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#### **REVIEW ON THE BLACKLEG DISEASE IN DOMESTIC ANIMALS**

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#### SUMMARY

Blackleg is an acute disease of cattle and sheep caused by the spore forming, rod shaped, gas producing bacteria Clostridium chauvoei (feseri). It is characterized by emphysematous swelling (production of gas in the animal's tissues), usually in the heavy groups of the rump and loin. Blackleg occur worldwide at rates that differ between and within geographic areas, which suggests a soil reservoir or climatic or seasonal factors yet to be defined. Typical blackleg of cattle has a seasonal incidence, with most cases occurring in the warm months of the year. In cattle the disease usually occurs without a history of trauma but in sheep is almost always a wound infection. The disease is controlled by minimizing hard and dry feed feeding as it causes injury through which the spore gets entry to the body. Mineral feeding not to lick the soil. Burn the carcass or bury it deeply with lime. Blackleg vaccine used to stimulate active immunity against. Generally, blackleg is almost entirely preventable by vaccination.

Key -Words: Black leg, Clostridium chauvoei, Control, Prevention

#### **INTRODUCTION**

Ethiopia stands first in livestock population in Africa, ranking ninth in the world. According to the CSA report in 2017, the country has about 59.5 million cattle populations. However, Ethiopia has high livestock population which provides draught power, milk, meat, fuel and fertilizer and foreign currency from hide and skin, our country is not using from her livestock as much expected due to many animal diseases circulating in animal population (Zeleke, 2009).

Animal diseases such as blackleg which is also called quarter ill or black quarter cause the major limitation to the livestock agriculture of the country and affect livelihood through their effect on animal health and impact on the production. Blackleg also called quarter ill or black quarter is an acute specific infectious disease of cattle, sometimes of sheep and pigs characterized by the rapidly increasing presence of swellings containing gas, and occurring in the region of the shoulder, neck, thigh, quarter, and sometimes in the diaphragm. Young cattle between the ages of 6 months and 2 years are also susceptible (Boden, 1998).

Blackleg is an infectious disease of cattle and rarely other ruminants, produced by *Clostridium chauvoei*, and characterized mainly by necro hemorrhagic myositis. C. chauvoei is an anaerobic, gram-positive bacillus that persists in the soil as resistant spores (Cooper and Valentine, 2016). The spores of the organisms can live in the soil for many years. The spores are resistant to heat, cold, drought, UV radiation and chemical disinfectants. It is characterized by emphysematous swelling (production of gas in the animal's tissues), usually in the heavy groups of the rump and loin. It is primarily a disease of young cattle (less than 3 years) (Hirsh and Zee,1999). The bacteria enter to the host by ingestion and then gains entrance to the body small punctures in the mucous through membrane of the digestive tract. Cattle that are on a high plane of nutrition, rapidly gaining weight and between 6 months and 2 years of age are most susceptible to the disease. The disease is not transmitting directly from sick animal to healthy animals by contact (Boden, 1998).

Contaminated pasture from feaces and carcass of animal's dead of the disease appear to be the source of infection (Bagge *et al.*, 2009). In areas where the disease has not been known to exist soil disturbances such as soil excavation or drainage can suddenly initiate outbreaks, probably by turning up, or by creating the conditions necessary for activation of latent spores (Scott *et al.*, 2011). Blackleg occur worldwide at rates that differ between and within geographic areas, which suggests a soil reservoir or climatic or seasonal factors yet to be defined (Radostits *et al.*, 2007).

In cattle the disease usually occurs without a history of trauma but in sheep is almost always a wound infection. The disease is not transmitted directly from sick animals to health animals by mere contact. These infections usually cause a per-acute or acute disease which is characterized by focal, gangrenous, myositis and associated localized cellulitis. True blackleg is a myositis of the skeletal and sometimes cardiac muscles with an associated toxaemia and a high case fatality rate. Death is caused by the local and systemic effects of toxins elaborated by *C.chauvoei* (Coetzer*et al.*, 1994).

