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# Reviewon Botulismin Cattle

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**Abstract:** Botulism is a poisoning caused by a toxin produced from bacterium *Clostridium botulinum*. The toxin may be taken up orally (intoxication) or produced inside the digestive tract (infection). Cattles are one of the most susceptible species to the botulinum neurotoxins due to extremesensitiveness to the effects of the toxin, meaning that ingestion of very small amounts of toxin can result in clinical disease. The absorbed toxins from the intestine or wound of affected animal can enter to the nerves through the blood stream and inhibit release of acetylcholine. The cattle affected by the disease manifest paralysis of the tongue, throat and unable to drink. In addition, lack of appetite, dehydration, depression, muscular weakness and lameness can be observed. Detection of antibody and necropsy findings isused for diagnosis. Botulinum neurotoxin signs should be differentiated from other neurological diseases by symmetric motor paralysis formation. Treatment may involve the antitoxin injection and supportive treatment; however, the later has more importance than others. Correction of dietary deficiency, vaccination, removal of carcass and bones and supplementary feeding of cattle are taken as control and preventive measures. In Ethiopia, deadtortoisesarethe source of botulinum toxin (type C and D) on the cattle.

**Key words:** Vaccination · Poisoning · Botulinum · Neurotoxin

## INTRODUCTION

Botulism is a neuro-paralytic illness resulting from the action of a potent toxin produced by organism *Clostridium botulinum*. This microbe was first described in 1897 by E.van Ermengem [1] after his investigation of a food borne outbreak in Ellezelles, Belgium. The name Botulism was derived from the Latin word `Botulus`, meaning sausage and the disease, with the first designated name of the organism, Bacillus botulinus. Four distinct forms of botulism can occur, depending on the mode of acquisition of the toxin. The first one is Food borne botulism that results when there is the ingestion of food that contain preformed toxin.Food borne botulism is rare but it may kill rapidly and contaminated products may expose many persons. Food borne botulism therefore represents a medical and a public health emergency that places a premium on rapid, effective communication between clinicians and public health officials. Wound botulism is the second form of botulism that caused by the organisms that multiply and produce toxin in contaminated wound. Infant botulism which is due to the production of toxin by germinating spores of *Clostridium botulinum* in the intestine of infant and the fourth one, Child or adult botulism from intestinal colonization is represented by those cases in which no food vehicle can be identified, there is no evidence of wound botulism and there is the possibility of intestinal colonization in a person older than one year of age [2-5].

*Clostridium botulinum* is a group of culturally distinct organisms that are alike only in that they are clostridia and produce antigenically distinct neurotoxins with similar pharmacologic action.They are straight to slightly curved, Gram-positive (in young cultures), motile, anaerobic rods and 0.5-2.0µm in width 1.6-22.0µm in length with oval, sub terminal spores. Clostiridial spores are resistant to heat, light, drying, radiation and specific conditions are necessary for germination [4, 6]. The heat resistance of spores varies from type to type and even from strain to strain with in each type; although some strains will not survive at 80°. Spores of many strains require temperatures above boiling point to ensure destruction. The thermal resistance of spores also increases with higher pH and lower salt content of the

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medium in which the spores are suspended [7- 9]. The intoxication raises when the feed source is contaminated by organism or its spores.In many cases, it is impractical or undesirable to treat a food product in manner to eliminate all *C.botulinum* spores. As a result, most control methods focus on the inhibition of growth and toxin production. The main limiting factors for growth of *C.botulinum* in food are temperature, pH, water activity, redox potential, food preservatives and competing microorganisms. All of these factors are interrelated and so, changing one factor influences the effect of other factors.The interaction of factors may have a positive or negative effect on the inhibition of *C.botulinum*[6, 10].

Botulinum is seen mainly in ruminants, horses, mink and fowl, particularly water fowl.Cattle in intensive dairy feeding systems that relay on stored feed are at risk of botulismbecause, at some stages the feed may be contaminated with a dead animal or some rotting plant material. Only a relatively small amount of contamination of the feed source with the botulinum toxin may result in an outbreak. Toxin could come from the carcass of small animals (*e.g*. mouse rat or snake) being accidentally processed with the feed.Cattle are very susceptible to botulism toxin due to extremely sensitiveness to the effects of the toxin, meaning that ingestion of very small amounts of toxin can result in clinical disease. Toxin production occurs in an anaerobic environment, with moisture and an optimum temperature of around 23°C (15-35°C). All these conditions can be found in a rotting carcass. Toxin can last for a year at 30°C and is rapidly inactivated at 37°C, so the amount of toxin present is not constant. Not all carcasses are necessarily toxic but the proportion of toxic carcasses can be very high and it easily harms the cattle population that ingests the contaminated feed [11- 13].

