FEED POISONING IN HOLESTEIN CATTLE ASSOCIATED WITH CLOSTRIDIUM BOTULINUM TOXIN (TYPE - D)

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ABSTRACT

Botulism is a rapidly fatal disease caused by a toxin of the spore forming anaerobic becteria Clostridium botulinum (Cl. botulinum). The current study was conducted to put an acceptable program of investigations, treatments and control of an outbreak of botulism in a cattle farm. In spring 2004, an outbreak of suspected botulism occurred in large private farm of 5000 Holestein cattle (dairy cattle, heifer and calves) which were distributed in their specific yards. The total mixed ration of concentrates and mineral mixture are the feeding stuffs of the animals, corn-silage lately used for feeding 18 yards of dairy cattle and heifers. 3-5 days post silage feeding a disease syndrome was appeared where 295 cattle and heifer become diseased, 55 animals were died or slaughtered. The important clinical signs were lack of appetite, abdominal respiration, recumbency, dullness and death. General congestion and petechial haemorrhages of internal organ were the prominent necropsy findings. The drinking water, corn-silage, blood and the liver were subjected to various investigations as : bacteriological identification of pasteurella, listeria and anaerobes for all samples, silage mycotoxin (Alfatoxin-B1), pesticide poisoning of Diazinon and malathion organophosphorus compounds (in all samples) and toxic elements determination of iron, cupper, zinc and manganese (in water). The Cl. botulinum toxins (types C and D) were investigated in all samples. Results indicated that Cl. botulinum (type-D) anaerobic organism was isolated from silage, liver and abomasum content. The Cl. botulinum toxin (type - D) was detected from serum, abomasum, liver and corn-silage. 240 diseased animals were treated with antitoxin (antiserum) types C and D with supportive medical and nutritional treatments. All the remaining healthy animals were vaccinated with bivalent toxoids (vaccines) (types C and D) of Cl. botulinum. It could be concluded that the corn silage may be the source of botulism in cattle and the all feeding stuffs should be periodically examined for Cl. boltulinum organisms and their toxins, and the healthy animals should be annually vaccinated against botulism by bivalent toxoids (C and D types) as the cattle are susceptible to C and D clostridium toxins.

INTRODUCTION

Botulism is a rapidly fatal and motor paralytic disease caused by Cl. botulinum anaerobic organism **(Blood et al., 1979)**. Clostridia are widely spread in the environment, soil, dust and water, and 120 described strains of clostridia are present, although few can cause diseases **(Baldassi, 2005)**.

The clinical signs of botulism are anorexia, depression and reduce in milk production **(Gray and Bulgin, 1982)**, weakness of hind limbs, ataxia and recumbency **(Abbitt et al., 1984)**, paralysis of the tongue and chest muscle and abdominal respiration (Kelch et al., 2000), constipation alternating with diarrhoea, oedema and apathy **(Bohnel et al., 2001)**, profuse salivation and sternal recumbency **(Wenzel et al., 2005)**, difficult gait, low rectal temperature, high pulse rate and low blood hydrogen ion concentration (PH) **(Braun et al., 2005)**. The differential diagnosis with botulism in cattle included: hypocalcaemia, hypomagnesaemia, carbohydrate overload, and several toxicoses by: mycotoxins, nitrate, organophosphates, atropine and atropine-like alkaloids **(Kelch et al., 2000)**.

The post mortem (necropsy) findings include: hyperaemia of abomasal mucosa and gas filled large intestine **(Gray and Bulgin, 1982)**, congestion of the small intestine **(Abbitt et al., 1984)**, and congestion of parenchymatous organs in case of visceral botulism **(Bohnel et al., 2001)** Who hypothesized the visceral botulism as "when long lasting absorption of low quantities of Cl. botulinum toxin, that it may be interferes with the neurophysiology of the intestine. Lately, Baladassi (2005) divided the boltulism into: neurotropic disorders (affecting the nervous system), enterotoxaemias (affecting intestine and parenchymatous organs) and gas gaugrene (myonecrosis with toxaemia).