Prevention of blackleg is the only practical approach, and is fortunately very effective. There are a variety of bacterins or bacterin/toxoid combinations that will induce immunity to the various Clostridial diseases. Because of the common occurrence of a number of Clostridial infections in an area, it becomes a common practice in recent years to use multivalent bacterin toxoids containing *Clostridium chauvoei*, *Clostridium septicum*, *Clostridium novyi*, *Clostridium serdolli*, and *Clostridium perferingens* types C and D. The vaccine appears to be highly effective and, in situation where the extra expense can be justified, worthy of recommendation. For this reason, cattle should be immunized regularly (Coetzer *et al.*, 1994). Therefore, the objective of this paper is to review on the blackleg disease in domestic animals.

# GENERALDESCRIPTIONOFCLOSTRIDIUMCHAUVOEIMorphology and Staining Reactions

Clostridium chauvoei is seen in tissues and cultures as straight, round-ended rod about 0.6µm wide and from 3 to 8µm long. The organism is a rod-shaped bacillus longer than the anthrax bacillus but slenderer. It can be highly pleomorphic. It usually appears singly or in chains of three to five organisms in the peritoneal exudates of inoculated guinea pig, а configuration that is useful in distinguishing it from C.septicum and other anaerobic bacilli, which usually occur in long chains and are frequently found in specimens from animals suspected of having blackleg. Spores are oval and appear eccentrically, swelling the rods into lemon-shaped structures. Organisms in very young cultures are motile by means of peritrichous flagella. Pleomorphic cells stain somewhat unevenly. When young, organisms are positive to Gram's stain but stain erratically after they are a few days old (Hagan and Bruner's, 1988).

#### **Cultural and Biochemical Features**

Clostridium chauvoei is a little more exacting in its cultural requirements than are most of the clostridia in the tissueinvading and enterotoxigenic group. It is strictly anaerobic and will not grow on ordinary glucose agar except carried when tissues are over in the inoculums. The addition of blood or tissue favors its growth in ordinary broth and agar. It will grow luxuriantly on all media made with a liver infusion base without enrichment. It has a high requirement for cystine (Hagan and Bruner's, 1988).

Colonies grow in deep agar are delicate and compact, being irregularly spherical. When blood is present, there is evidence of slight hemolysis, but definite zones are not digesting it. Cultures of this organism give off a characteristic odor, which experienced workers can use to identify the species. The principal fermentation products are butanol and acetic and butyric acid (Quinn *et al.*,2000).

## Antigens and Toxins Produced by *Clostridium* chauvoei

*C. chauvoei* strains have flagella antigens, somatic, and spore antigens. Most strains share the same spore, somatic and flagellar antigens (Gyles *et al.*, 2004). The spore antigen is also

shared with *C. septicum*. Toxin produced include alpha toxin with hemolytic and necrotizing activity, hyaluronidase, and deoxyribonuclease (Hagan and Bruner's, 1988). Formalized wholebroth cultures, usually alum precipitated, are used to produce life-long immunity. Recovery from disease renders animals immune for life. *C. chauvoei* is antigenically heterogeneous, although there is considerable cross protection among strains (Carter and Chengappa, 1991).

#### ETIOLOGY

True blackleg, the Clostridial myositis of skeletal muscles, associated with *C. chauvoei*, a Grampositive, spore forming, and rod-shaped bacterium. The spores are highly resistant to environmental changes and persist in soil for many years (Radostits *et al.*, 2007).

False blackleg may be associated with C.septicum and C.novyi but this disease is more accurately classified as malignant edema. Mixed infections with C. chauvoei and C. septicum are not uncommon but the significance of C. septicum as a cause of the disease is debated (Radostits et al., 2007). The relatively small genome of C. chauvoei as compared to other Clostridium species, such as C. difficile i.e. 4.2 million base pairs, reflects its adaptation to a restricted host range (bovine, caprine and ovine),

where C. chauvoei can replicate and to cause disease (Frey and Falquet, 2015).