Now days, sporadic incidences and high amount of outbreaks with cattle botulism have been reported from different parts of the world.The disease in cattle represents a serious environmental and economic concern because of the high mortality during the outbreak. However, there is scarcity of reliable information concerning the effect of Botulinum toxin among the cattle[14- 16].Therefore; the objective of this reviewwasto review on the available literature of botulism in the cattle.

**Etiology:** Botulism is caused by a toxin produced by the bacterium *C. botulinum* which multiplies rapidly producing a highly lethal toxin. *C. botulinum* is Gram positive, spore forming, saprophytic, anaerobic rod that is distributed in soil worldwide. All warm blooded animals can be paralyzed by the botulism toxin. This toxin prevents nerve functions that eventuate on paralysis. It is one of the most toxic poisons known and cattle are one of the most susceptible species.*C.botulinum* produces eight different types of neurotoxins; A, B, $C_{\alpha}$, $C_{\beta}$, D, E, F and G [6, 12, 17, 18]. The Production, mode of action and effects of *C. botulinum* neurotoxins are explained in Table 1 below.

Botulism in cattle is most often caused by ingestion of the preformed BoNT B, C and D.The usual source of the toxin is decaying carcasses or vegetable materials such as decaying grass, hay, grain, or spoiled silage.Phosphorous deficient cattle chew any bones with accompanying bits of flesh that they find on the range; if these came from animal that had been carrying type D strains of *C. botulinum*, intoxication is likely to result. A gram or so of dried flesh from such Caracas may contain enough toxins to kill a mature cow. Any animal eating such material also ingest spores, which germinate in the intestine and, after death of the host, invade the musculature, which in turn becomes toxic for other cattle in a similar fashion [20]. The chain of events most commonly required for botulism poisoning isdescribed in detail at Fig. 1as follows.

**Epidemiology**

**Occurrence:** *Clostridium botulinum* pathogens occur worldwide and are associated with soil, decaying and rotting vegetative material or cadavers. The geographic occurrence of botulism cases in temperate countries closely parallels the prevalence of *C.botulinum* in the soil, including the compatibility of disease causing strain types with soil type. Study in USA shows that type A was found in neutral or alkaline soils in the west, while type B and E were in damp or wet soil, all over, except that type B was not found in the south. Type C was found in acid soils in the gulf cost and type D in alkaline soil in west. Microorganisms capable of inhibiting *C. botulinum* were present with or without the clostridia in many soils. Type B is also common at the soils in UK and in Europe. Types C and D are more common in warm climate and Type D botulism occurs more commonly in South Africa and South America when phosphorous-deficient range cattle chew on bones of decaying carcasses [3, 22].

**Source of Infection**

**Forage Botulism:** The disease is caused by ingestion of food in which *C.botulinum* has grown, producing preformed toxin. Poisoning has also been known to occur by consuming water or feed that has been contaminated

Table 1: Production, mode of action and effects of *C. botulinum* neurotoxins.

| Feature of neurotoxin | *Clostridium botulinum* |
|---|---|
| Site of production | In carcasses, decaying vegetation, Occasionally in wounds or in intestine (toxico infection) |
| Genes which regulate production | Usually in genome (in bacteriophages for types C and D) |
| Antigenic type | Eight antigenically distinct toxins, types A -G |
| Mode of action | Inhibition of neuromuscular transmission |
| Clinical effects | Flaccid paralysis |

Source:-Adapted from Quinn *et al*. [19]