The large molecular weight (M. wt.) of Cl. botulinum toxins (types B and C), in part, are responsible for botulism in cattle than that of the medium, or small M. wt. molecules, due to the more stability of large molecules of the toxins **(Kozaki and Notermans, 1980)**. The mouse protection test bioassay is used for the tentative diagnosis of botulism toxins, where the injection of mouse by the maize extract killed the non protected mouse by the specific antitoxin **(Gray and Bulgin, 1982 and Kelch et al., 2000)**. The ELISA technique specific for type-C toxin gives positive result with the extract of rumen content, weakly positive to liver extract and negative with milk sample **(Galey et al., 2000)**. Botulism-D in cattle induced neutrophilic leucocytosis (all affected animals), proteinurea (most animals) and low serum inorganic phosphorus (some animals) **(Abbitt et al., 1984)**.

The aim the current study is to put an acceptable program of investigations, treatments and control of botulism outbreak in cattle farm according the available data (clinical and necropsy

signs, and circumstances of animal feeding and housing and laboratory results) to overcom such outbreaks and for easy (in the future) controlling and preventing such outbreaks in cattle farms.

MATERIAL AND METHODS

A) Material and Animals:

1- Animals: 5000 heads of Holestein cattle (3800 dairy cattle + 1200 heifer and calves for meat production) are reared in their particular yards of the farm.

2- Rations: total mixed ration: of concentrates and mineral mixture, and corn silage.

3- Bacteriological media and instruments for cultivation of aerobic and anaebrobic species as : 5% sheep blood agar, tween AT Albumin and listeria selective agar (for Listeria), sheep blood agar, trypose agar and brain heart infusion agar (for Pasteurella multocida), blood agar anaerobic culture and chocolate agar (for Clostridia).

4- The high performance liquid chromatography (HPLC) for measuring the mycotoxin (Aflatoxin-B1).

5- Atomic absorption for determination of elements (iron (Fe), cupper (Cu), Zinc (Zn), and Manganese (Mn)) in water.

6- Antibiotics (penicillin, streptomycin and oxytetracycline), antiallergic drugs (histakel - antihistaminic), anti-inflammatory drugs (cimadil - non-steroidal anti-inflammatory), supportive liquids (saline and dextrose) and vitamines (AD3E) and source of protein and phosphorus supplementation.

7- Clostridium Botulinum, antitoxins (types C and D).

B) Methods :

a- The dairy cattle, and heifer of 18 yards were fed with corn silage.

b- The animals of the other yards were fed on the total mixed ration (without silage).

c- Diseased cases were appeared in 6 yards three-days after feeding with the silage, where the clinical signs are appeared in 295 animals. 55 animals are died and the remaining animals receiving different types of medical treatments and supportive treatments before the diffinit diagnosis. After diagnosis of botulism, the treatments with the specific antitoxin (antiserum). The bivalent vaccines (toxoids, types C and D) was given to the remained healthy animals **(Lobato et al., 1999)**.

d- The clinical signs and the necropsy findings were recorded from the diseased and died animals respectively.

e- Laboratory investigations:

The corn silage, water, blood, liver and abomasal content were subjected to different types of investigations as follow: bacteriological examination for pathogenic bacteria (pasteurella, and listeria) **(Cruichshank et al., 1975)**, Mycotoxins (AFB1) in silage and diazinon and malathion organophosphorus compounds in all samples **(Association of Analytical Chemists, 1980)**, toxic elements (as cupper, manganese, iron, and Zinc) in the water according to **(American Public Health Association, 1971)**, anaerobes (Clostridium botulinum) in all samples **(Quinn et al., 1994)** and the mouse protection bioassay used for detection of toxins of Clostridium botulinum (C and D) **(Gray and Bulgin, 1982)**. The investigated samples from died animals were compared with that of normally slaughtered healthy animals that frequently slaughtered for meat production in the same farm.

f- Symptomatic treatments were tried by antibiotics, saline - dextrose solution, anti-inflammatory, antiallergic, vitamins (AD3E injection), protein and phosphorus supplementations. Cooling time was increased to 10 hours/day, and only two milkings instead of three times / 24 hours, all these trials of disease treatments were carried out before difinit diagnosis of the botulism.

