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Enteritis Caused by Type A *Clostridium perfringens* Producing α-Toxin in a Dog

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Abstract : A 6-year-old, female, Siberian husky was referred with mucous diarrhea. On fecal examination, numerous clustered and individual large epithelial cells and rod-shaped, spore-forming bacteria were examined. By bacterial culture and molecular typing, the bacteria was identified as *Clostridium perfringens* (*C. perfringens*), and by toxin analysis of *C. perfringens*, production of α-toxin was confirmed. Based on these results, the dog was diagnosed as enteritis caused by *C. perfringens* producing α-toxin, and was treated with amoxicillin/clavulanate. After 1 week, the diarrhea was disappeared and no spore-forming bacteria were examined on fecal examination. This report shows that the rapid and exact diagnosis keeps a effective treatment for enteritis caused by *C. perfringens* producing α-toxin in dogs.

Key words **:** dog, enteritis, *Clostridium perfringens*, α-toxin, amoxicillin/clavulanate.

Introduction

Clostridium perfringens (*C. perfringens*) is a gram-positive, anaerobic bacteria that is responsive of enteritis and fatal enterotoxemia in domestic animals (1,4,15). The pathogenesis of this organism is associated with exotoxins and enterotoxin (8,11). Based on the synthesis of four major lethal toxins, alpha, beta, epsilon and iota, *C. perfringens* is classified into five types, A, B, C, D and E (19). Type A produces only alpha toxin, type B produces alpha, beta and epsilon toxin, type C produces alpha and beta toxin, type D produces alpha and epsilon toxin and type E produces alpha and iota toxin. Enterotoxin is most often produced by type A; however it may be produced by all of the toxin types (6). Thus, detection of the four major toxins plays an important role in diagnosis of *C. perfringens*-induced enteric disease (14).

This report describes a case of type A *C. perfringens*-induced enteritis in a dog. This report shows that a rapid identification of the bacteria and its toxin, and treatment is effective for type A *C. perfringens*-induced enteritis in a dog.

Case

History

A 6-year-old, intact female, Siberian husky was referred to the Veterinary Medical Center at the College of Veterinary Medicine, Chungbuk National University with mucous diarrhea. The diarrhea was firstly detected 1 week previously, and was slowly progressive. Before the onset of clinical sign, the dog had received a surgery for intranasal foreign bodies and had received oral cephalexin and prednisolone for 1 month. The dog was alert and responsive, and no other abnormalities were found on physical examination.

Laboratory examination

Because of mucous diarrhea, wet-mount and direct smear of the feces were prepared for microscopic examination. The feces appeared grossly yellowish and mucoid. The smears contained numerous clustered and individual large epithelial cells and rod-shaped bacteria (Fig 1). More than 70% of bacteria contained a spore in its body. Highly degenerated neutrophils and broken RBCs were also observed. The CBC and serum biochemical analysis showed no abnormalities except highly elevated ALP concentration (1,260 U/L; reference range 29-97 U/L). Because of the cell clusters and many bacteria, the enteritis caused by spore-forming bacteria was suspected.

Culture and Molecular identification of the bacteria

The feces was cultured for bacteria at 37°C for 3 days in a Muller-Hinton broth (Becton Dickinson, USA) in aerobic and anaerobic conditions. Under anaerobic conditions, the culture showed growth of gram-positive rod that were similar to those observed in microscopy of stained feces.

The bacteria was identified by molecular typing. The genomic DNA was extracted by using Dynabeads™ DNA Direct Universal (Invitrogen Life Technologies, Inc., USA), and was amplified using the primers CIPer1 and CIPer2 as described

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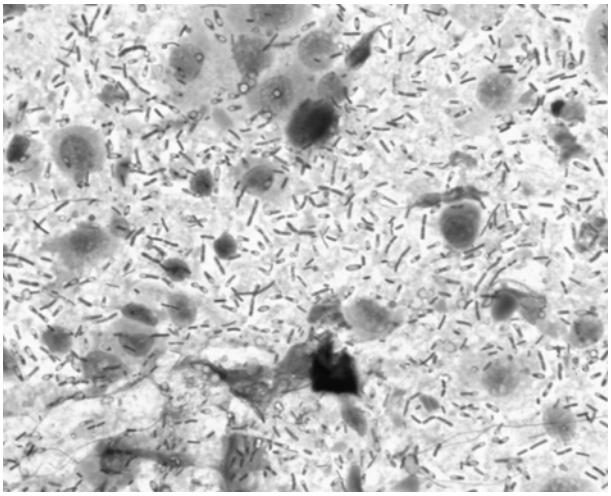

Fig 1. Microscopic examination of the feces showing clustered and individual large epithelial cells and rod-shaped, spore-forming bacteria. Diff-Quik, ×1000.

