of its rapid nature. Disease at flock level, however, is usually sporadic, and can be prevented with clostridial bacterin toxoid vaccines administered to animals at risk on a routine basis.

Enterotoxaemia with type A *Clostridium perfringens*

Traditional associations with yellow lamb disease are strains of *C. perfringens* type A that release high-level of alpha toxin. Anecdotal evidence suggests a few cases occurred in South America, but the condition has been discovered mainly in the United States and Europe. As a result of the hemolytic lecithinase (phospholipase) alpha toxin, yellow lamb disease is associated with hemolysis and jaundice and characterized by depression, anemia, icterus, and hemoglobinuria, with sudden death occurring in rare cases. No specific findings can be identified during necropsy in animals with yellow lamb disease. In addition to generalized icterus and hydropericardium in the heart, red urine in the bladder, and an enlarged, pale and friable spleen are the most commonly defined gross findings. Upon microscopic examination of the liver, hepatocellular necrosis has been noted in the mid-zone central to the mid-zone areas and bile stasis has been observed in the bile canaliculi. The kidneys appear microscopically to contain multiple hemoglobin casts in the tubular lumens. In addition, the kidney's proximal and distal convoluted tubules are filled with eosinophilic, multifocal, and granular intracytoplasmic hyaline droplets. As of now, vaccines are not currently able to prevent yellow lamb disease; however, there is a chance of protection from vaccines meant for different types of *C. perfringens*.

Enterotoxaemia with type B *Clostridium perfringens*

Infections with *C. perfringens* type B have been reported in the Middle East, Europe, and South Africa. Usually, it infects lambs up to the second week of their life, calves of the comparable age, and foals in their earliest few days of life, causing lamb dysentery. Unlike cattle or horses, lambs suffer from an extremely acute disease, which often results in death without warning. There is usually watery bloody diarrhea, reluctance to suckle, and abdominal pain normally accompanying the illness. An older lamb may be suffering from an extremely chronic form of the disease, exemplified by depression, conditional loss, and reluctance to suckle. Opisthotonus, blindness, and absence of coordination are occasionally seen as neurological signs. It is characterized by deep-seated necrohemorrhagic or ulcerative enteritis with deeply penetrating, irregular, well-defined, mucosal ulceration bordered by a rim of hyperemia, with a fibrinous pseudomembrane overlying the peritonitis. Hyperemic intestinal mucosa and coagulative necrotic yellow necrotic areas may reach the muscularis as well as serosa and are usually non-sporulated, large, Gram-positive rods in the lumen. Blood usually stains the intestinal contents because of thrombosis of mucosal blood vessels. Occasionally, animals stay alive for a few days without hemorrhaging in more chronic cases. Possibly there will be a little blood or serous-stained fluid in the peritoneal cavity. In the epicardium and endocardium, there are petechiae and ecchymoses, as well as hydropericadium. Vaccination of the dam just before parturition, which lasts approximately 4 to 6 weeks, is an essential measure due to the high mortality rate among animals at very early ages. Afterward, a double vaccination should be given four to six weeks apart, followed by an annual booster.

Enterotoxaemia with type C *Clostridium perfringens*

Struck is a *C. perfringens* infection of type C, which causes hemorrhagic inflammation in the intestine with ulcerations, ascites, and peritonitis in adult feedlot ruminant early in the spring and winter. Associated with *C. perfringens* type C, enterotoxic hemorrhagic enteritis can occur in piglets within the first eight hours of life, causing the entire litter to die whereas calves, lambs, and foals may show no symptoms or suffer from bloody diarrhea during their first few days. During necropsy, hemorrhagic or necrotizing enteritis, frequently with gas inside the lumen as well as within the walls, may be found. Alpha and beta exotoxins are produced by *C. perfringens* type C, and neonates are predisposed to beta toxin since colostrum contains trypsin inhibitors. Consequently, excessive milk consumption in a protease-deficient digestive system may allow clostridial organisms to rapidly thrive, attach to villus tips, lyse the cells and produce beta toxin causing necrosis to enterocytes, which then extends downward leading to hemorrhage, necrosis, and edema of the lamina propria, and finally death due to diarrhea or secondary bacteremia and toxemia. As a result of the severity of the disease, treatment is usually ineffective, but if attempted, specific hyperimmune sera and oral antibiotics may be used. Vaccinating pregnant dams during the last third of pregnancy, first with two shots one month apart, then annually thereafter, is the best way to control the disease. In livestock born to unvaccinated mothers, antisera should be administered immediately after birth when outbreaks occur.