RESULTS

A- Clinical Signs of Botulism in Cattle were : Off food , slow movements, weakness of hind limbs, recumbancy after 8 hrs from starting the clinical signs, abdominal respiration, normal body temperature and pulse rates, constipation, marked decrease of milk yield, dullness, death (55 died from 295 total diseased animals), all these symptoms appeared 3-5 days post silage feeding.

B- Post mortem (Necropsy) findings: Gelatinous texture of the body fats, congestion of kidneys and heart with petechial haemorrhages (especially in heart), congestion of abomasum, liver, intestine and lungs, and enlargement of the gall bladders.

C- Laboratory examinations: The results of the bacteriological investigations showed the presence of Cl. botulinum (type-D) which detected in corn silage and abomasals content. The results of mouse protection bioassay is the presence of Cl.botulinum neurotoxin (type-D).

D- The other different examined parameters (other pathogenic bacteria, Aflatoxin-B1, organophosphorus compounds, toxic elements) are negative or within permicible limits. The results are tabulated in table (1).

E- Results of treatments of Botulism in Ccattle:

1- Treatment with antiserum:

a- Clostridium botulinum (types C and D) horse-antitoxins for treatment of cattle which were suffering from botulism, as cattle are susceptible to the C and D clostridium toxins. The antiserum was prepared and supplied by the Veterinary Research Institute (Ondersteopoort, 0110, South Africa).

b- The antitoxin-D (5ml) was mixed with the antitoxin-C (5ml) and given intravenously by single dose of 10ml (types C and D) in the early stage of Botulism which was usually adequate dose, a 2nd injection (within 18 hours) may be needed if clear clinical improvement is not apparent. Further treatment with antitoxin after the 2nd antitoxin treatment should not be taken under any condition.

c- Antiallergic drug (Histakil - antihistaminic) and the cimadil (non-stroidal - anti-inflammatory) drug were administered parallel with treatment with the antitoxin (antiserum).

d- All cattle suffered from botulism (in its early stage) and treated with antitoxins (C, D) showed clear improvement, but the treatment with the antitoxin was non-effective in cases of the late stage of the disease.

e- Supportive (saline-dextrose) liquid therapy, vitamines AD3E, mineral mixtures (especially phosphorus) and protein rich feed were also supplied paralled with the antiserum.

2- Vaccination with toxoids (Botulism vaccines):

a- Formalinized aluminum hydroxide gel adsorbed bivalent toxoids of Cl. botulinum (types C and D) used for immunization of cattle against botulism, the vaccine was prepared and supplied by Onderstepoort Biological Products (Onderstepoort, 0110, South Africa).

b- All the healthy cattle were vaccinated with the botulism vaccine by sterilized syringes without any disinfectant, and with a separate needle for each animal, the dose is 2ml (s/c)/ animal. The vaccine will protect the animals against the botulinum toxin.

c- The vaccination with the toxoids were carried out twicely (4-7 weeks apart), then after, the vaccination of all herd will carried out (only one injection annually). All vaccinated animals

may fed the contaminated silage with botulinum toxins without appearance of any clinical signs of botulism.