In the tissues of the living animal it forms spores which distend the bacillus and may either be in the middle giving it a lemon-shape appearance, or terminal producing a tennis-racket shape. It is mainly a tissue parasite, and is found in greatest abundance in the emphysematous swelling which is characteristic of the disease. It is there in the inflammatory exudates, in the substance of the muscular fibers, and in the connective tissue. This inflammatory swelling is the only constant seat of the organism. It occasionally invades the blood stream, but it does not occur in such numbers as to make it possible to find it by microscopic examination. When there are exudations in the serous cavities of the body it is usually found there (Gross et al., 2009).

#### **EPIDEMIOLOGY**

#### Occurrence

Blackleg affects mainly nonvaccinated cattle between 6 month and 2 years of age with occasional cases occurring in animals outside this age range. The disease affects mostly animals in good nutritional condition, often on pasture (Uzal, 2012). Occurrence of the disease is worldwide, although it tends to be localized, even to certain farms or to certain pastures. Because of this localization, it is assumed that C. chauvoei is soil borne, but likely does not grow in soil. The bacteria grow readily in the intestinal tract of cattle and may be recycled through fecal contamination of the soil. Once exposed to the environment, C. chauvoei readily forms spores, which may survive for long periods (many years) in the soil (Van Vleet and Valentine, 2007). The disease occurs usual for a number animal to be affected within the space of a few days. The disease is endemic areas, especially when they are subject to flooding such as area may vary in size form group of forms to on individual field are created late in black leg approaches 100% (Radostatis *et al.*, 2007).

#### **Source of Infection**

Blackleg is a soil-borne infection. The portal by which the organisms enter the body is still in disputes. It is presumed that the portal of entry is through the alimentary mucosa after ingestion of contaminated feed or associated with erupting teeth. The bacteria may be found in the spleen, liver, and alimentary tract of normal animals, and contamination of the soil and pasture may occur from infected feaces or decomposition of carcasses of animal dead of the disease. True blackleg develops when spores that are not lodged in normal tissues are caused to proliferate by mechanisms such as trauma or anorexia (Radostits *et al.*, 2007).

#### Transmission

In cattle the disease usually occurs without history of trauma but in sheep is almost always a wound infection. Infections of skin wounds at shearing and docking and of the navel at birth may cause the development of local lesions (Viel et al., 2011). Infection of the vulva and vagina of the ewe at lambing may cause serious outbreaks and the disease has occurred in groups of young ewes and rams up to a year old, usually as a result of infection of skin wounds caused by fighting. Occasionally, outbreaks have occurred in vaccination sheep after against enterotoxaemia. Presumably the formalized vaccine causes sufficient tissue damage to permit latent spores of the organisms to proliferate. A special occurrence is seen in fetal lamb. The disease is not transmitted directly from sick animals to healthy by mere contacts (Seifert, 1996).

#### **Risk Factors**

#### Environmental risk factors

Typical blackleg of cattle has a seasonal incidence, with most cases occurring in the warm months of the year. The highest incidence may vary from spring to autumn, depending probably on when calves reach the susceptible age group. In some areas there is an increased prevalence in years of high rainfall. Outbreaks have occurred following excavation of soil, which suggests that disturbance of the soil, may expose and activate latent spores (Naz *et al.*, 2005).

#### Animal risk factors

True blackleg is usually thought as disease of cattle and occasionally sheep but out breaks of the disease has been recorded in deer and in one case in a horse. In cattle the disease is largely confined to young stock between the age of 6 month and 2 years. In the field the disease appears to occur most frequently in rapidly growing cattle on a high plane of nutrition. Elevation of the nutritional status of sheep by increased protein feeding increases their susceptibility to blackleg. In sheep there is no restriction to age group (Andrews and Blower, 1992).

