Kafkas Univ Vet Fak Derg
24 (6): 905-908, 2018
DOI: 10.9775/kvfd.2018.19952

Kafkas Universitesi Veteriner Fakultesi Dergisi
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Case Report

Enterotoxemia Caused by *Clostridium perfringens* Type E in a Calf

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Article Code: KVFD-2018-19952 Received: 16.04.2018 Accepted: 16.09.2018 Published Online: 17.09.2018

How to Cite This Article

Eroksuz Y, Otlu B, Calicioglu M, Eroksuz H, Akdeniz Incili C, Karabulut B, Abayli H: Enterotoxemia caused by *Clostridium perfringens* type E in a calf. *Kafkas Univ Vet Fak Derg,* 24 (6): 905-908, 2018. DOI: 10.9775/kvfd.2018.19952

Abstract

Clostridial enterotoxemia caused by *Clostridium perfringens* type E was diagnosed postmortem in a 2-months-old calf in a family farm containing 20 cattle at different ages. Varying degrees of severity of segmental fibrino-hemorrhagic and necrotic enteritis was present at the necropsy. *Clostridium perfringens* was isolated from the intestinal tissue and intestinal content and the genes encoding alpha and iota toxins were detected by polymerase chain reaction (PCR). Histopathological examination showed the presence of adherent Gram-positive rods on the surface of villi and in poly morphonuclear leucocytes in the lamina propria of the intestinal mucosa.Overall, the results of the present study suggest that *C. perfringens* type E should be considered at differential diagnosis in fibrino-hemorrhagic enteritis and sudden deaths in post weaned calves.

Keywords: *Bovine, Clostridium perfringens type E, Enterotoxemia*

Bir Buzağıda *Clostridium perfringens* Tip E Nedenli Enterotoksemi Olgusu

Öz

Farklı yaş gruplarındaki 20 sığırdan oluşan bir çiftliğe ait 2 aylık buzağıda, postmortem olarak *Clostridium perfringens* tip E ye bağlı enterotoksemi tespit edildi. Nekropside, değişen derecelerde fibrino-hemorajik ve nekrotik enteritis tespit edildi. *Clostridium perfringens,* bağırsak dokusundan ve içeriğinden izole edildi, alfa ve iota toksinlerini kodlayan genler polimeraz zincir reaksiyonu (PZR) ile tespit edildi. Histopatolojik incelemede, bağırsak villuslarında, adherent Gram pozitif rodlar ve polimorfnükleer lökositler görüldü. Sonuç olarak, *C. perfringens* tip E'ye bağlı tokseminin, sütten kesilmiş buzağılardaki ani ölümlerin ve fibrino-hemorajik enteritislerin ayırıcı tanısında düşünülmesi gereken bir hastalık olduğunu söylemek mümkündür.

Anahtar sözcükler: *Sığır, Clostridium perfringens tip E, Enterotoksemi*

INTRODUCTION

Clostridium perfringens (CP) is a non-motile and ubiquitously distributed Gram-positive microorganism [1]. It is normally present in animals and humans intestinal contents but sometimes causes infection and proves highyl pathogenic regarding intestinal diseases [2]. Most of the infections caused by CP biotypes are mostly encountered in herbivores and human. Clostridial diseases of the intestinal tract are often evaluated under an umbrella term so called enterotoxemia, characterized by the intestinal and histotoxic tissue injury arising from 4 major exotoxins including alpha, beta, epsilon and iota together with 13 minor toxins. Each of the major toxin types encoded by different genes causes specific disease syndromes. There are 5 strain types of CP as classified by letters A, B, C, D, and E. These strain types are differentiated based on the 4 major antigenic lethal exotoxins that they produce. Alpha toxin is a lecithinase that affects cell membranes, causes hemolysis or cell necrosis and is produced by all 5 strain types [1]. The structure of the beta toxin is unknown. This toxin is produced by B and C strains, and causes enteritis. It has necrotic effect, leading to paralysis in the intestine. Epsilon toxin is activated by enzymatic digestion, and