Enterotoxaemia with type D *Clostridium perfringens*

Overview

"Overeating disease" or pulpy kidney infection, produced by *C. perfringens* type D toxins, is a serious infectious globally distributed condition of sheep, goats, and calves. Grain overload (starch) and sudden dietary changes may contribute to the disease that affects the small intestine (Quinn et al. 2002). Historically, focal symmetrical encephalomalacia (FSE) was used to refer to the diseases that are one form of type D enterotoxemia of sheep, and sometimes it is yet used today when discussing these forms.

Etiology and Epidemiology

In small intestine enterotoxemia, *C. perfringens* type D produces a variety of toxins, among them the Epsilon toxin, which is the 3rd powerful clostridial toxin following botulinum and tetanus toxin, and it causes vascular damage and neuropathy characteristic of this condition (Niilo 1980; Jemal et al. 2016). Typically, lambs are the most susceptible, calves and goats also commonly contract the disease, horses are less vulnerable while adult cattle, deer, and domesticated camels, are rare cases. Between the ages of 3 and 10 weeks in lambs, enterotoxemia is most common, and the same risks apply to calves between the ages of 1 and 4 months. Foraging on lush, fast-growing grass or young cereal crops, intense grain nutrition, and abrupt dietary changes are among the conditions that favor the disease (Uzal et al. 1994). Under these conditions, it has been called 'overeating disease'. Following heavy rains, the disease often manifest within 5-14 days of a flock being introduced to lush pastures.

Pathogenesis

Despite large numbers of *C. perfringens* type D ingested, many are obliterated in the rumen and abomasum. However, a few make it to the duodenum alive, where they multiply and produce an epsilon prototoxin that is triggered by trypsin/chymotrypsin to produce an epsilon toxin. A number of conditions must be met before the disease can begin, such as excessive amounts of starch rich nutrient passing into the duodenum after sheep eat too much grain or are abruptly switched from a largely roughage-based ration to one that is mostly grain-based; furthermore, slowing of alimentary tract movement that allows excess toxin accumulation. Microvascular endothelial impairment caused by Epsilon toxin of *C. perfringens* type D results in boosted vascular permeability and harsh vasogenic edema. A part of the toxin may enter the brain after the blood-brain barrier breaks down, damaging neurons directly. As well, increased intracranial pressure and significant neurologic impairment are the consequences of generalized edema in the brain.

Clinical Findings

Lambs are frequently found dead within 2 to 12 hours without any previous signs of illness. Close observation of a flock may reveal symptoms such as yawning, dullness, depression, and loss of interest in feeding. Those who survive exhibit green, pasty diarrhea, staggering, recumbency, opisthotonos, frothing at the mouth and severe clonic convulsions. Most adult sheep will survive up to 24 hours, during which they may show staggering and knuckling, salivation, convulsions, muscle tremor, rapid, shallow and irregular breathing, and bloat in the terminal stages.

The symptoms exhibited in cattle are comparable to those in sheep and are primarily neurological in nature. Acute cases show bellowing, mania, and persistent convulsions, whereas peracute cases die without any signs of illness. Cases that are subacute tend to be calm, docile and go blind for 2 to 3 days, then recover (Blood and Henderson 1974; Jemal et al. 2016).