#### **Economic Important**

Blackleg is a cause of sever financial loss to cattle raisers in many parts of the world. For the most part major outbreaks are prevented by vaccination, although outbreaks still occur occasionally in vaccinated herds of cattle in completely vaccinated. When the disease occurs, it is usual for a number of animals to be affected within the space of a few days (Songer, 1997).

#### PATHOGENESIS

The detailed pathogenesis of blackleg is still somewhat uncertain, but many of the critical points in the following proposed sequence of events have been confirmed in the natural disease and in experimental infections in cattle (Cooper and Valentine, 2016). It is believed that blackleg in cattle develops when latent spores within large muscle groups germinate and multiply. When these muscles are traumatized, resulting in localized areas of low redox potential. The vegitative cells grow, ferment muscle glycogen, digest protein, and produce exotoxins and gas. The role of toxin produced by C. chauvoei in the pathogenesis of the disease remains uncertain. The lesions, which usually remain localized and in most animals are situated in one of the large muscle groups, expand along facial planes, but bacteremia also develops, particularly terminally. Occasionally, latent spores in the myocardium caused by increased blood level of cortisol and catecholamine as a response to stress. The exotoxins and certain metabolites produced by the bacteria during their multiplication, and those which arise from tissue damage, are absorbed from the affected muscles into the systemic circulations, causing toxemia and death. In cattle and sheep atypical outbreaks of sudden death occur in which the lesion is cardiac myositis (Irisk, 2007).

#### **CLINICAL FINDINGS**

#### Cattle

If animal is observed before death there is sever lameness, usually with pronounced swelling of the upper part of the affected leg (Irisk, 2007). On closer examination the animal will be found to be very depressed and have complete anorexia and ruminal stasis, and a high temperature 41  $C^0$ and pulse rate (100 to 120/minute) (Gyles et al., 2004). Pyrexia is not present in all cases. In the early stages the swelling is hot and painful to the touch but soon becomes cold and painless, edema, emphysema and if pressed it crackles as if filled with screwed up tissue paper (Irisk, 2007). There blowing is respiration, accompanied by grunting. As the condition advances the breathing becomes more labored the patient becomes prostrate, and the temperature falls normal to or slightly subnormal, to be quickly followed by death. In the rare non-fatal cases in cattle a deep-seated abscess forms which is very slow in healing, causing great loss of condition and emaciation. An animal so affected is not an economical asset, and is best destroyed (Gross et al., 2009). The skin is discolored and soon becomes dry and cracks (Coetzer et al., 1994). Although the lesions are usually confined to the upper part of one limb, occasional cases are seen where the lesions are present in other locations such as the base of the tongue, the heart muscle, the diaphragm muscles, the brisket, and the udder (Cynthian, 2005).

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Feet or legs, and the tongue are often the predilection site (Shoshtary *et al.*, 2007). Lesions are sometimes present in more than one of these locations in one animal. The conditions develop rapidly and the animals die quietly 12 to 36 hours after the appearance of signs. Many animals die without signs having been observed (Songer, 1997).

#### Sheep

When blackleg lesions occur in the limb musculature in sheep, there is stiff gait and the sheep is disinclined to move because of sever lameness in one limb or, more commonly, in several limbs. The lameness may be severe enough to prevent walking in some animals but be only moderate in others. Subcutaneous edema is not common on and gaseous crepitation cannot be felt before death. Discoloration of the skin may be evident but skin necrosis and gangrene do not occur (Radostits et al., 2007). In sheep the wool over the affected part is easily pulled out. When the swelling is cut into, the muscles and other tissues are dark red, or almost black, and have a more or less porous appearance, and are rather dry-looking. This is due to separation of the fibers by gas produced by the organisms. In other parts there is a bloodstained exudates and hemorrhage into the muscular tissue. In sheep and goats, the dark lesion usually occurs at the

site of the wound and entry of the organism (Lewis, 2007).