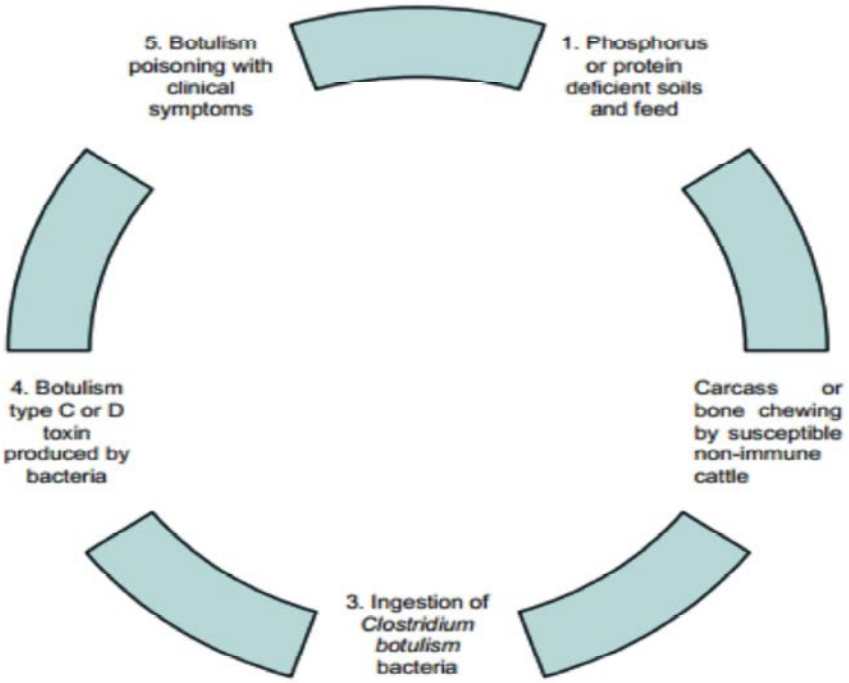


Fig. 1: The chain of events most commonly required for botulism poisoning [12]

by animal carcasses and rotting feed (moldy hay or silage and decomposing grass tussocks). Botulinum neurotoxin C and D are mostly associated with the ingestion of carcasses and broiler litter while, BoNT B is connected with rotten vegetation or feed stuff [23-25].

**Carrion Associated Botulism:** Bones and carrion of decaying cattle and fly maggots are the best sources of toxin. Cattle with pica tend to chew on cadavers and bones in order to balance their mineral deficiency which means a high risk of BoNT ingestion. Direct carrion ingestion can occur where cattle subsist on a phosphorous deficient diet and manifest osteophagia, with subsequent ingestion of carrion. The disease is likely to occur in outbreak form [22, 26]. Carrion-associated botulism is commonly associated with type C and D strains, occurring when the carcass of a dead animal is invaded by *C. botulinum*and toxin is produced, contaminating feedstuff. Type C strains are usually associated with a decomposing carcass, for example, cats, or water fowl, or with poultry litter.Botulinum toxin can persist in carrion for at least one year [10, 18].

**Wound Botulism:** Wound botulism is a disease resulting from the growth of *C. botulinum* spores in a contaminated wound with *in-vivo* toxin production. It is a rare acquired neuromuscular junction disease with descending flaccid paralysis due to botulinum neurotoxins (BoNTs), produced after infection of wounds by Clostridium botulinum [27].

**Toxico Infection Botulism:** Toxico infectious botulism is the name given to the disease in which *C. botulinum* grows in tissue of a living animal and produces toxins there. Another form of botulism, toxico infectious botulism, occurs when animals consume actively growing bacteria from gastrointestinal tract of cattle where the toxin is subsequently produced. The toxins (described in

Table 2: Toxins of *C. botulinum*

| Toxin | Source |
|---|---|
| Type A | Meat, carcasses, toxico infection. |
| Type B | Meat, carcasses, toxico infection. |
| Type C | Dead invertebrates, maggots, rotting vegetation and carcasses of poultry, ensiled poultry litter, hay or silage contaminated with rodent carcasses |
| Type D | Caracas, bones, feed contaminated with carcasses |
| Type E | Dead invertebrates, sludge in earth bottomed ponds, fish |
| Type F | Meat, fish |
| Type G | Soil contaminated food |

Source: - Adapted from Quinn and Markey [29]

Table 2 below) are distributed via the blood stream, travel retrograde along the nerve cells to the neuromuscular junction and interfere with the release of acetylcholine [20, 28].

**Risk Factors**

**Animal Risk Factors:** Botulism is most common in birds, particularly the domestic chicken and wild waterfowl. Cattle, sheep and horse sire are susceptible but pigs, dogs and cats appear to be resistant. The horse appears to be particularly susceptible to type B toxin. Cattle and sheep are usually affected by types C and D [22].

**Environment Risk Factors:** Botulism in range animals has seasonal distribution outbreaks that most likely to occur during drought periods when feed is sparse, phosphorus intake is low and carrion is plentiful. Silage-associated botulism is also seasonal with feeding of silage. The variation that occurs in the geographical distribution of varies types and in carrion versus none carrion associated botulism is an important factor when considering prophylactic vaccination programs [22].