Gross and Microscopic Lesions

A typical examination of the dead animal shows fecal dirtiness of the perineum besides quick decay of the carcass, the presence of a clear, straw-colored pericardial and thoracic fluid that clots as soon as exposed to air; petechial hemorrhages are observed in the cardiac epicardial layer, endocardial layer; and there are patches of congestion in the abomasal and intestinal mucosa. In freshly examined specimens, the "pulpy kidney" lesion may not be noticeable. It is not considered to be a useful investigative finding (Itodo et al. 1986; Blackwell et al. 1992; Radostits et al. 2007; Jemal et al. 2016). On microscopic examination of the brain tissue, encephalomalacia is evident with necrotic and degenerated neuronal and neuroglial cells, spongy neuropil, axonal swelling, increased gitter cells and lymphoplasmacytic infiltration together with vascular changes including hypertrophic endothelium, hyalinized arterial walls, lymphoplasmacytic perivascular cuffing, capillary hemorrhage.

Treatment, Prevention and Control

Sheep are too acutely ill for effective treatment to be effective. It may be possible to treat goats with antitoxin plus sulfadimidine if the course lasts longer. As soon as an outbreak begins, you can administer antitoxin to all sheep, which will provide protective levels of circulating antitoxin for 21-29 days. When ewes are vaccinated twice a month, with the last vaccination occurring around one month before lambing, they will develop good passive immunity in young lambs and have positive levels of protective antibodies by the time they are eight weeks old. When lambs are between four to ten weeks old, they need to be vaccinated with toxoid and then again, one month later. While waiting for vaccination immunity to develop, decreasing food intake is an effective means of short-term control measure (Pawaiya et al. 2020).

REFERENCES

Abreu CC et al., 2017. Blackleg in cattle: a case report of fetal infection and a literature review. Journal of Veterinary Diagnostic Investigation 29: 612-621.

Abreu CC et al., 2018. Pathology of black leg in cattle in California, 1991-2015. Journal of Veterinary Diagnostic Investigation 30(6): 894-901.

Acke E et al., 2004. Tetanus in the dog: review and a case-report of concurrent tetanus with hiatal hernia. Irish Veterinary Journal 57: 593-597

ACMSF, (Advisory Committee on the Microbiological Safety of Food), 2006b. Ad hoc group on botulism in cattle. Report on botulism in cattle. London: Food Standards Agency

Ahourai P et al., 1990. Bovine bacillary hemoglobinuria (clostridium haemolyticum) in Iran. Journal of Veterinary