In those cases where infection occurs through wounds of the skin, vulva, or vagina there is an extensive local lesion. Lesion of the head may be accompanied by sever local swelling due to edema and there may be bleeding from the nose. Ewes exposed to infection at shearing develop typical lesions but ewes treated in latter group show distended abdomens. weakness. or recumbency due to edema and gas formation in the fetus, from which C. chauvoei can be isolated. In all instances there is high fever, anorexia, depression, and death occur very quickly. Sheep and cattle with cardiac myositis associated with C.chauvoei are usually found dead (Radostits et al., 2007).

#### Horses

The clinical syndrome in horse is not well defined. Pectoral edema, stiff gait, and incoordination are recorded. The localized gas gangrene as a consequence of wound infection with *C. chauvoei* rarely occurs. However, in cases in which this does occur, the infection may spread and involve large areas adjacent to the initial lesion. Most affected horse die but the affected tissues may slough in those that do recover, following intensive antibiotic treatment,

leaving large cavities which heal slowly (Radostits *et al.*, 2007).

#### DIAGNOSIS

Blackleg infection should be suspected when calves and yearlings in known blackleg districts die suddenly, showing gassy swelling of the muscle while on pasture, or when sheep develop symptoms after shearing or docking (Coetzer *et al.*,1994).

Further evidence of blackleg is found if the carcass is examined few hours after death (Robson and Wilson, 2007). It will usually show rapid and excessive bloating, which causes the legs on the upper side of the carcass to extend out straight and which forces bloody-colored foam to escape from the natural body openings. One or more gaseous swellings located on the region of the hip, shoulder, neck, jaw, brisket, or loin may be observed. They emit a crackling sound on pressure (Gyles *et al*, 2004).

If swelling is lanced, a frothy, dark-red fluid with a characteristic sweetish sour, rancid odor like that of butyric acids is discharged. The body lymph glands draining the affected areas may be enlarged and inflamed. On postmortem examinations of the affected animals, congestion and ulceration of the gut mucosa, with gas pockets and yellow fibrin deposits were observed. The tongues were swollen, with pale and dry muscle and accumulation of gas (Harwood *et al.*, 2007). The body cavities may contain bloody exudates, but the internal organs usually show little or no alterations. In contrast to anthrax, which is sometimes confused with blackleg, the spleen is not enlarged, and the coagulability and color of the blood are normal (Coetzer *et al.*, 1994).

In sheep the muscle lesions are more localized and deeper and the subcutaneous edema is not so marked, except around the head (Cynthian, 2005). Gas is present in the affected muscles but not in such large amounts as in cattle. When the disease has resulted from infection of skin wounds. the lesions are more obvious superficially, with subcutaneous edema and swelling and involvement of the underlying musculature. When invasion of the genital tract occurs, typical lesions are found in the perineal tissues and the walls of the vagina and occasionally the uterus. In the special case of pregnant ewes, typical lesions may involve the entire fetus and abdominal distention in ewe (Radostitis *et al.*, 2007).

Histologically, blackleg causes feature myonecrosis, edema, emphysema, and unimpressive neutrophilic cellulitis. Organisms may be few in number but can usually be seen in tissue sections. The isolation and identification of C.chauvoei and C.novyi is difficult because of the fastidiousness of these species in culture and rapid postmortem contamination of tissues by Clostridial species from the gastrointestinal tract. Thus, it is essential that tissues be examined a soon after death as possible (Radostits et al., 2007). The standard method of *C.chauvoei* detection is culture biochemical and identification, which requires a pure culture. In most muscel samples from cattle died from blackleg the amount of *C.chauvoei* in sample is high and the bacterium can be easily be cultured, although some samples may be contaminated (Bagge et al., 2009).

Fluorescent antibody technique has been used as a rapid and high efficiency method for differentiation and identification of *C. chauvoei and C. septicum* from infected animals and bacteriological cultures. The preparation of fluorescent labeled antibodies to *C. chauvoei* and *C. septicum* was also reported (FAO,1991).

