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RESEARCH ARTICLE

Determination of *Clostridium botulinum* Toxins in Dairy Cows with Abomasal Displacement

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Summary

A new form of bovine *Clostridium botulinum* toxicity was described as visceral botulism. Long-lasting absorption of very low quantities of botulinum toxin may interfere with neurological control of intestinal physiology instead of acute or subacute lethal effects. The objective of this study was to investigate the presence of *C. botulinum* toxins and their types in abomasal content and serum of dairy cows diagnosed with abomasal displacement. Abomasum displacement was confirmed in 17 cows by laparoscopic examination and abomasal content and serum samples were collected during surgical correction. The mouse toxicity and neutralization bioassay was used to detect and identify the *C. botulinum* toxins in samples. *Clostridium botulinum* toxin type C was detected in 2 of abomasums contents and in 1 of sera. The toxin detected animals were fed with silage. Although the identification ratio (17.6%) of *C. botulinum* type C toxin in this study seems so farther to correlate AD with C. botulinum toxins; we are of the opinion that *C. botulinum* toxins, especially type C, may be associated with AD because AD is considered to be a multifactorial disease.

Keywords: *Cow, Abomasal displacement, Clostridium botulinum, Toxin*

Abomasum Deplasmanlı İneklerde *Clostridium botulinum* Toksinlerinin Araştırılması

Özet

Clostridium botulinum toksikasyonunun yeni bir formu 'visceral botulizm' olarak tanımlanmaktadır. Botulinum toksininin uzun sürede ve çok düşük miktarlarda emilmesi, akut ya da sub-akut ölümcül patolojiler yerine, intestinal organların nörolojik kontrolünde bozukluklara neden olmaktadır. Sunulan çalışma, abomasum deplasmanı tanısı konmuş ineklerin serum ve abomasum içeriğinde *C. botulinum* toksinlerinin varlığını ve olası tiplerini belirlemek amacıyla yapıldı. Abomasum deplasmanı tanısı laparaskopik olarak doğrulanmış 17 inekten, cerrahi tedavi sırasında abomasum içeriği ve serum örnekleri toplandı. Örneklerde *C. botulinum* toksinlerinin varlığı ve tiplendirilmesi, fare toksisite ve nötralizasyon testleri ile yapıldı. Abomasum içeriği örneklerinden 2'sinde ve serum örneklerinden 1'inde C tipi *C. botulinum* toksini tespit edildi. Toksin belirlenen ineklerin silaj ile beslendiği belirlendi. C tipi toksin varlığının %17.6 oranında tespit edilmiş olması, abomasum deplasmanı gelişiminde C tipi *C. botulinum* toksininin etkili olduğu şeklinde yorumlanamaz. Ancak abomasum deplasmanı'nın multifakröriyel etiyolojili bir hastalık olduğu dikkate alınırsa bu toksinlerin olası varlığı göz ardı edilmemelidir.

Anahtar sözcükler: *İnek, Abomasum deplasmanı, Clostridium botulinum, Toksin*

INTRODUCTION

Abomasal displacement (AD) in dairy cattle has recently become more common. The AD is characterized by the abomasum filled with gas floating in the dorsal part of the abdomen. Abomasal atony is considered to be the primary dysfunction in this condition [1-3]. When motility of the abomasum is inadequate, gas accumulation may occur [2,4,5]. The vagal nerve plays a predominant role in abomasal motility [1,2]. Major risk

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factors were reviewed as feed intake, negative energy balance and calcium related effects on the abomasal functioning, with respect to motility and production of gas [2,4,6]. Geishauser et al.[1] have investigated *in vitro* abomasal motility in dairy cows diagnosed with displaced abomasum and suggested that AD is associated with malfunctions at the level of the intrinsic nervous system combined with impaired cholinergic muscle responses.

Botulism is caused by the neurotoxins (botulinum neurotoxin; BoNT) produced by the anaerobic bacteria, *Clostridium botulinum* [7]. These toxins are named A to G according to the corresponding types of *C. botulinum.* Each of these toxins affects different species of animals and is usually found in different environments. Cattle appear to be most susceptible to toxin type B, C, and D. The most common way of botulism intoxication in cattle is through the ingestion of toxin contaminated feed sources. After absorption in the intestines, BoNT is carried by the bloodstream to the neural synapses. This toxin then affects the nerve endings at the neuromuscular junction preventing the release of acetylcholine. Death is usually due to paralysis of the muscles of the diaphragm leading to respiratory arrest [7,8].

