Enterotoxemia Caused by Clostridium perfringens Type E in a Calf

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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, 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 highly 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

Bir Buzağıda Clostridium perfringens Tip E Nedenli Enterotoksemi Olgusu

Öz

Anahtar sütçü: Sığır, Clostridium perfringens tip E, Enterotoksemi
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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 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 mucosa 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...
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 \textit{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.

\textbf{PCR for the Detection of Toxin Genes}

The \textit{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).

\textbf{DNA and RNA Isolation for Bovine Herpesvirus and Bovine Virus Diarrhea Virus}

\textbf{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.

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

\textbf{Table 1. Sequences of the PCR primers}

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence 5’-3’</th>
<th>Product Size (bp)</th>
<th>Gene</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>cpb F</td>
<td>GCGAATATGCTGAATCATCTA</td>
<td>196</td>
<td>cpb</td>
<td>[7]</td>
</tr>
<tr>
<td>cpb R</td>
<td>GCAGGAACATTATATATCTTC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cpe F</td>
<td>GGGGACCCCTCAGTAGTTTCA</td>
<td>506</td>
<td>cpe</td>
<td></td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cpi F</td>
<td>AAAGCGATTTAAGGCTCACACC</td>
<td>293</td>
<td>iap</td>
<td>[8]</td>
</tr>
<tr>
<td>cpi R</td>
<td>CTGCATAACCTGGAATGGCT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cpa F</td>
<td>GTTGTATGCGCCAAGCATGTTAAG</td>
<td>402</td>
<td>cpa</td>
<td>[9]</td>
</tr>
<tr>
<td>cpa R</td>
<td>CATGTAATCTGTCGGTTCCAGCATC</td>
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</tbody>
</table>
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 of 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-1 were 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 \(^{[12]}\) 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.

**REFERENCES**