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exerts its effects especially in the brain and kidneys. The iota toxin which increases the permeability of the cell membrane is released as a prototoxin and activated by proteolytic enzymes. CP type E causes intestinal disease in calves, lambs and rabbits. Illnesses with type A and E develop in the same way, but types B and C mostly affect newborns and have a much simpler pathogenesis [1,3]. In all of the enterotoxemia cases, the bacteria rapidly multiply in the intestine and the disease develop depending on the bacterial exotoxin. CP Type E isolates secrete two of these toxins, alpha toxin, single polypeptide with phospholipase C, sphingomyelinase, hemolytic, and lethal properties and iota toxin, a binary toxin consisting of two non-covalently associated components. The latter toxin is an uncommon and unique toxin that produced by type E *C. perfringens* isolates [1,4]. The disease caused by type E isolates of *C. perfringens* are the most poorly understood of all *C. perfringens* enteritis. The role of the iota toxin in pathogenesis is unknown [4] and it has rarely been reported in calves. The aim of this report is to diagnostic workup confirming the case of CP type E infection in a 2 months old calf.

CASE HISTORY

Two months old, male, Black Swiss calf was referred to Veterinary Teaching Hospital with the clinical signs of depression, diarrhea, anorexia and high body temperature (42°C) for treatment. The calf died before the clinical examination and the necropsy was carried out within 1 h of death. The calf was from a family farm containing 20 cattle at different ages. There was no any clinical finding of illness for the rest of animals.

Gross Findings

The gross examination revealed varying degrees of severity of segmental fibrino-hemorrhagic and necrotic enteritis *(Fig. 1A)*. The most severely affected segment was the ileum and the jejunum in which there were intraluminal fibrin tangles, hemorrhagic exudate, and necrosis *(Fig. 1B)*. Mezenterial lymph nodes were swollen approximately 2 times of normal size and were congested in cut surfaces *(Fig. 1C)*. The visceral organs including liver and lungs were pale in color, whereas kidneys showed diffusely cortical and medullar congestion.

Histopathological Findings

Tissue samples of small and large intestines, liver, lungs, spleen, pancreas, brain, heart, pericardium and kidneys were collected and fixed by immersion in 10% neutral formalin. All tissues were processed routinely by histological techniques for the production of 4-5 µm sections and stained with hematoxylin-eosine (H&E). Selected tissue sections were stained with Brown-Brenn's method. Histopathologically, there was necrotizing hemorrhagic enteritis at various severity *(Fig. 1D)*. Gram-positive rods were attached on the surface of villi and in polymorphonuclear leucocytes in lamina propria of muscosa were also observed. Follicular lymphoid hyperplasia, histiocytic hyperplasia and interstitial hemorrhage were detected in mesenteric lymph nodes. In liver, there was multifocal, moderately dense periportal lymphohistiocytic infiltrations.