DISCUSSION

The current study revealed that the diseased cattle in the farm showed the following clinical signs: off food, decreased movement and activity, abdominal respiration, constipation, marked decrease of milk yield, normal temperature and high pulse rate, recumbency (6-8 hrs from starting the signs) and death (2-4 days from starting the signs). Such clinical signs of botulism were recorded by **Gray and Bulgin (1982), Abbitt et al. (1984), Kelch et al. (2000), Bohnel et al. (2001), Wenzel et al. (2005) and Braun et al. (2005).**

The necropsy findings of died cattle of the present study were congestions of the internal organs and petechial haemorrhages on the organs especially on heart, such necropsy finding could be recorded by **Gray and Bulgin (1982), Abbitt et al. (1984),** and **Bohnel et al. (2001).**

The mortality rate in diseased cattle with botulism was 18.6% (55 died animals from 295 total diseased ones). A higher mortality rates could be reported by other authors, with various percentages may attributed to the different factors and circumstances, it may be 62.7% with toxin-D **(Abbitt et al., 1984),** 77.8% despite treatments **(Gray and Bulgin, 1982),** 30.8% with toxin-D **(Herdt et al., 1991)** and 96.8% with type-C toxin **(Galey et al., 2000).**

The current investigation revealed presence of Cl. botulinum toxin (type D) in the corn silage feed, blood, liver and abomusum content of diseased cattle. Similar study on botulism of cattle due to feeding on infected grass-silage could be reported by **Notermans et al. (1981).** Cattle are usually affected by types D and C toxins. Outbreaks of botulism in cattle are most likely to occur during drought periods when feed is sparse, phosphorus intake is low and carrion is plentiful **(Radostits et al., 2000),** so that the antitoxins (antiserum) of types D and C were injected to diseased cattle in the early stage of botulism due to the susceptibility of cattle to these two types of toxins and as a precautional program, also the vaccination of healthy cattle was achieved in the present study by the C and D toxoids (vaccines) against both the botulinum toxins (C and D), two vaccinations (21 days apart), then one vaccination annually. The antibody levels must be checked two times yearly. The antibody levels against botulisnum toxins were determined using the Serum Neutralization Test in mice. The monovalent vaccine induced higher antibody levels than the bivalent vaccine. Vaccination with type-D induced higher antibody levels than that of the type-C toxoid **(Lobato et al., 1999),** but the vaccination with bivalent toxoids (C and D types) should be practiced in enzootic areas **(Radostits et al., 2000).**

The supportive treatment by sufficient quantities of (saline-dextrose), vitamins (AD3E) and phosphorus, and protein rich feed, then the anti-inflammatory and the antiallergic drugs are the medications used for treatment of diseased cattle, along with the specific antidote (antitoxin) as followed in the current study. The correction of dietary deficiencies by supplementation with phosphorus and protein should implemented if conditions permit. Some local reactions are encountered after giving toxoid or antitoxin, but they are seldom serious, so the antiallergic and anti-inflammatory drugs should be administered along with the antitoxin therapy **(Radostits et al., 2000)**.

Based on the current work, it could be concluded that the corn silage may be the source for botulism in Holestein cattle, and this fact should put in consideration in such outbreaks. Antitoxin (antiserum) types C and D of Cl. botulinum should be administered in the early stage of botulism outbreaks. Annual vaccination of the cattle with bivalent vaccines (of types C and D) should be one of the vaccination program in cattle farms with intermittent checking for antibody levels against the Cl.botulinum polypeptide neurotoxins. Also, periodical examination of silage, hay and other feeding stuffs and water for botulinum toxins and other toxins should be carried out, the animal feeding stuffs should be in balanced manner and rich with phosphorus and protein to make the animals partially non-susceptible to botulism.

Table (1): The results of laboratory examinations of water, silage, blood, abomasal content and liver.

Examined materials	Bacteriological examination	Aflatoxin B_1 (AFB_1)	Organophosphrus cpds (Diazinon and Malathion)	Toxic Elements (Cupper, Iron, Manganese, Zinc)	*Cl. botulinum*	*Cl. botulinum toxins*
Water	N.P.	N.D	-ve	Cu=0.33 ± 0.063 ppm Fe=0.228 ± 0.03ppm Mn=0.12 ± 0.018ppm Zn= 0.14 ± 0.031ppm	-ve	-ve
Corn silage	Anaerobes *(Cl.botulinum)*	8 ± 0.63 PPb	-ve	N.D	+ve (type-D)	+ve (type-D)
Blood	N.D.	N.D	-ve	N.D	-ve	+ve (type-D)
Abomasal content	Anaerobes *(Cl.botulinum)*	N.D	-ve	N.D	+ve (type-D)	+ve (type-D)
Liver	Anaerobes *(Cl.botulinum)*	N.D	-ve	N.D	+ve	+ve (type-D)