**Pathogenesis and Clinical Findings in Cattle**

**Pathogenesis:** The *C. botulinum* neurotoxins produce functional paralysis without development of histological lesions. Botulinum toxins are absorbed from intestinal tract or the wound and carried via blood stream to the peripheral cholinergic nerve terminals including neuromuscular junction, postganglionic parasympathetic nerve ending and peripheral ganglia. Flaccid paralysis develops and the animal dies of respiratory paralysis. The heavy chain of the toxin is responsible for binding to receptors and translocation in to the cell and the light chain of the toxin for resultant blockage due to release of acetylcholine as neuromuscular junction [30].

**Clinical Findings:** The course of the disease in cattle varies from per acute to chronic intoxication (days), or as long-lasting infection (weeks to months) with sometimes final acute outcome. The signs of botulism are caused by muscle paralysis and include progressive motor paralysis, disturbed vision, difficulty in chewing and swallowing, with generalized progressive weakness. Death is usually due to respiratory or cardiac paralysis. No characteristic lesions develop and pathologic changes may be ascribed to the general paralytic action of toxin on any particular organ. Epidemics have been occur in dairy herds in which up to 65% of adult cows developed clinical botulism and died 6-72 hour after the onset of recumbence. Visual symptoms of botulism poisoning can vary dramatically depending on the amount of toxin ingested, pre-existing immunity and the stage of poisoning.The period of time for the ingested toxin to show effect on the animal usually varies between three to seven days, depending on the quantity of toxin ingested. In very severe cases the animal can die in less than 24 hours withoutsigns of illness. Animals may ingest low doses of toxin and not be affected, especially if they have antibodies to the toxin. The toxin binds to nerve endings and prevents nerve impulses to muscles. This leads to a floppy orflaccid paralysis, which usually progresses throughout the body. Early symptoms result from the paralysis of the tongue, throat and the stomach. Often the earliest sign of the botulism poisoning is refusal to drink and a lack of appetite, related to the paralysis of the throat. This leads to dehydration that is evident by sunken eyes, hollow paunch, unplayable skin and strange behavior. The tongue may protrude and the animal may drop cud and drool saliva. The animals' voice may alter or fail. The first observed signs may be depression, muscular weakness and incardination which makes the animal appear lame. The animal may become aggressive and often attempt to charge anyone who comes close. On falling down, the beast may appear very weak and have great difficulty in rising. It may not even be able to lift its head (limber neck). Initially the beast lays in a normal resting position with its head on the ground or turn towards the flank (sternal recumbence). As the paralysis worsens, the animal may go in to semi-conscious state and when it goes on to its side (lateral recumbence), it is unlikely to gate up again [31- 33].

**Diagnosis:** Diagnosis in sick animals is based mainly on history and clinical investigation findings and may be supported by laboratory diagnosis; that is, proof of BoNT or toxigenic bacteria in the gastrointestinal tract [32].

**Laboratory Diagnosis**

**Elisa-Enzyme-Linked Immunosorbent Assay:** This test was used to sow that an animal has anti bodies to the toxin in its blood serum. Anti-bodies may arise from natural exposure to a toxin or from vaccination. The test can identify the type of toxin involved (type C or D) with natural infection and the level of antibodies in the animal. Because of cross reactions following vaccination, it is not possible to differentiate between type C and D vaccination titers. This test is useful for assessing the success of vaccination program and can be done on any group of animals where there is some doubt about their vaccination history.It is however an expensive test. Use of ELISA methodology for detection of the toxin makes it feasible to test large number of sample, increasing the chances of diagnosis confirmation [11, 12].

**MPT (Mouse Protection Test) (Toxin Neutralization Test):** This test relies on paralyzing mice with an injection of a toxic bacterial growth or toxic serum from an affected beast and then protecting them with specific type C or D botulism antiserum. The test is good for identifying the presence of toxic botulism bacteria and is used with the ELISA test. However, it is not so useful in proving that a paralyzed beast has botulism. This is because only very low doses of toxin are present for short periods in the serum and the mouse is relatively resistant to the toxin compared to cattle [2, 12, 13].

**Culture for the Bacterium:** The beast samples are taken from lower intestinal contents and maggots from carcasses. Once the organism is grown in the laboratory, tests are carried out to show that it is *C. botulinum* and to identify the type [12].