Diagnostic Investigation 2: 143-144.
Alemu B and Ayele M, 2018. Review on Botulism in cattle. Applied journal of Hygiene 7(2): 17-25.
Aoki KR et al., 2010. Mode of action of botulinum neurotoxins: current vaccination strategies and molecular immune recognition. Critical reviews in immunology 30: 167-187.
Aronoff DM, 2013. Clostridium novyi, sordellii and tetani: mechanisms of disease. Anaerobe 24: 98-101.
Aslani MR et al., 1998. Outbreak of tetanus in lambs. The Veterinary Record 142: 518-519.
Barnes DM et al., 1975. Differential diagnosis of clostridial myonecrosis. Canadian Veterinary Journal 16: 357-359.
Beran GW, 1994. Bacterial, rickettsial, chlamydial and mycotic zoonosis. In: Handbook of zoonosis, 2nd ed. ; Pp:361-366.
Blackwell TE et al., 1992. Enterotoxemia in the goat: the humoral response and local tissue reaction following vaccination with two different bacterin-toxoids. Canadian Journal Comparative Medicine 47: 127-132.
Bleck TP, 1991. Tetanus: pathophysiology, management, and prophylaxis. Disease-a- Month 37: 545-603.
Blood DC and Henderson JA, 1974. Veterinary Medicine. 4th edn. Baillieretindall London, UK; p: 132.
Braun U et al., 2005. Clinical finding and treatment of 30 cattle with botulism. Veterinary Records 156: 438-441.
Bohnel H and Gessler F, 2010. Neurotoxigenic clostridia. In: Gyles CL, et al., Pathogenesis of Bacterial infections in Animals, 4th ed. Wiley- Blackwell Ames, 189-202.
Boyd NA et al., 1972. The prevention of experimental clostridium novyi and Cl. perfringens gas gangrene in high velocity missile wounds by active immunization. Journal of Medical Microbiology 5: 467-472.
Butler HC, 1998. Black leg of the fetus in ewes. Journal of the American Veterinary Medical Association 128: 401-402.
Cebra C and Cebra M, 2012. Diseases of Hematologic, Immunologic and Lymphatic systems (Multisystem Diseases) in Sheep and Goat Medicine ,2nd ed.
Cebra C and Cebra M, 2002. Diseases of the hematologic, immunologic, and lymphatic systems (multisystem diseases). In: Pugh, DG, ed. Sheep & Goat Medicine. 1st ed. Elsevier; Pp 359-391.
Chao HY et al., 2004. Immune polymerase chain reaction assay for clostridium botulinum neurotoxin type A. Toxicon 43: 27-34.
Choi YK et al., 2003. Clostridium perfringens type A myonecrosis in horse in Korea. Journal of Veterinary Medical Science 65: 1245-1247.
Coetzer JA et al., 1994. Infectious disease of livestock. Oxford University press, London 1325- 1330.
Constable PD et al., 2017. A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs, and Goats, 11th ed., Elsevier Ltd; pp. 1431-1432.
Cooper BJ and Valentine BA, 2016. Jubb, Kennedy and palmer's Pathology of Domestic Animals 6th ed.
Costa JLN et al., 2007. Outbreak of malignant edema in sheep caused by clostridium sordellii, predisposed by routine vaccination. Veterinary Records 160: 594-595.
Crowe SP and Moss EW, 1989. Alberta. Bacillary hemoglobinuria in a beef herd. Canadian Veterinary Journal 30: 681
Cullen JM and Stalker ML, 2016. Necrotic hepatitis (black disease). In: Maxie MG, ed. Jubb, Kennedy and Palmer's Pathology of Domestic Animals. 6th ed., Vol. 2. Philadelphia, PA: Elsevier, 316.
Desta S et al., 2016. Botulinum Toxin and Its biological significance: A review. World Applied Sciences Journal 34: 854-864.
Driemeier D et al., 2007. Outbreaks of tetanus in beef cattle and sheep in Brazil associated with disophenol injection. Journal of Veterinary Medicine series A 54: 333-335.
Farias LD et al., 2014. Acute myonecrosis in horse caused by *Clostridium novyi* type A. Brazilian Journal of Microbiology 45: 221-224.
Fitzpatrick S and Katherine, 2006. Botulism poisoning in cattle in the Northern territory. Serial No. 651, Ag dex No. 420/654.
Frey J and Falquet L, 2015. Patho-genetics of Clostridium chauvoei. Research in Microbiology 166: 384-392.
Ettinger SJ and Feldman EC, 2004. Text book of veterinary Internal Medicine disease of the dog and cat, 6th ed.; 1: 629-631.
Galey FD et al., 2000. Type C botulinum in dairy cattle from feed contaminated with a dead cat. Journal of Veterinary Diagnostic Investigation 12: 204-209.
Groseth PK et al., 2011. Large outbreak of blackleg in housed cattle. Veterinary Record 169: 339.
Hassel B, 2013.Tetanus: pathophysiology, treatment, and the possibility of using botulinum toxin against tetanus-induced rigidity and spasms. Toxins (Basel) 5:73-83.
Hjerpe CA, 1990. Clostridial disease vaccines. The Veterinary Clinics of North America. Food Animal Practice 6: 222-234.
Hogarth, 2000. Animal microbiology, 6th ed., Lippincott A, D, London, UK, pp. 117.
Hussein HA et al., 2013. Bacillary hemoglobinuria in dairy cows: clinical, hematological, biochemical, and pathological alterations. Comparative Clinical Pathology 22: 1137-1143.
Irisk MB, 2007. Blackleg in cattle. Journal of University of Florida 165: 1- 2
Jasmin AM, 1947. Isolation of Clostridium hemolyticum from bones. American Journal of Veterinary Research 8(29): 341.
Itodo AE et al., 1986. Toxin-types of Clostridium perfringens strains isolated from sheep, cattle and paddock soils in Nigeria. Veterinary Microbiology 12: 93-96.
Jemal D et al., 2016. Review on pulpy Kidney disease. Journal of Veterinary Medicine and Technology 7(5) 2:6.
Kahn CM and Line S, 2005. The Merck Veterinary Manual 9th Edition. Merial, USA.; Pp. 487.
Das AK et al., 2011. Tetanus in a buffalo calf and its therapeutic management. Insta Polivet 12: 383-384.
Lewis CJ, 2011. Control of important clostridial diseases of sheep. The Veterinary Clinics of North America. Food Animal Practice 27: 121-126.
Linnenbrink T and Macmichael M, 2006. Tetanus: pathophysiology, clinical signs, diagnosis, and update on new treatment modalities. Journal of Veterinary Emergency and Critical Care 16: 199-207.
Lobato FCF et al., 2013. Clostridial infection in farm animals. Veterinária e Zootecnia 20: 29-48.
Lotfollahzadeh S et al., 2018. Tetanus outbreak in a sheep flock due to ear tagging. Veterinary Medicine and Science 5(2): 146-150.
Maria S et al., 2009. Braxy (Bradsot) in lambs: A case report. Lucrări Ştiinłifice Medicină Veterinară XLII (1).
Morris WE et al., 2002. Malignant oedema associated with