1- N.P. = non pathogenic bacteria, 2- -ve = not present (negative result),
3- + ve = present (positive result), 4- N.D. = not done, 5- permicible value of AFB_1 in feed = > 20 PPb

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الملخص العربى

التسمم الغذائى فى البقر الهولستين المصاحب لسم الكولستريديوم بوتيولينم - نوع د

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مرض التسمم الغذائى (البوتيوليزم) مرض سريع وقاتل متسبب عن سم ناتج من البكتريا اللاهوائية (الكولستريديوم بوتيولينم) وتم وضع برنامج مقترح من الفحوص والعلاجات والتحصينات للحيوانات المصابة والغير مصابة بالبوتيوليزم، الذى ظهر فى أحد المزارع الخاصة المكونة من خمسة آلاف رأس من البقر الحلوب والعجلات الإناث وعجول التسمين المرباة فى أحواش التربية الخاصة بكل منهم، وتم تغذية حيوانات المزرعة على مخلوط المواد الغذائية والمكون من المركزات مع مخلوط الأملاح المعدنية، وتم إعطاء سيلاج الذرة فى الفترة الأخيرة لحيوانات ثمانية عشرة من أحواش التربية، حيث بعد ٣-٥ أيام من إعطاء السيلاج ظهرت حالات مرضية فى ٢٩٥ حيوان من ستة أحواش تربية، وقد نفق أو ذبح منهم ٥٥ بقرة وعجلة، وكانت الأعراض الأساسية للمرض عبارة عن : قلة الحيوية والحركة وقلة الإقبال على الغذاء وحدوث رقود ثم اتلنفوق مع إحتقان حشوى عام وأنزفة نقطية فى الأعضاء الداخلية للحيوانات النافقة، وقد تم أخذ عينات من ماء الشرب، والسيلاج والدم ومحتويات المعدة الرابعة والكبد، وقد تم الفحص البكتريولوچى للاهوائيات والباستيريللا والليستيريا لجميع العينات وفحص السموم الفطرية (AFB_1) وفى الدم وسموم المركبات الفسفورية العضوية (المالاثيون والديازينون) لجميع العينات، والعناصر السامة (النحاس والزنك والحديد والمنجنيز) فى الدم، وسموم الميكروب اللاهوائى كولستيريديوم بوتيولينم لجميع العينات. أوضحت نتائج الفحوص أنه تم عزل الميكروب اللاهوائى الكولستريديوم بوتيولينم (النوع D) من سيلاج الذرة ومن المعدة الرابعة والكبد وكذلك تم الكشف عن سم هذا الميكروب (نوع D) بهذه العينات أيضاً وفى المصل.

وتم إعطاء علاج المصل المضاد للبوتيولينم (نوعى C,D) مع إعطاء علاجات سوائل مدعمة وفوسفور وبروتين ومضادات للحساسية والالتهاب، وتم تحصين باقى القطيع السليم بطعم ثنائى ضد نوعى سم الكولستريديوم بوتيولينم (C,D).

وبناء على هذه الدراسة أمكن إستنتاج أن سيلاج الذرة يمكن أن يكون مصدر التسمم الغذائى بالبوتيوليزم فى البقر، وأن إعطاء المصل المضاد لسم ميكروب (الكولستريديوم بوتيوليزم C,D) فعال فى المراحل الأولى للمرض، وأن إعطاء طعم واقى (toxoid) ضد نوعى السم الميكروبى (Bivalent vaccine, C and D) يجب أن يكون أحد برامج التحصين بمزارع الأبقار.