#### **DIFFERENTIAL DIAGNOSIS**

False blackleg may be associated with *C. septicum* and *C.novyi* but this disease is more accurately classified as malignant edema. Mixed infections with *C.chauvoei* and *C. novyi* are uncommon but the significance of *C. septicum* as a cause of the disease is debated (Radostits *et al.*, 2007).

Blackleg may be difficult to distinguish clinically from other peracute or acute disease, particularly anthrax. Because most affected animals die suddenly and rapidly become bloated and putrified, with blood stained fluid oozing from orifices. blackleg is difficult natural to distinguish from anthrax. For this reason, when confronted with animals that have died acutely, it is advisable to examine suitable stained blood smears in order to exclude anthrax before proceeding with necropsy (Songer, 1997). Lesions at necropsy are generally easily distinguished from those caused by most other infectious disease, except those due to some other Clostridial infections (C.perfringens, C.septicum, C.novyi, C.serdolli and C.carnis) that cause gas gangrene or malignant edema. In cases where other Clostridial infections are concerned, the identification of causative species by application of fluorescent antibody techniques are pre-requisites for identification (Cynthian, 2005).

### TREATMENT AND CONTROL

#### Treatment

Treatment of affected animals with penicillin and surgical debridement of the lesion, including fasciotomy, is indicated if the animal is not

moribund. Recovery rates are low because of the extensive nature of the lesions. Large doses (44,000 IU/kg BW) should be administered, crystalline commencing with penicillin intravenously and followed by longer-acting preparations. Blackleg antiserum is unlikely to be of much value in treatment unless very large doses are given (Constable et al., 2017). According to Cockcroft (2015); treatment of clostridial myositis is rarely successful due to the rapid course. Antimicrobials (drug of choice procaine penicillin) around affected tissues, aggressive surgical debridement to allow aeration along with supportive treatment can be of value. Majority of cases show poor prognosis. Treatment of affected animals with penicillin is logical if animal not moribund but results are generally any fair because of the extensive nature of the lesion. Large dose should be administered, commencing with crystallin penicillin intravenous and followed by longer acting preparations, some of which should be given in to the affected tissue if it is aquesible. Blackleg antiserum is unlikely to be of much value in treatment unless very large does are giver (Radostatis et al., 2007).

#### Control

Blackleg can be prevented by vaccination, a procedure that is an important component of the health management of many cattle-producing

operations. Conventional blackleg vaccines are bacterins, prepared from formalin-treated cultures of C. chauvoei, that are generally available in polyvalent formulations together with other clostridial components. The evidence for the efficacy of these vaccines is mostly anecdotal or based on measurement of antibody titers in vaccinated animals. However, the literature on clinical trials of these vaccines in cattle is surprisingly scant (Uzal, 2012; Falquet et al., 2013). The vaccine should be delivered just under the skin not in to the muscle. Draw pinch of skin and insert the needle between the skin and the muscle. The loose skin of the neck is convenient. Do not save unused parts of bottles or containers of vaccines for future use, become contaminated with they can as undesirable organisms and/or lose their potency. Destroy and vaccine not used within 24 hours of opening (Sarah and Wilson, 2007). Modern vaccines are produced under conditions of strict quality control by reputable manufactures. Occasionally, reports are received of apparent failure or vaccines. When investigated, most of these vaccination failures due to: In correct dosing, faulty technique, using time- expired vaccine, vaccine having been subjected to high temperature during storage or transportation.

#### CONCLUSION AND RECOMMENDATIONS

The bacteria enter the calf by ingestion and then gains entrance to the body through small punctures in the mucous. The disease is not transmitted directly from sick animals to healthy by mere contact. Outbreaks of blackleg have occurred in cattle on farms in which recent excavations have occurred or after flooding. The animal usually dies in 12 to 48 hours. Although treatment is usually fails, if attempted, appropriate doses of penicillin may prove helpful. Do not conduct any biopsy on animal dead of the disease as the agent exposed to oxygen and form spore. Calves less than 6 weeks should not be vaccinated as passive immunity passes from the dam. Generally, blackleg is almost entirely preventable by vaccination.