**Detection of Toxin:** Toxin production or carrion contamination can potentially occur in a number of feeds, however the majority of outbreaks are associated with contamination in hay or silage and suspect feeds should be tested mice for toxin. To get around the problem of lack of the sensitivity with the mouse test, suspect feed has been feed to experimental cattle. Alternatively one can make an infusion of the feed sample and use this as the sole drinking water supply for experimental animals. The problem with all feeding experiments that; the botulinum toxin is likely to be very patchy in its distribution in the feed. Failure to produce the disease in animals vaccinated against botulism when deaths are occurring in the unvaccinated controls has also been used as a diagnostic procedure [22, 34].

**Demonstration of Spore:** Demonstration of toxin in feed stuffs, fresh stomach contents, or vomits supports a diagnosis of botulism. Toxin is extracted from suspect material (unless fluids) over night with saline. The mixture is centrifuged and the clear portion filter sterilized and trypsinized (1% at 37° for 45 minutes) [13].

**Isolation of the Bacterium:** In affected animals, sampling should be performed from the affected parts of the digestive system. In healthy animals, fecal pellets would be the most appropriate. Isolation of *C.botulinum* from suspect feeds or tissue begins with heating suspect materials for 30 minutes at 65° to 80° to induce germination.Type E spores require, in addition, treatment with isoenzyme (5mg/ml of medium)Culture anaerobically on blood agar plates. Immunofluorescence is used to identify some cultural groups. Primers designed to amplify the genes encoding varies toxins (by polymerase chain reaction) can be used to support the demonstration that *C.botulinum* has been isolated [16, 35].

**Detection of Antibodies:** In chronically affected animal and at risk herd mates by an ELISA test has been used to support a diagnosis in outbreak of type C and type D botulism. It has been used in range cattle where actually affected clinical case or fresh animals for postmortem toxin testing are not available, cattle exposed to Sublette and subclinical amount of toxin with sufficient immunogenicity develop specific antibody response. An increased antibody prevalence overtime or increased antibody prevalence in affected group compared with similar group nearby suggests exposure to toxin [36, 37].

**Necropsy Findings:** There are no specific changes detectable at necropsy. Also the presence of suspicious feedstuffs in the fore stomach or stomach maybe suggestive. There may be none specific sub endocardial and sub epicardial hemorrhage and congestion of the intestines. The presence of toxin in the liver at post mortem examination is taken as evidence that the disease has occurred. In addition to traditional bioassay such as the mouse protection test, newer methods for toxin detection include ELISA techniques and a recently described immune polymerase chain reaction (PCR) assay [38].

**Differential Diagnosis:** A presumptive diagnosis is made on the clinical signs and history, occurrence in unvaccinated animals and the ruling out of other diseases with a similar clinical Presentation. The symmetric motor paralysis of botulism with muscle paralysis that progresses to recumbence in 1-4 days is a major differential diagnosis for botulism from other causes of neurological dysfunction in large animals. In ruminants, tick paralysis, paralytic rabies poisoning by phalaris aquatic, organophosphate/carbamate poisoning but clinically and at necropsy the disease resembles parturient paresis in cattle [22].

**Zoonotic Implication:** Botulism is one of thediseases ofconcern toboth humans and animals with extremely small toxic dose. Botulism toxin is identified as possible agent for bioterrorismeither by contaminating food and/or feed stuffs or in an aerosol form. The ingestionof preformed toxins in foods causes foodpoisoning in human that mostly related to A, B, E and F botulinum toxin types. However, Intoxicationofhuman beings by toxin types A, B and E. is common and Furthermore, pathogenicity and zoonotic potential of toxin type C botulism are minimum [2, 21, 22].

**Treatment:** There is very little that can be done to treat cattle suffering from botulism. Physical removal of the toxin in the early stages may reduce the effect. Good nursing may be of assistance in mild cases of the disease. Once a beast has absorbed botulinum toxin and has become affected, there is nothing that can be done to speed up recovery. Antiserum is very expensive to produce and only available in very small quantities. The antitoxin can lessen the severity and duration of symptoms of botulism by the toxin that has not yet bound to nerve endings. A fundamental imperative of treatment in all forms of botulism is supportive care to preclude complications of respiratory failure, which is usual cause of death [3, 12, 39].