A new form of bovine *C. botulinum* toxication was described as visceral botulism by Böhnel et al.[9] and Schwagerick [10]. Böhnel et al.[9] have stated that long-lasting absorption of very low quantities of botulinum toxin may interfere with neurological control of intestinal physiology instead of acute or subacute lethal effects. Schwagerick [10] has reported an outbreak of a previously unknown disease of dairy cows observed in 3 regions of Mecklenburg (Germany). The common recorded signs were ataxia, somnolence, and paralysis of the smooth and skeletal musculature, non-infectious chronic laminitis, digestive disturbances and displacement of the abomasum. The presence of *C. botulinum* and its toxins in the intestinal flora of these animals has been confirmed by laboratory tests.

The specific cause(s) of AD has not been elucidated. Although the different fields of research have positive contributions to the understanding of the pathogenesis of AD, contradictions in different studies are present. Data are lacking with regard to the impact of *C. botulinum* toxins on the development of abomasal hypomotility and the incidence of AD. Therefore, we conducted a study to investigate the presence of *C. botulinum* toxins in the abomasum contents and sera of the dairy cows diagnosed with AD.

MATERIAL and METHODS

Animals

The study was carried out on 17 dairy cows with AD, surgically treated at animal hospital in Faculty of Veterinary Medicine, Burdur, Turkey. Of 17 cows, 8 had right displaced abomasums and 9 had left displaced abomasums. The cows were of Black and White Holstein Friesian breed and 2-8-year old. The cows were fed with hay and commercial pelleted feed. Some of cows were fed with maize silage or white beet silage. All cows were from the farms around Burdur. The cows were examined clinically and the abdomen was examined for the presence of auscultable “pings” at right or left flank. The cows which were positive for typical “pings” sounds were prepared for aseptic laparoscopic examination to confirm the diagnosis of AD. Laparoscopic examination was carried out with a 10 mm 0º laparoscope (Karl Storz, Tutlingen, Germany) and mobile videoendoscopic laparoscopy unit (Lemke Vision GmbH, Ludwigsstadt, Germany) as described previously [11]. Confirmed left abomasal displacement (LAD) cases were treated by laparoscopic abomasopexy technique [12]. Right abomasal displacement cases were surgically treated by right paralumbar laparotomy and subsequent abomaso-pexy. Data were collected at the start of the operation and the records of cows -in relation to age, type of AD, duration of AD and fed with silage- were listed in *Table 1*.

Sampling

For detection of BoNT, jugular blood samples (20 ml, before starting surgery) and abomasal contents (20 ml, during surgery) were collected from all the surgically treated cows. The abomasal content was aspirated by a silicone tube (length 100 cm, diameter 4 mm) which was inserted into the insufflation cannula during deflation of the abomasum in LAD. In RAD cases, the abomasal content was aspirated from the abomasum by inserting a large-gauge needle attached to a silicone tube. All specimens were refrigerated and examined as quickly as possible after collection.

Mouse toxicity and neutralization bioassay

The mouse toxicity and neutralization bioassay [13] was used to detect and identify the BoNT in sera and abomasal contents of cows with AD. The experimental protocol was approved by the Animal Use Committee of Pendik Veterinary Control and Research Institute (acceptance number: 01208). The abomasal contents

were mixed with equal volumes of phosphate buffer, pH 6.2, and held overnight at 5°C; after centrifugation, 0.5 ml of the supernatants was injected intraperitoneally into two 4-week-old white mice to detect the presence of any botulinum toxin. One part of the supernatant was heated to 80°C for 20 min. and injected intraperitoneally into two mice, as a negative control. Serum samples (0.5 ml) were directly injected into two 4-week-old white mice.

When mice died within 96 h of inoculation, neutralization test was carried out by adding to separate samples of supernatants and sera of the polyvalent antitoxin against type A through F toxins and monovalent antitoxins against type B, C and D toxins (10 IU/ml; Pendik Veterinary Control and Research Institute, Turkey). The samples to be examined were diluted 5:1 with antitoxins and incubated for 30 min at 37°C. Then two mice were injected with 0.5 ml of each antitoxin-sample mixture. Survival of the group injected with one of the neutralized samples and the heated supernatant established the final identification of the toxin.