Based on the above conclusion the following points are recommended:

- New born animals should have to get colostrum few hours after born in order to get passive immunity from the dam.
- Opening of dead animals should be avoided and the carcasses must bury and/or burned carefully.
- Grazing infected spot areas should be omitted or graze only during rainy season.

- The animals should not be deprived of mineral deficiency and hard and dry feed feeding have to be minimized.
- Calves at the age of 6 8 months with interval of 6 weeks should be vaccinated as booster dose.

#### REFERENCES

- Andrews A.H. and Blower, R.W. (1992): Bovine medicine, A text book of disease and husbandry of cattle, black well, UK, pp. 344-347.
- Bagge, E., Lewerin, S. S. and Johansson, K. L. (2009): Detection and identification by PCR of *Clostridium chauvoei* in clinical isolates, Bovine feaces and substrates from biogas plant. *Journal of National Veterinary Inistitute*, **51**: 1
- Boden, A.D (1998): Diagnostic procedure in veterinary bacteriology and mycology. (5th edn), Pp. 229-251.
- Carter, G.R. and Chengappa, M.M. (1991): Essentials of Veterinary Bacteriology and Mycology (4<sup>th</sup> Edition), Lea and Febiger, Philadelphia, Pp 133- 134
- Central Statistical Agency. 2017. Report on Livestock and Livestock Characteristics, Volume II (585), Addis Ababa.
- Cockcroft, P. (2015): Bovine Medicine (3rd edn), John Wiley & Sons, pp. 575.

- Coetzer, J.A., Thomson, G.R. and Tustin, R.C. (1994): Infectious disease of livestock. Oxford University press, London, Pp 1325-1330
- Constable, P.D, Hinchcliff, K.W, Done S.H. and Grunberg, W. (2017): A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs, and Goats, (11th edn), Elsevier Ltd pp. 1431–1432.
- Cooper B.J. and Valentine, B.A. (2016): Muscle and tendon, In: Maxie MG (Eds.), Jubb, Kennedy, and Palmer's Pathology of Domestic Animals. (6th edn), Elsevier, pp. 164-249.
- Cooper BJ, Valentine BA. Muscle and tendon.
  In: Maxie MG, ed. Jubb, Kennedy, and Palmer's Pathology of Domestic Animals.
  6th ed. Vol. 1. St. Louis, MO: Elsevier, 2016:230–233.
- Cynthian, M.K. (2005): Merck Veterinary Manual. (9<sup>th</sup> Edition), Merck and Co. Inc. Whitehouse Station, N. J. USA, Pp 1689-1690
- FAO (Food and Agricultural Organization) (1991): Manual for the production of anthrax and blackleg vaccine: Animal production and healthy paper 87th, Rome, Italy, 65 - 105

- Frey, J and Falquet, L. (2015): Patho-genetics of Clostridium chauvoei, Res Microbiol 166(4): 384-392.
- Gross, L.W., Barbarin, R.E. and Haines, A.W. (2009): Some characterization of *Clostridium chauvoei. Journal of Infectious Disease*, **29**, 615- 629
- Gyles, C.L., John, F.P., Glenn, S. and Charles,
  O.T. (2010): Pathogenesis of Bacterial Infections in Animals. (4<sup>th</sup> Edition),
  Wiley-Blackwell, UK, Pp 205- 206
- Gyles, C.L., Prescott, J.F., Songer, J.G. and Thoen, C.O. (2004): Pathogenesis of Bacterial Infection in Animals (3<sup>rd</sup> Edition), Blackwell, Ltd, Pp 125- 128.
- Hagan and Bruner's (1988): Microbiology and Infectious Disease of Domestic Animals. (8<sup>th</sup> Edition), Newyork state Cornell University press; Comstock publishing, USA, Pp 233-234.
- Harwood, D.G., Higgins, R.J. and Aggett, D.J.
  (2007): Outbreak of intestinal and lingual *Clostridium chauvoei* infections in two years old Fresian Heifers. Veterinary Record; *Journal of the British Veterinary Association*, **161**: 307
- Hirsh, D.C. and Zee, C. (1999): Veterinary Microbiology. Blackwell, Ltd., London Pp 238- 239.
- Irisk, M.B. (2007): Blackleg in cattle. *Journal of University of Florida*, **165:** 1- 2.