navel infection in a Merino lamb. Journal Arquivo Brasileiro de Medicina Veterinariae e Zootecnia 54 (4): 448-449.

Navarro M and Uzal FA, 2016. Infectious necrotic hepatitis. In: Uzal FA, et al., eds. Clostridial Diseases of Animals. 1st ed. Ames, IA: Wiley Blackwell, Pp: 275-279.

Navarro MA, 2017. Pathology of Naturally occurring Bacillary Hemoglobinuria in cattle, Journal of Veterinary Pathology 54 (3): 457-466

Niilo L, 1980. Clostridium perfringens in Animal Disease: A Review of Current Knowledge. Canadian Veterinary Journal 21: 141-148.

Nusair SD et al., 2009. A mini review of available pharmacotherapy and potential immunotherapy for the toxicity of 32 mainly encountered substances. Middle-East journal Scientific Research 4: 263-266.

Nyaoke AC et al., 2017. Infectious necrotic hepatitis caused by Clostridium novyi type B in a horse: case report and review of the literature. Journal of Veterinary Diagnostic Investigation 1-6.

Odani JS et al., 2009. Malignant edema in postpartum dairy cattle. Journal of Veterinary Diagnostic Investigation 21: 920-924.

Oliver O and Staempfli H, 1999. Bacillary hemoglobinuria, braxy, and black disease. In: Howard JL (ed) Current veterinary therapy 4: food animal practice. Saunders, Philadelphia, pp 386-387.

Oliveria Junior CA et al., 2020. Gas gangrene in mammals: A review. Journal of Veterinary Diagnostic Investigation 32(2): 175-183.

Parish SM et al., 2019. Clostridial myonecrosis. In: Smith BP, et al., eds. Large Animal Internal Medicine 6th ed. St. Louis, Mo: Elsevier, 1432-1434.

Pawaiya RS et al., 2020. The challenges of Diagnosis and Control of Enterotoxaemia caused by Clostridium perfringens in small ruminants. Advances in Microbiology 10: 238-273.

Peek SF et al., 2003. Clostridial myonecrosis in horses (37 cases 1985–2000). Equine Veterinary Journal 35: 86-92.

Pires PS et al., 2017. Intracellular survival of *Clostridium chauvoei* in bovine macrophages. Veterinary Microbiology 199: 1-7.

Popoff MR and Bouvet P, 2009. Clostridial toxins. Future Microbiology 4: 1021-1064.

Popoff MR, 2020.Tetanus in animals. Journal of Veterinary Diagnostic Investigation 32(2): 184-191.

