Original Research Article

Serological Investigation of a Botulism Outbreak in Cattle in Bolu, Turkey

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ABSTRACT

Botulism is a disease caused by toxins, which produced by Clostridium botulinum. The aim of the study is to asses results of serologic (ELISA antibody) evaluation in clinically normal, randomize selected cattle living where outbreak area and selected clinically normal cattle from Bursa, different area. All of the blood samples from outbreak area (n:60) and control blood samples (n:10) were tested in two different laboratory in Germany for anti-BoNT antibodies using antibody ELISA which used purified A, B, C and D BoNT antigens and separately ruminal fluids, sera samples from 2 animals suffering from suspected Botulism were analyzed in Istanbul. The diseases were determined in four different villages. Hundred sixty six cattle were died during 2.5 year. In outbreak area, clinical findings included a paralyzed tongue, decreased rumen contractions, decreased muscle tones, incoordination, lateral recumbency, progressing to weakness of extremities and progressively paralysis was back to front. In the study, BoNT/C and BoNT/D were predominant serotype. In some cattle, we found positive in same animal both of BoNT/C and BoNT/D. It may be related with BoNT/C-D mosaic gene serotype. The outbreak illustrates that botulism is increasingly encountered disease in our country and will be important issue in future. Therefore, we should develop control strategies and further comprehensive studies are needed.

Keywords: Botulism, Cattle, Toxin

INTRODUCTION

Botulism is a disease caused by toxins, which are produced by Clostridium botulinum. Cl.botulinum is an anaerobic organism with 8 serotypes (A, B, Ca, Cb, D, E, F and G). (Radostits et al. 1989, Smith 1990). Serotypes B, C and D cause disease in cows. Animal carcasses contain serotype C (such as poultry waste) and D, but not A or B. (Neill et al., 1989). The toxins may remain in such carcasses for more than one year. Cl.botulinum spores are resistant to environmental conditions; thus, they could live for many years (30 years) (Smith 1990).

The sources of infection are rotting vegetables, meat and fish. The agent may also be found in animal and bird intestines. In animals, botulism has been diagnosed throughout the world, primarily in ruminants, horses and many bird species. (Böhnel 1999).

The course of the disease depends on the amount of ingested toxin. Initial symptoms may be slight or overt in ruminants and are typically observed between 1 and 14 days after ingesting the toxin. Clinical signs typically include stiff walking, muscle weakness, miscoordination, lateral recumbency, paddling movements, progressive dyspnea and muscle paralysis. Animals may be found dead or die within a few hours due to respiratory failure (Smith 1990, Radostits et al. 1989).

Botulism cannot be definitively diagnosed through clinical findings; a laboratory confirmation is essential for diagnosis. A botulism diagnosis requires an ELISA (toxin and antibody), additional immunological tests, toxin detection through a mouse bioassay and molecular techniques, such as PCR (Böhnel et al. 2001, Thomas 1991).

The aim for this study was to assess serological results (ELISA antibody) to evaluate clinically normal, randomly selected cattle living in an outbreak area and clinically normal cattle from Bursa, which is a different area.

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MATERIALS AND METHODS

Göynük (40°23′59″N 30°47′07″E) is a town and district in Bolu Province in the Black Sea region of Turkey. The Göynük economy and that of another district in Bolu depend on broiler breeding, which involves contract manufacturing or large enterprise. Further, many agricultural areas and stream beds include randomly scattered poultry litter and carcasses. In 2010-2012, a disease outbreak in cattle began in Bolu, Göynük, Turkey. Similar reports were also received from a neighbouring city during the same period. Four villages were involved, and the number of animals with clinical signs was 217; certain affected animals had died or had an unknown status. The number of affected animals and villages is shown in Table 1.

Table 1. Mean ELISA OD value for the studied animals.

BoNT/serotype	Base line $\overline{\times}$ (n=22)	Positive animals $\overline{\times}$ (n=7)	Negative animals $\overline{\times}$ (n=25)	Control animals $\overline{\times}$ (n=10)
BoNT/A	0,08 ± 0,01	0,15 ± 0,03	0,05 ± 0,006	0,05 ± 0,01
BoNT/B	0,15 ± 0,02	0,27 ± 0,06*	0,10 ± 0,02	0,15 ± 0,05
BoNT/C	0,20 ± 0,04	0,42 ± 0,07*	0,09 ± 0,01	0,09 ± 0,02
BoNT/D	0,18 ± 0,03	0,34 ± 0,06*	0,092 ± 0,009	0,06 ± 0,01

*Define statistically importance (p<0.005)

Clinical findings for animals observed by a veterinarian and local authorities were recorded. Based on previously noted clinical findings, Botulism was suspected. We randomly selected animals from the outbreak area. Then, blood samples were the collected through jugular venipuncture with (2 ml) (Hema&Tube®, Turkey) plain vacuum tubes for serologic tests. Until the test could be performed, the samples were stored at -20 C. Samples from animal feed, drinking water, liver, sera samples, and intestinal and ruminal fluids from 2 animals that likely had Botulism were sent to the Pendik Control and Research Institute, Anaerob Diagnostic Laboratory, Istanbul. The blood samples were tested in Miprolab and Leipzig University in Germany for anti-BoNT antibodies.