- Lewis, C.J. (2007): Disease of Sheep (4<sup>th</sup> Edition), Blackwell Science Ltd., London, Pp156-157.
- Naz, S., Ahmed, S. and Bhatti, J.I. (2005): Isolation and Identification of *Clostridium chauvoei* from Cattle and Buffaloes. *Pakistan veterinary journal*,25:101-102.
- Parish, S.M. and Valberg S.J. (2009): Clostridial myonecrosis. In: Smith BP (Eds.), Large Animal Internal Medicine, (4th edn), St. Louis, Mosby. 1400-1402.
- Quinn, P.J., Markey, B.K., Carter, M.E., Donnelly, W.J.C., Leonard, F.C. and Maghire, D. (2000): Veterinary Microbiology and Microbial Disease. BlackwellScience, Ltd., London, Pp 191-208.
- Radostits, O.M., Clive, C.G., Kenneth, W.H. and Cliff, P.D. (2007): Veterinary Medicine.
  A textbook of the disease of Cattle, Sheep, Goats, Pigs and Horses (10<sup>th</sup>Edition), Saunders, Elsever, London, Pp 828- 830.
- Robson, S. and Wilson, J.M. (2007): Blackleg in cattle. Journal of British Veterinary Association, 433, 1325-1330
- Sarah Robson and Wilson, J.M. (2007): Blackleg in cattle.

- Scott, P.R., Colin, D.P. and Alastair, I.M. (2011): Cattle Medicine. Manson University press, USA, Pp 242- 243.
- Seifert, H.S. (1996): Tropical Animal Health. (2<sup>nd</sup> Edition), Kluwer Academic Publishes, Netharland, Pp 288- 289.
- Shoshtary, M.M., Pilehchian, L.,
  Abdolmohammid, R. and Jabbari, A.L.
  (2007): Preparation of Concentrated
  Blackleg Vaccine. *Journal Archives of Razi Inistitute*,62:165-169.
- Songer, J.G. (1997): Clostridial Disease of Small Ruminants.London academic press, Pp 153-182.
- Useh, N.M., Andrew, J.N., Najume, I. and King, A.N. (2012): Anemia in *Clostridium chauvoei* masked by haemoconcentration. *Journal Veterinariski Archive*, Ahmadu Bello University, Zaira, Nigeria, **82**:433-447.
- Useh, N.M., Ibrahim, N.D., Nok, A.J. and Esieve, K.A. (2006): Relationship between outbreaks of blackleg in cattle and annual rainfall in Zaira, Nigeria. Veterinary record: *Journal of British Veterinary Association*, **158**:100.
- Uzal FA. Evidence-based medicine concerning efficacy of vaccination against *Clostridium chauvoei* infection in cattle. Vet Clin North Am Food Anim Pract 2012;28:71–77.

- Van Vleet, J.F, and Valentine, B.A. (2007):
  Blackleg. In Maxie MG (Eds.), Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, I, Philadelphia, WB Saunders Co, 1(5): 261-264.
- Vilei, E. M., Johansson, A., Yvonne Schlatter, Keith Redhead and Joachim Frey (2011): Genetic and functional characterization of the NanA sialidase from *Clostridium Chauvoei. Journal of Inistitute of Veterinary Bacteriology*, **42**:1-2.

Zeleke, T. 2009. Common defects of sheep and goat skins in Ethiopia and their causes.In: Ethiopia sheep and goat productivity improvement program. Technical Bulletin, 19, 1–5.

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