Pugh DG and Baird AN, 2012. Sheep and Goat Medicine, 2nd ed. Saunders, an imprint of Elsevier. Inc: Philadelphia, PA.

Quinn PJ et al., 2002. Veterinary Microbiology and Microbial disease. 2nd edn, Blackwell Publishing Company, USA 66: 92-93.

Quinn PJ et al., 2011. Clostridium species. In: Quinn PJ, et al., eds. Veterinary Microbiology and Microbial Disease. 2nd ed. West Sussex, UK: Wiley-Blackwell, 233-241.

Radostits OM et al., 1994. Veterinary medicine, (8th edn), Baillier Tindall, London, UK, pp. 608-610

Radostits OM, 2000. Diseases caused by bacteria—II. pp. 767–769. In: Veterinary Medicine. 9th ed. (Radostits, O.M. and Gay, C.C., Blood, D.C., Hinchcliff, K.W., eds.) Philadelphia, Pennsylvania: WB Saunders Company Ltd

Radostits OM, 2007.Veterinary medicine: a textbook of the diseases of cattle, horses, sheep, pigs, and goats. (10th edn), W.B. Saunders, Philadelphia, USA.

Rings DM, 2004. Clostridial disease associated with neurologic signs: tetanus, botulism and enterotoxaemia. Veterinary Clinics of North America: Food Animal Practice 20: 379-391.

Sherein IA, 2013. Bacterial Causes of Sudden Death in Farm Animals. Life Science Journal 10: 1188-1201.

Shinozuka Y, 2011. Bacillary hemoglobinuria in Japanese Black cattle in Hirshima, Japan: A case study. Journal of Veterinary Medical Science 73(2): 255-258

Silva ROS et al., 2016. Chapter 20 Gas gangrene (malignant edema). In: *Clostridial* Diseases of Animals. Hoboken, NJ: Wiley, Pp: 243-254.

Smith MC and Sherman DM, 2009. Goat Medicine. 2nd ed. Wiely-Blackwell: Hong Kong, Printed in Singapore.

Smith GW, 2015. Black disease. In: Smith BP, ed. Large Animal Internal Medicine. 5th ed. St. Louis, MO: Mosby Elsevier, Pp: 849-850

Snider TA and Stern AW, 2011. Pathology in practice. Myocarditis and epicarditis. Journal of American Veterinary Medical Association 238: 1119-1121.

Songer JG, 2004. Histotoxic clostridia. In: Gyles CL, Prescott JF, Songer JG, Thoen CO (Eds.), Pathogenesis of Bacterial Infections in Animals, 3rd ed., Ames, Blackwell, UK, pp. 127.

Sprott KR, 2008. Generalized tetanus in a Labrador retriever. Canadian Veterinary Journal 49:1221-1223.

Sunagawa K and Sugitani M, 2017. Post-mortem detection of bacteremia using pairs of blood culture samples. Legal Medicine (Tokyo) 24: 92-97.

Tolera T et al., 2019. Review on Blackleg in Cattle. Dairy and Veterinary Science Journal 9(5): 555771.

Useh NM et al., 2006. Relationship between outbreaks of blackleg in cattle and annual rainfall in Zaria, Nigeria. Veterinary Record 158: 100-101.

Uzal FA et al., 1994. An Outbreak of Enterotoxaemia Caused by Clostridium perfringens Type D in Goats in Patagonia. Veterinary Record 135: 279-280

Van Kampen, KR and Kennedy, PC. Experimental bacillary hemoglobinuria, II: pathogenesis of the hepatic lesion in the rabbit. Pathol Vet. 1969;6(1):59–75.

Wernery U et al., 2004. Tetanus in a camel (Camelus dromedaries)- a case report. Tropical Animal Health and Production 36: 217-224.

Whitfield LK et al., 2015. Necrotic hepatitis associated with *Clostridium novyi* infection (black disease) in a horse in New Zealand. New Zealand Veterinary Journal 63: 177-179