**Prevention and Control**

**Prevention:** Prevention is better than cure and the most practical way to prevent loses from botulism poisoning is by vaccination. properly storing animal feed, inspecting water sources, removing died carrions from animal feed store, avoiding poultry liter use in cattle feeding area are important to prevent the rouse of the diseases. The three steps recommended to prevention of botulism poisoning are:-Vaccination with bivalent botulism vaccine following a recommended program, Supplementary feeding of cattle with phosphorus and protein and Removal of all carcasses and bones[2, 12].

**Vaccination:** Vaccination with a bio valent (type C and D) botulism vaccine is the most effective long-term prevention strategy. There is a range of botulism vaccines available. Conventional vaccines involve an initial two shot program, one month apart, or a single shot followed by an annual booster shot. An alternative long-acting vaccine involves an initial single shot followed by a booster shot every three years. Both vaccines produce a similar level of protection and the decision on which vaccine to select will depend on cost and management practices. All vaccines require booster shots to maintain protective levels of immunity [12, 31].

**Supplementations:** The supplementation of non-protein nitrogen (*e.g*. urea) and phosphorous is a well-recognized management practice. However, even the best supplementation programs will not completely prevent carcass or bone chewing [12].

**Carcass Removal:** Removal of carcasses is not always an option under extensive range conditions where paddocks are large and the checking of stock is infrequent. However, the removal of all carcasses from areas of stock congregation (*e.g*. watering points) is important. Carcasses can be burnt, buried, locked up in the turkey nest enclosure or at least taken a considerable distance away [12].

**Control:** Commercial canned foods are heated to a sufficient temperature and for sufficient time to kill the spores. Unheated commeritial foods in cans or jars can be made safe by acidification or other manipulation that inhibit the growth of the organisms (*e.g*., addition of phosphoric acid to garlic in oil). Occasionally, commeritial foods still cause botulism if they are prepared in a way that permits toxin production. Botulinum toxin can be inactivated by heating to 176°(80°). Therefore, heating home-canned foods before consumption can reduce the risk of botulism intoxication. In animals, correction of dietary deficiencies by supplementation with phosphorus or protein should be implemented if condition permit. Using high level disinfection is important to kill the spore of *clostridium* species. Hygienic disposal of carcasses is advisable to prevent further pasture contamination but may not be practicable under range conditions [27, 40].

**Status of Cattle Botulism in Ethiopia:** Dead tortoises putrefied under field condition were found to contain spores and bacilli of *Clostridium botulinum* with toxins identified as biotypes C and D. Two mature Arsi (zebu) cows were drenched with 3 liters of the supernatant fluid,

two were allowed to lick tortoise shells and the remaining two were controls. The supernatant fluid was also injected intraperitoneally at 1 ml/kg body weight to one calf, a mature sheep and a goat and at 1 ml per animal to 4 mice. A duplicate group of animals received the same treatment but using material boiled for 30 minutes before injection. Cattle, sheep and mice died, but goats did not. The post mortem examinations revealed hemorrhagic changes on the omasum, abomasum and intestinal lining. None of the animals inoculated with the boiled material died or developed clinical signs. Examination of soil collected from the area where tortoises were found, revealed only traces of phosphorus. It is concluded that phosphorus deficiency forced the cattle to consume tortoise tissues [41].

## CONCLUSIONS

Botulism is a severe intoxication caused by *C.botulinum* which produces neurotoxin in an aerobic condition. This pathogen occurs worldwide and its distribution depends on food storage, feeding and management practice thorough the world. The contamination of food or silage by decaying Caracas of died small animals, that contain *C. botulinum* spores may be the source of the toxin and it is risky to cattle feeding on contaminated silage. Supportive treatment and antitoxin injection are considered to be essential for cure success. Eradication of the disease is difficult because of ubiquitous nature of *C.botulinum* but necessarily controlled by vaccination. In Ethiopia, the cattle had been affected due to the consumption of putrefied tortuous flesh that contains the *C. botulinum* spores, but there were no significant epidemiological study on the botulinum disease.In light of the above conclusion, the following recommendations are forwarded;

- Minerals should be supplemented in feed stuffs of cattle to correct dietary deficiency.
- Epidemiological surveillance should be done; hence the botulinum disease in the cattle is prevalent.
- Hygienic disposal of carcass should be practiced.
- Vaccines should be given in areas where there is seasonal outbreak of botulism and should be seriously consideredupon feeding stored feed.
- Considering botulism as a risk when buying or storing feed for cattle are recommended.

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