Bacterial Isolation, Identification and Determining Antimicrobial Susceptibility

A 25 g of the affected intestinal sample was pummeled

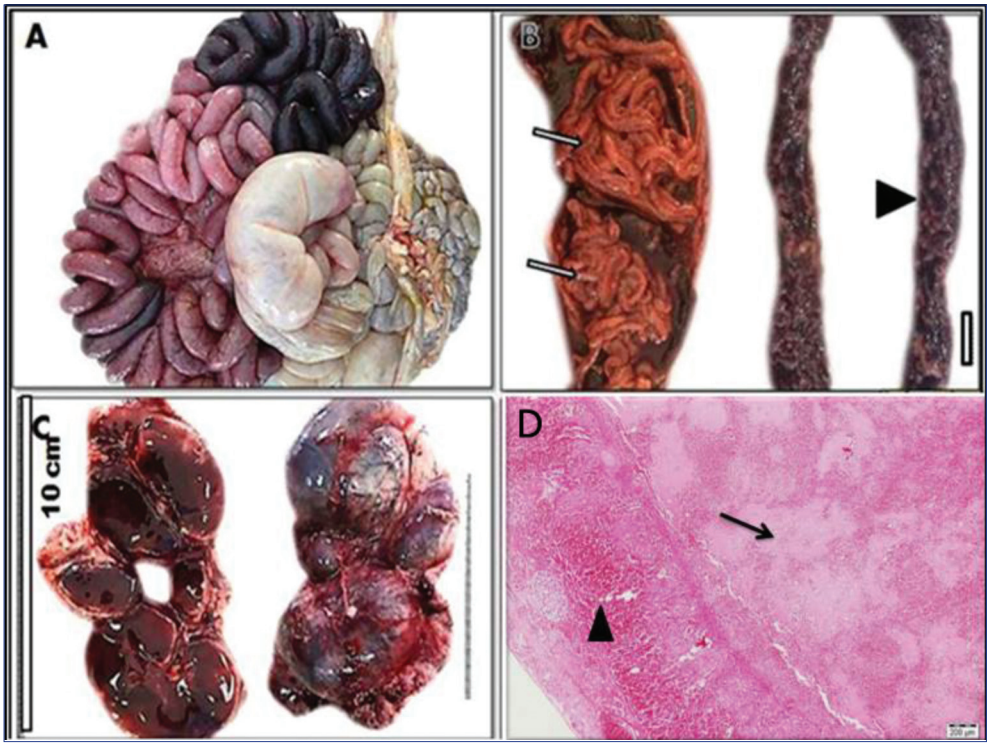

Fig 1. Gross and histopathological findings. A: Gross appearance of intestinal tract showing segmental, moderate to severe enteritis. B: Intraluminal fibrinous *(arrow)* and hemorrhagic exudation in cecum and necrotic changes *(arrow head)* in ileum. C: Cut and external surface of a cranial mesenteric lymph node having hyperemic and edematous appearance. D: Severe necrotic *(arrow)* and hemorrhagic enteritis *(arrow head)* with focal neutrophilic and few histiocytic infiltrates

within 225 mL of sterile peptone water for 2 min in a stomacher. Decimal dilutions were made from the resulting homogenate and pour-plated to Tryptose Sulphite Cyloserine (TSC) agar. The petri plates were incubated at 46±1°C for 20-24 h under anaerobic conditions (anaerobic jar with Anaerocult). Five colonies were selected at random among the typical black colored colonies and purified for identification [5].

The suspected isolates were in grown in Brucella blood agar and the Thioglycolate broth (enriched with 5% sheep blood, hemin (5 mg/L) and vit K1 (1 mg/L) (Becton Dickinson, Heidelberg, Germany) at 37°C for 72 h in a jar with an anaerobic gas-pack (Thermo Fisher Scientific, USA). The colonies were subjected to Matrix assisted laser desorption ionization-time of flight-mass spectrometry (MALDI-TOF MS) based VITEK MS (database v2.0) (Bio-Mérieux, France) system identification. Results showed that the isolates were identified as *C. perfringens* with a high score value (99.90%).

Antimicrobial susceptibility test was performed on Brucella agar by modified Kirby-Bauer disk diffusion method [5,6]. The isolate was susceptible to imipenem, vancomycin, tetracycline, florfenicol, teicoplanin, linezolid, chloramphenicol, cefepime, but resistant to penicillin, ampicillin, trimethoprim-sulfamethoxazole, streptomycin, levofloxacin, erythromycin and clindamycin.

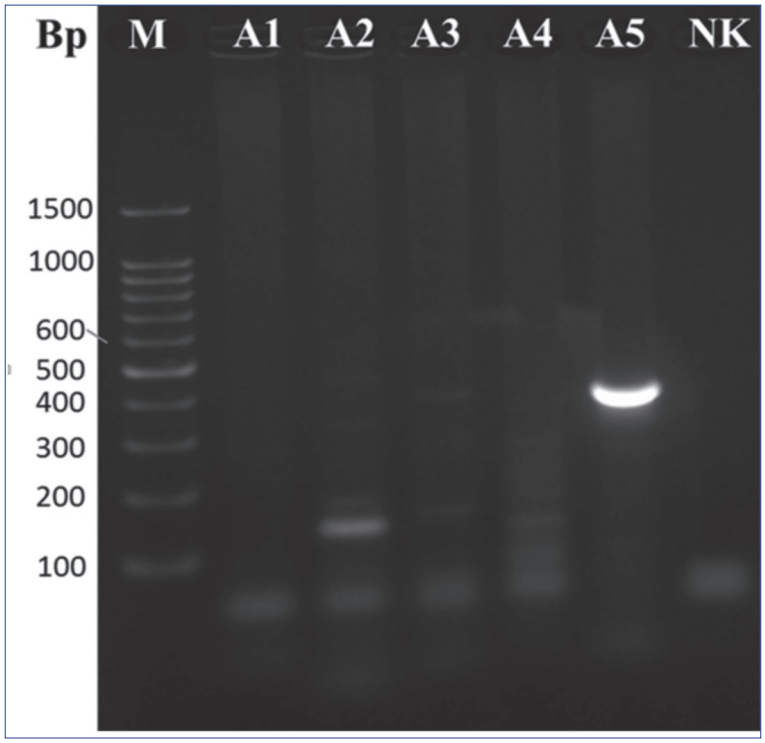

Fig 2. PCR result of C. perfringens' toxin genes. M: 100 bp DNA Ladder (NEB, USA), NK: Negative Control, A1: Enterotoxin; Negative, A2: cpi - iota toxin; Positive, A3: cpb-beta toxin; Negative, A4: cpe - epsilon toxin; Negative A5: cpa - alpha toxin; Positive