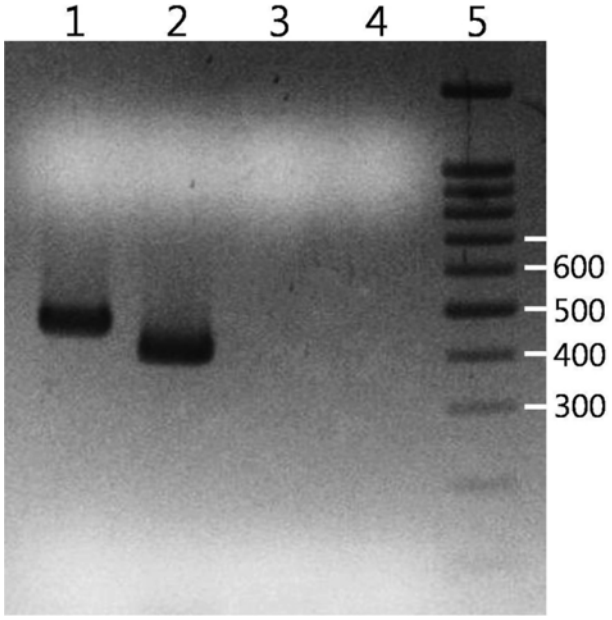

Fig 2. The results of molecular analyses for the identification of *C. perfringens* and its toxin typing. Lane 1: 18S rRNA gene of *C. perfringens* (481 bp), lane 2: multiplex PCR for α (402 bp), β (236 bp),ε (541 bp) and τ-toxin (317 bp), lane 3: β2 toxin (573 bp), lane 4: enterotoxin (233 bp), lane 5: 100 bp ladder.

previously (16). The PCR amplification was performed in a total volume of 50 μL. The final reaction conditions were as follows: 50 mM KCl, 10 mM Tris-HCl (pH 8.3, 25°C), 1.5 mM $MgCl_2$, 200 μM of each dNTP, 100 ng of each primer, and 5 units of Taq polymerase (iNtRON Biotechnology, South Korea). The PCR was performed in a TaKaRa Thermal Cycler Dice (Takara Bio Inc., Japan) under the following conditions: an initial denaturation at 94°C for 2 min 30 s, followed by 30 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 1 min, and a final run at 72°C for 5 min. The PCR product was separated by electrophoresis for 50 min at 100 V in a 2% agarose gel and was stained with ethidium bromide for visualization under ultraviolet light. The amplicon was sequenced using an ABI Prism® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit v3.1 (PE Applied Biosystems, USA). A comparison with the gene sequences deposited in GenBank revealed that the sequence of the isolated bacteria were 99% similar to the *C. perfringens* sequence that had been deposited in Japan (GenBank accession number AB045284). Thus, we identified the bacteria isolated from the dog as *C. perfringens*.

Toxin analysis

To verify whether the bacteria produce its toxin, we analyzed bacterial toxin using DNA extracted from the feces collected on first presentation. α, β, β2, ε, τ, and enterotoxin of *C. perfringens* were analyzed as previously described (7). The final reaction conditions were same with that of bacterial identification, and the PCR was performed under the following conditions: an initial denaturation at 94°C for 2 min 30 s, followed by 30 cycles of 94°C for 30 s, 55°C (multiplex PCR for α, β, ε, and τ-toxin) or 53°C (for β2-toxin) or 48°C (for enterotoxin) for 30 s, and 72°C for 1 min, and a final run at 72°C for 5 min. The PCR product was separated by electrophoresis for 50 min at 100 V in a 2% agarose gel and was stained with ethidium bromide for visualization under ultraviolet light. The electrophoresis revealed that the bacteria produced α-toxin (Fig 2). Based on the results of bacterial identification and toxin analysis, the case was diagnosed as enteritis by *C. perfringens* producing α-toxin.