The data were analysed using the Mann-Whitney U test for the control group and the outbreak area. The statistical analyses were performed using Sigma Stat 3.1 for Windows statistical package (Systat Software, Inc., Point Richmond, CA, USA).

RESULTS

Samples from four different villages were tested for the disease (Orencik 24, Cubuk 7, Degirmenozu 2, Karacalar 4). One-hundred and sixty-six cattle died within 2,5 years.

For the outbreak area, the clinical findings included paralysed tongues, decreased rumen contractions, decreased muscle tone, miscoordination, lateral recumbency, progressive weakness in the extremities and progressive paralysis from the back to front.

The box plot and mean values for the BoNT types analysed by Mipro Lab are shown in Figure $1_{a, b, c}$ and Table 1 and 2. Seven animals from the outbreak area were positive (31,8%). The ratio of animals that were positive for BoNT to the total number of animals in outbreak area were *BoNT/A-0,5%, BoNT/B-9,09%, BoNT/C-31,8,* and *BoNT/D-22.7,* respectively. However, the control group did not include any positive animals. There was no statistical significance between the outbreak and control groups.
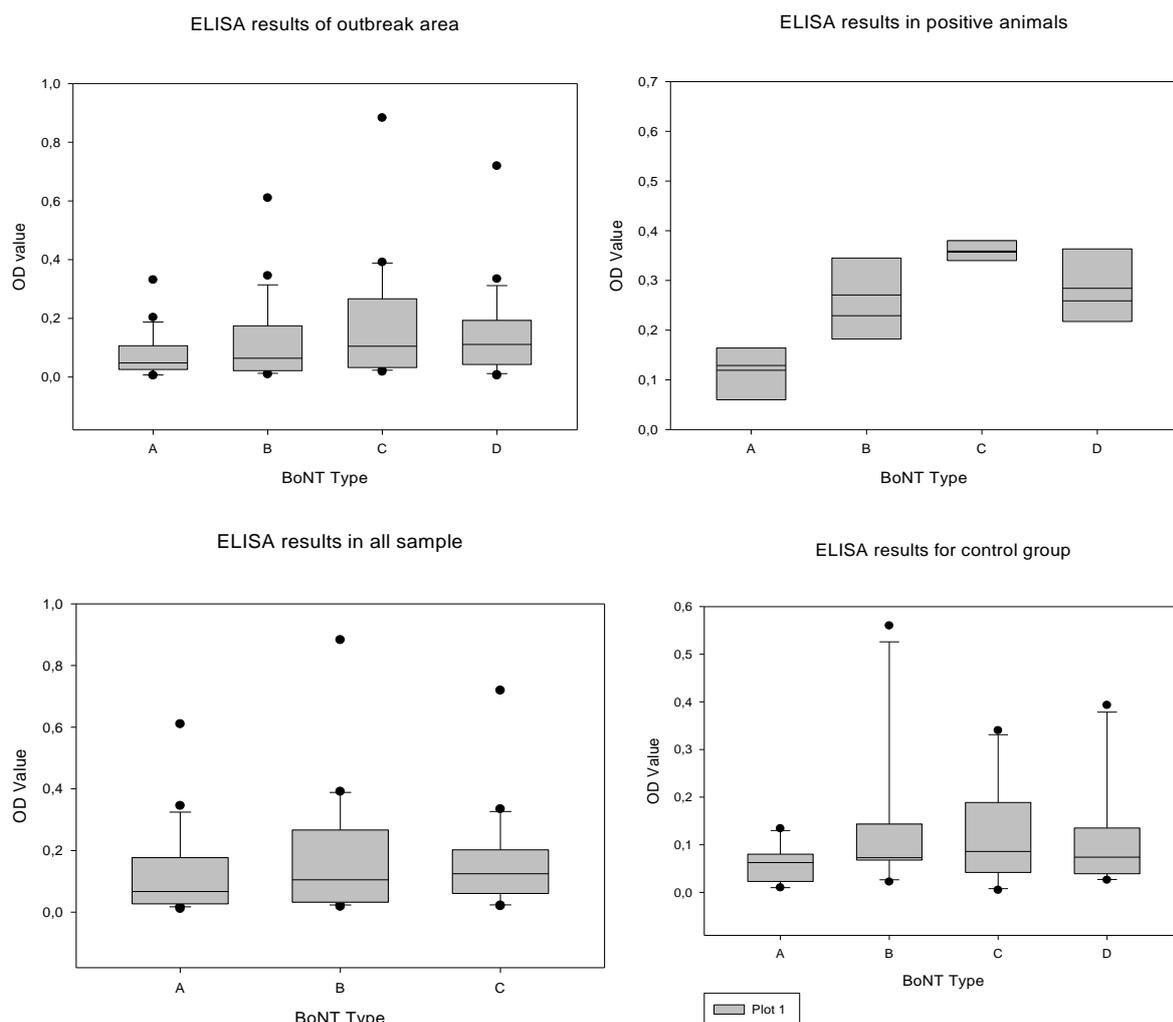
ELISA results of outbreak area

