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Diagnosis of *Clostridium botulinum* intoxication in Bovine in field condition

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ABSTRACT

In an outbreak of Clostridium botulinum in dairy cattle, 121 cattle affected over a period of three months with the following symptoms anorexia, adipsia, restlessness, reluctance to move and animals when forced to walk showed stumbling, knuckling and staggering. The clinical samples like rumen fluids, dung, blood, peripheral blood smear and urines collected from all symptomatic cattles. The suspected cattle could be treated with intravenous administration of 10 to 15 litres of lactated Ringers solution and 5% dextrose per day and oral administration of activated charcoal at a rate of 1 g/kg, b.wt, PO After about 30 minutes, sodium sulphate (1 g/kg b.wt, PO) could be administered.

Key words: *Clostridium botulinum* in dairy cattle – Staggering-Rumen fluid- Activated charcoal

INTRODUCTION

Botulism is an intoxication caused by exposure to botulinum neurotoxins (BoNTs), one of the most potent toxins known, produced by the bacteria *Clostridium botulinum*. It can affect all mammals. Recent years, an increased frequency of new form of bovine botulism has been observed. *Clostridium botulinum is a* Gram-positive spore-forming, strictly anaerobic bacillus present in soil, dust, dung of cattle, poultry manure and slaughter house waste. *The* strains are grouped I to IV. There are seven antigenically distinct toxin types (A, B, C, D, E, F, G). Intoxications are usually the result of ingestion of decaying material. Feeding cattle with poultry litter contaminated with chicken carcasses have also been associated with visceral form of disease (Bohnel *et.al.,*2001).

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Botulism may occur when silage, which is usually, harvested from fields with large concentrations of *C. botulinum* organisms is not fermented properly when the pH is maintained above the inhibitory pH of 4.5 and which allows for the organism to grow and produce toxin (Wilson *et.al*.,1995). The bacterium occurs commonly in the soil and is also found in the digestive tract of about 20% of normal cattle and other herbivores. Bones and flesh of decaying animal, bird carcasses and fly maggots are also best sources of toxin. Spores of *Clostridium botulinum* can survive for 30 years and under warm and humid conditions the organism multiplies rapidly and produce its toxin (Radotitis et.al.,2000). The prevalence of *Clostridium botulinum* in cattle can be determined by detection of botulinum neurotoxin (BoNTs) or *Clostridium botulinum* vegetative bacteria or spores in the gastro intestinal tract or organs like liver, kidney, lungs and muscles (Kataria *et.al.,* 2005)

MATERIALS AND METHODS

Clinical observation and laboratory diagnosis:

In an outbreak of *Clostridium botulinum* in dairy cattle, 121 cattle affected over a period of three months with the following symptoms anorexia, adipsia, restlessness, reluctance to move and animals when forced to walk showed stumbling, knuckling and staggering. Rumination and rumen movement were reduced and animal showed shallow and abdominal breathing. After 2-3 days animal came into sternal recumbency, tongue protruded out and death was seen during 4-5 days. Some animal showed

hind quarter paralysis, reduced reflex of hind legs, tail paralysis, sluggish tail tone, grinding of teeth, engorged veins, oedema of legs,laminitis with normal body temperature and scanty defecation and urination were noticed. The clinical samples like rumen fluids, dung, blood, peripheral blood smear and urines collected from all symptomatic cattle. Feed and water samples also collected.

The laboratory diagnostic test like direct microscopic examination of rumen fluid and dung samples for the presence of organism, protozoan motility in rumen fluid, rumen fluid pH, peripheral blood smear examination, biochemical analysis of blood plasma and urine samples, anaerobic propagation of microorganism in cooked meat medium and *Clostridium botulinum* isolation agar were performed. Seven field investigation and three post mortem investigation were carried out.

The suspected animals rumen fluid and dung were collected in hygienic way by using stomach tube and per rectum method. The collected samples were pooled and mixed with water. 1 ml of pooled sample mixed with 1 ml of ethanol and incubated at 37°C for 2 hours. Then 100 ml of processed sample inoculated in sterile pre warmed cooked meat medium and *Clostridium botulinum* isolation agar. The inoculated culture tubes and agar plate kept in side anaerobic jar. The anaerobic gas pack kept inside and the cap was tightly closed and the culture were maintained in strict anerobic condition at 37°C for eight days. After eight days the culture were analyzed by physical appearance of colony and staining and biochemical method.