Treatment

The patient was treated with amoxicillin/clavulanate (Kuhnil Pharm, South Korea) 30 mg/kg, PO, bid, for 1 week. By the seventh day, the diarrhea was disappeared, and there were no spore-forming bacteria on fecal examination.

Discussion

This report describes a case of enteritis caused by type A *C. perfringens* in a dog. α-toxin is an enzyme, chemically known as phospholipase-C (lecithinase-C), which hydrolyzes lecithin into phosphorylcholine and a diglyceride (12). As the membranes of most cells consist of lipoprotein complexes containing lecithin, α-toxin leads to their destruction. The resultant biological effect is hemolysis, necrosis or death, depending on tissues accessible to the toxin. In dogs, *C. perfringens* has been associated with 28-34% of diarrheic cases, ranging in severity from a mild self-limiting diarrhea to a potentially fatal acute diarrhea according to the toxin (3,10,13). Enterotoxemia due to type A *C. perfringens* infection causes sudden death as a result of systemic toxemia in many animals (5,12,13). In this study, the dog was diagnosed as enteritis caused by type A *C. perfringens*, and was treated successfully with amoxicillin/clavulanate. It shows that the rapid diag-

nosis and proper treatment are effective in dogs with enteritis caused by type A *C. perfringens* producing α-toxin.

In animals, *C. perfringens* usually forms a part of the normal microbial flora (12). To exclude the possibility that *C. perfringens* was cultured selectively during the bacterial culture, we additionally performed the bacterial identification using DNA extracted from the feces collected on the first presentation. In the PCR for 16S ribosomal RNA (16S rRNA) gene (18) and direct sequencing, we confirmed that only one product (confirmed as *C. perfringens* in direct sequencing) was amplified. As the primers we used in additional test is a universal primer for bacterial 16S rRNA gene (18), this result indicates that *C. perfringens* was only bacteria in the feces, along with the result of the bacterial culture. Thus, we confirmed that the dog has enteritis caused by *C. perfringens*.

Classically, toxin typing of *C. perfringens* is performed with toxin neutralization test in guinea pigs or mice (9). This procedure is complex and time-consuming, and also consumes a lot of antisera and experimental animals. Recently, there have been several reports of immunoassay, including immunoelectrophoresis, latex agglutination, immunodiffusion, and ELISA (14). These are, however, also complex and time-consuming. Instead, molecular analyses such as PCR have been used to type *C. perfringens* (2,9,14,17,20). Primers derived from the sequence for several toxin-producing genes, such as *cpa*, *cpb*, *etx*, and *i*A, have been successfully detected in PCR assays. In this study, we performed molecular analyses for toxin typing, and confirmed that the bacteria produces α-toxin, that can induce a fatal enterotoxemia in dogs (13).

In conclusion, this report describes a case of type A *C. perfringens* diagnosed by molecular analyses of the bacteria and its toxin type. Although the high pathogenicity, this report indicates that the rapid diagnosis and proper treatment are effective in dogs with enteritis caused by type A *C. perfringens* producing α-toxin.