ELISA results in positive animals

ELISA results in all sample

ELISA results for control group

Figure 1. a, b, c ELISA results in the outbreak area and for the control group.

For the animals that were positive for *BoNT/C*, one animal was positive for both *BoNT/B* and *BoNT/C*, four animals were positive both *BoNT/C* and *BoNT/D*, one animal was positive for *BoNT/A*, and one animal was positive for each type (Table 1) using the Miprolab investigation.

We detected 6 positive results among 38 different samples. Three were seropositive for the C/D type; the ELISA were 150, 315, and 1368%, respectively. Three were seropositive for the A/B/E type; the ELISA results were 217, 140, and 102%, respectively, using the Leipzig University investigation.

Table 2. ELISA OD value for the positive animals from the outbreak area using Miprolab's investigation.

Serotype	Cl.Botilinum type A	Cl.Botilinum type B	Cl.Botilinum type C	Cl.Botilinum type D
Positive 1	0,068	0,068	+ 0,251	0,247
Positive 2	0,134	0,220	+ 0,340	+ 0,393
Positive 3	0,151	0,182	+ 0,382	+ 0,259
Positive 4	0,124	+ 0,345	+ 0,391	0,188
Positive 5	0,203	0,229	+0,374	+0,334
Positive 6	+ 0,331	++ 0,610	+++ 0,883	+++ 0,719
Positive 7	0,036	0,242	+ 0,340	+ 0,252

DISCUSSION

It is well known worldwide that many botulism outbreaks arise from poultry litter (Neill et al. 1989, Hogg et al. 1990, Payne et al. 2011). In the case examined here, the outbreak area contract manufacturers. There is also a river that is utilised by all of the animals and which is used to irrigate the fields in the outbreak area. Local authorities and the public indicate that many poultry carcasses have been observed in the riverside. In the outbreak area, we detected dogs that scavenge from poultry litter or dead hen pits. We have not detected toxin in food and water stored in this area, but the outbreak is most likely related to animals eating contaminated food from poultry litter or carcasses in the grazing area.

Clostridium botulinum strains have been detected worldwide. However, animal carcasses have been shown to cause BoNT/C and BoNT/D botulism in livestock (Galey et. al. 2000, Jean et al. 1995). Also, outbreaks of Botulisms have been reported in Turkey (Duru, 2014; Senturk, 2007). Recent studies have reported the BoNT gene and protein sequence for certain serotypes. For the types BoNT/C and BoNT/D, certain strains have the C/D mosaic BoNT (Nakamura et al. 2010, Woudstra et al. 2012). In this study, types C and D were the predominant types in the endemic area. Five animals were also positive for both C and D. These observations may be related to the D/C or C/D mosaic neurotoxin produced by certain strains (Nakamura et al., 2010; Woudstra et al., 2012). We also detected both BoNT/A and BoNT/B in two animals. There are insufficient data regarding the mosaic neurotoxin gene for BoNT/A and BoNT/B or a potential cross-reaction among BoNTs, but further comprehensive studies are necessary (Dover et al. 2009, Franciosa et al. 2004).

In animals, subclinical or tentative clinical signs that are characteristic of botulism are an important issue for animals (Hunter et al. 1999, Böhnel 2001). Further, ingestion of the toxin by an animal may not yield a positive diagnosis through conventional diagnostic methods; it is difficult to detect positive results using a botulism mouse experiment. While ELISA is a good diagnostic tool, seroconversion may be problematic. In this study, the positive cattle did not show clinical signs. In contrast, the lack of clinical signs in these animals may be due to immunotolerance related to receiving small levels of toxin gradually.

In conclusion, botulism is a dangerous disease for animals and humans. Thus, authorities must take prophylactic measures. This disease should be controlled through extensive vaccination, especially for the C/D type. This study suggests that farms located near broiler industries are at high risk for botulism. Further investigation may identify ways to decrease risks associated with Botulism in cattle.

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