PCR for the Detection of Toxin Genes

The *C. perfringens* isolate was analyzed for the presence of alpha, beta, epsilon and iota toxin genes by PCR using the primers and protocol as described previously [7]. The primers are shown in *Table 1*. Briefly, DNA was extracted from the isolate using a column-based DNA isolation kit (DNA mini kit, Qiagen, Germany). The PCR run was performed using the GeneAmp PCR System 9700 device (Applied Biosystems) and amplicons were photographed with UV illumination after electrophoresis.

The identities of the amplicons were confirmed by comparison of the sequence with previous reports obtained from Gen-Bank. The isolate was found positive alpha and iota toxins by PCR *(Fig. 2)*.

DNA and RNA Isolation for Bovine Herpesvirus and Bovine Virus Diarrhea Virus

Amplification conditions: Following initial denaturation step for 3 minutes at 94°C, 35 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 1 min, and a final elongation step at 72°C for 10 min.

Viral Genome Amplification: Tissue samples from mesenterial lymph nodes and liver were homogenized in which of 50 mg were taken

Table 1. *Sequences of the PCR primers*

Primer	Sequence 5'-3'	Product Size (bp)	Gene	Reference
cpb F	GCGAATATGCTGAATCATCTA	196	*cpb*	[7]
cpb R	GCAGGAACATTAGTATATCTTC			
cpe F	GGGGAACCCTCAGTAGTTTCA	506	*cpe*	[8]
cpe R	ACCAGCTGGATTTGAGTTTAATG			
cpi F	AAACGCATTAAAGCTCACACC	293	*iap*	[8]
cpi R	CTGCATAACCTGGAATGGCT			
cpa F	GTTGATAGCGCAGGACATGTTAAG	402	*cpa*	[9]
cpa R	CATGTAGTCATCTGTTCCAGCATC			

for RNA and DNA isolation. DNA isolation was performed using DNAzol (ThermoFisher, USA), and RNA isolation using EZ-RNA Total RNA Isolation Kit (BI Biological Industries, Israel). Positive control bovine herpesvirus type 1 (BHV-1), bovine virus diarrhea virus, and Colorado strain (BVDV), strain NADL with DNA and RNA isolation was performed.

In order to determine the presence pestivirus from the suspected tissue, the isolated RNAs and their panpestivirus gene-specific primers of RT-PDiscCR was performed according to a previously defined procedure [10] to determine the presence of BHV-1 from the suspicious tissue using BHV-1 specific primers. The PCR analysis was carried out as described earlier [11].

No evidence for the presence of pestivirus or BHV-1were found in RT-PCR and PCR analysis (data not shown).

DISCUSSION

Infection caused by *C. perfringens* type E has not been reported in calves in Turkey. However, a case of enterotoxemia due to *C. perfringens* type A in a cow was reported earlier with only microbiological findings [12]. *C. perfringens type E* was found to be responsible for 4.0% (45 out of 1113 strains) of all clostridial enterotoxemia cases in calves in the USA [3]. Absence of complete immunoprophylaxis and lack of drug treatment are the issues that make the type E enterotoxemia important in animals. The important factor in the pathogenesis of clostridial enterotoxemia is the presence of starch forming a suitable environment for the propagation and proliferation of such saccharolytic bacteria in the small intestine [1,4]. When the calves were weaned, enough intestinal nutrients might pass through the intestines as it takes time for the rumen flora adaptation. This assumption might explain the higher prevalence of toxin type E in weaning calves as in the presented case. For differential diagnosis and/or concurrent occurrence of BVD and herpesvirus were excluded by PCR examination as these viruses have immunosuppressive feature.

Overall, toxinotype E could be considered in differential diagnosis in fibrino-hemorrhagic enteritis and sudden deaths in calves. Pathological examination would be highly important to reveal the importance of type E toxinotype in cattle industry. Further studies are necessary to clarify the mechanisms involved in the pathogenesis of enteritis caused by this toxinotype, and the role of iota toxin during the disease.

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