RESULTS

Treatment and Discussion:

The suspected cattle could be treated with intravenous administration of 10 to 15 litres of lactated Ringers solution and 5% dextrose per day and oral administration of activated charcoal at a rate of 1 g/kg, b.wt, PO After about 30 minutes, sodium sulphate (1 g/kg b.wt, PO) could be administered (Braun *et.al.,*2005) In addition, 0.005 mg/kg neostigmine could be given subcutaneously. There is the risk of aspiration pneumonia, antibiotics can be injected intramuscularly to each animal everyday for five days. However antibiotics should not be routinely administered in animals with botulism unless a secondary infection such as aspiration pneumonia is suspected. Antibiotics that have been associated with neuromuscular weakness, such as aminoglycosides, tetracyclines and penicillin, should be avoided. The treatment is usually supportive and may include gastric lavage to remove some of the toxin. There are also some reports of success with guanidine hydrochloride. The cow with indigestion symptoms,3-5 lit of fresh rumen juice mixed with 100 g of yeast, 200g of sodium propionate and 100 g of trace element mixture were given per orally for 3-4 days (Dirksen2002a). The prognosis is poor in large animals that are recumbent. In cattle, death generally occurs within 6 to 72 hours after sternal recumbency. There is an Antitoxin available that helps by binding to any toxin that is still in the bloodstream. Once the toxin has bound to the nerve cells, it will not be removed by antitoxin. Among 121 symptomatic cattle, 2 cows recovered, remaining animals were culled by the owners. Among 121 cows, The *Clostridium botulinum* infected cattle showed progressive muscular paralysis of jaw, throat, head, neck and limb muscles. The tongue protrusion, drooling of saliva and unable to chew or drink, restlessness, in coordination, knuckling, ataxia and lie down in sternal recumbency. Some animals showed shallow and abdominal type of breathing and increased heart rate, grinding of teeth and respiratory distress are also present in some cases, decreased tail tone, normal temperature, sluggish urination and defecation noticed in some affected animals. The rumen pH and rumen fluid protozoa motility were normal. Blood protozoa and bacteria could not be detected in peripheral blood smear examination. Serum biochemical parameters revealed low level of calcium, potassium and phosphorous and elevated level of PCV and Hb. Cultural examination of rumen fluid, dung samples and poultry manure samples were revealed that three cases showed proteolytic changes in cooked meat medium. Only three cases showed photolytic changes in cooked meat medium and lecithenase activity in *Clostridium botulinum* isolation agar. The positive bacterial culture showed sub terminal spore in gram staining, acid- gas production in carbohydrate fermentation in lactose and sucrose medium, liquefaction of gelatine.

Exposure to botulinum toxin can occur through ingestion of preformed toxin, inhalation of preformed toxin, local production of toxin by *Clostridium botulinum* organisms in the gastrointestinal tract, local production of toxin by *Clostridium botulinum* organisms in devitalized tissue at the site of a wound. In ruminants, botulism mainly occurs in areas where phosphorus or protein deficiencies are found. Botulinum toxin enters the circulation and is transported to the neuromuscular junction (Rodostitis *et al*., 2000; V. Pranitha et al, 2014). At the neuromuscular junction, the toxin binds to the neuronal membrane and prevents release of acetylcholine and so without neuronal acetylcholine release, the affiliated muscle is unable to contract and becomes paralyzed. Death from botulism results acutely from airway obstruction or paralysis of respiratory muscles (Martins, 2003). Botulinum toxin apparently does not cross the blood-brain barrier, central nervous system functions remain intact. Differential diagnoses include hypocalcemia, hypomagnesemia, carbohydrate overload; several toxicoses including mycotoxin, lead, nitrate, organophosphate, atropine or atropine-like alkaloid, other diseases that have similar clinical signs, such as listeriosis and rabies are also to be considered in the differential diagnosis (Radotitis *et.al*.,2000; Swapna Gurrapu et al, 2007). Diagnosis of botulism is largely based on clinical signs suggestive of the disease. The definitive diagnosis however relies on identifying the toxin in feces, blood, vomitus, gastric aspirates, respiratory secretions or food samples. Feces are usually the most reliable clinical sample in food borne botulism (Bohnel *et.al.,*2001) The toxin can be identified by mouse

inoculation studies. This test relies on paralysis of mice with an injection of a toxic bacterial growth or toxic serum from an affected animal and then protecting them with specific type C or D botulism antiserum. In post mortem examination, there are no pathognomonic lesions can be noticed.

Conflicts of Interest

Authors declare that there is no conflict of interests regarding the publication of this paper.

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