RESULTS

Seventeen cows with AD were operated, 8 (47%) with right and 9 (53%) with left *(Table 1)*. No clinical symptoms of botulism or death were observed among the cows. In mouse toxicity bioassay, toxin was only detected in 2 (cows 1 and 12) of 17 (11.8%) abomasums contents and in 1 (cow 8) of 17 (5.9%) sera taken from cows. Toxins were detected in samples from cows feeding with silage. The neutralization bioassay was performed for toxin identification. *C. botulinum* type C toxin was identified both in the abomasum content of cows 1 and 12 and in the serum of cow 8. In the remaining 31 samples, *C. botulinum* toxins were not detected via the bioassays *(Table 1)*.

DISCUSSION

Although abomasal atony and gas production contribute to development of AD, the etiology and pathogenesis of abomasal displacement are unclear. In this study, we investigated whether BoNT was associated with development of AD among dairy cows, or not. BoNT was only detected in 2 of abomasums contents and in 1 of sera obtained from different animals. The toxin detected in the abomasums contents and serum was identified as *C. botulinum* type C. The identification ratio (17.6%) of *C. botulinum* type C toxin in this study seems so farther to correlate AD with *C. botulinum* toxins. However, the presence of *C. botulinum* toxins, especially type C, may be associated with AD with regard to Schwagerick [10] who concluded that *C. botulinum* toxins lead to AD in cows.

It has been stated that the use of animal models in understanding the pathogenesis of anaerobic infections is necessary [14]. Because *in vitro* methods for BoNT detection are under development and they are not validated, it has been stated that the only currently acceptable method for detection and identification of

Table 1. *Data and results of the mouse toxicity and neutralization bioassays from the cows diagnosed with abomasal displacement*
Tablo 1. *Abomasum deplasmanı tanısı konulan ineklere ait bilgiler ile fare toksisite ve nötralizasyon biyoanaliz sonuçları*

	Age (years)	Type of AD	Duration of AD (days)	Botulinum Toxins		Feeding with silage	
				Abomasums contents	Sera	Maize	White beet
1	4	RAD	9	Type C	-	-	+
2	2	LAD	10	-	-	-	
3	3	RAD	12	-	-	-	-
4	3	RAD	30	-	-	-	-
5	7	RAD	2	-	-	-	+
6	2.5	RAD	25	-	-	-	-
7	3.5	RAD	4	-	-	-	-
8	5	LAD	20	-	Type C	+	-
9	5	LAD	10	-	-	-	-
10	3	RAD	1	-	-	+	-
11	2	LAD	23	-	-	-	-
12	2.5	LAD	10	Type C	-	+	-
13	3.5	LAD	14	-	-	-	+
14	2.5	LAD	15	-	-	+	-
15	2.5	RAD	15	-	-	-	-
16	6	LAD	10	-	-	+	+
17	4	LAD	19	-	-	-	-

RAD: Right abomasal displacement, LAD: Left abomasal displacement

BoNT is the mouse toxicity and neutralization bioassay [13,15]. Suitable materials for examination for BoNT include serum, feces, gastric contents and suspected foods [13]. Therefore, in this study, mouse bioassay was used because the test is standard international method for detecting of the *C. botulinum* toxin in serum and abomasal or ruminal contents. There was no BoNT in sera and abomasum contents of 14 cows with AD. A negative mouse inoculation test does not exclude the presence of BoNT, because the toxin may be present at level below the threshold of detection, especially as BoNT can be rapidly biodegraded by bacteria in the rumen [8,16,17]. In addition, the fact that no circulating toxin was detected in the sera of the cows reflects that the cows in this investigation were not exposed to large amounts of toxin. Nevertheless, since no determination of the amount of circulating toxin was done, it is unclear whether this reflects a lower ingestion of toxin in cattle or a faster passage of the toxin to nerve endings. Likewise it has been reported that, since such small levels of toxin are present in the bloodstream, serum and blood samples often fail to identify toxin if present [15]. Therefore we thought that serum samples from cows for detecting of the BoNT seem very unlikely to be of diagnostic value.

Spores of the organism are commonly found in all soils and consequently will be found on most plant material [7,15]. Therefore silage can act as an ideal media source for *C. botulinum* to grow in and produce the toxin [6,17,18]. It has been reported that AD and other disorders of the abomasum were more frequent in herds that are fed a large proportion of maize silage [6,17,19]. In the presented study, 9 (5 with maize and 4 with white beet silage) of 17 cows were feeding with silage, and toxins were detected in samples from cows feeding with silage.

From the present work it can be concluded that the BoNT may influence the occurrence of AD as a possible predisposing cause. But it needs further research to determine the influence in cows fed with a control diet including different *C. botulinum* toxins, and also effects on the production of gas in the abomasum and on the contractility of the abomasal wall.

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