References

1. Bartholomew BA, Stringer MF, Watson GN, Gilbert GN. Development and application of an enzyme linked immunosorbent assay for Clostridium perfringens type A enterotoxin. J Clin Pathol 1985; 38: 222-228.
2. Baums CG, Schotte U, Amtsberg G, Goethe R. Diagnostic multiplex PCR for toxin genotyping of Clostridium perfringens isolates. Vet Microbiol 2004; 20: 11-16.
3. Cave NJ, Marks SL, Kass PH, Melli AN, Brophy MA. Evaluation of a routine diagnostic fecal panel for dogs with diarrhea. J Am Vet Med Assoc 2002; 221: 52-59.
4. Collins JE, Bergeland ME, Bouley D, Ducommun AL, Francis DH, Yeske P. Diarrhea associated with Clostridium perfringens type A enterotoxin in neonatal pigs. J Vet Diagn Inves 1989; 1: 351-353.
5. Dart AJ, Pascoe RR, Gibson JA, Harrower BJ. Enterotoxemia in a foal due to Clostridium perfringens type A. Aust Vet J 1988; 65: 330-331.
6. Fach P, Popoff MR. Detection of enterotoxigenic Clostridium perfringens in food and fecal samples with a duplex PCR and the slide latex agglutination test. Appl Environ Microbiol 1997; 63: 4232-4236.
7. Gholamiandekhordi AR, Ducatelle R, Heyndrickx M, Haesebrouck F, Van Immerseel F. Molecular and phenotypical characterization of Clostridium perfringens isolates from poultry flocks with different disease status. Vet Microbiol 2006; 113: 143-152.
8. Gkiourtzidis K, Frey J, Bourtzi-Hatzopoulou E, Iliadis N, Sarris K. PCR detection and prevalence of α-, β-, β2, ε-, τ- and enterotoxin genes in Clostridium perfringens isolated from lambs with clostridial dysentery. Vet Microbiol 2001; 82: 39-43.
9. Kalender H, Ertas HB, Cetinkaya B, Muz A, Arslan N, Kilic A. Typing of isolates of Clostridium perfringens from healthy and diseased sheep by multiplex PCR. Vet Med 2005; 10: 439-442.
10. Marks SL, Kather EJ, Kass PH, Melli AN. Genotypic and phenotypic characterization of Clostridium perfringens and Clostridium difficle in diarrheic and healthy dogs. J Vet Intern Med 2002; 16: 533-540.
11. Meer RR, Songer JG. Multiplex polymerase chain reaction assay for genotyping Clostridium perfringens. Am J Vet Res 1997; 58: 702-705.
12. Niilo L. Clostridium perfringens in animal disease: a review of current knowledge. Can Vet J 1980; 21: 141-148.
13. Sasaki J, Goryo M, Asahina M, Makara M, Shishido S, Okada K. Hemorrhagic enteritis associated with Clostridium perfringens type A in a dog. J Vet Med Sci 1999; 61: 175-177.
14. Songer JG, Meer RR. Genotyping of Clostridium perfringens by polymerase chain reaction is a useful adjunct to diagnosis of Clostridial enteric disease in animals. Anaerobe 1996; 2: 197-203.
15. Songer JG. Clostridial enteric diseases of domestic animals. Clinical Microbiol 1996; 9: 216-234.
16. Tonooka T, Skata S, Kitahara M, Hanai M, Ishizeki S, Takada M, Sakamoto M, Benno Y. Detection and quantification of four species of the genus Clostridium in infant feces. Microbiol Immunol 2005; 49: 987-992.
17. Uzal FA, Plumb JJ, Blackall LL, Kelly WR. PCR detection of Clostridium perfringens producing different toxins in faeces of goats. Lett Appl Microbiol 1997; 25: 339-344.
18. Vallianou N, Evangelopoulos A, Makri P, Zacharias G, Stefanitsi P, Karachalios A, Avgerinos PC. Vertebral osteomyelitis and native valve endocarditis due to Staphylococcus simulans: a case report. J Med Case Reports 2008; 2: 183.
19. Yamagashi T, Sugitani K, Tanishima K, Nakamura S. Polymerase chain reaction test for differentiation of five toxin types of Clostridium perfringens. Microbiol Immunol 1997; 41: 295-299.
20. Yoo HS, Lee SU, Park KY, Park YH. Molecular typing and epidemiological survey of prevalence of Clostridium perfringens types by multiplex PCR. J Clin Microbiol 1997; 35: 228-232.

개에서 α-Toxin을 생성하는 Type A *Clostridium perfringens* 에 의한 장염

한재익 · 장혜진 · 천행복 · 김지혜 · 김근형 · 장동우 · 나기정[1]

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요 약 : 6년령, 암컷 시베리안 허스키가 점액성 설사로 충북대학교 동물의료센터에 내원하였다. 분변 검사 결과 심한 장상피세포 박리와 함께 다수(> 70%)의 아포형성 간균 증식이 관찰되었다. 분자생물학적 세균 동정 결과 증식된 세균은 *Clostridium perfringens*로 확인되었으며, toxin 검사 결과 α-toxin이 세균에 의해 합성되고 있음을 확인하였다. 따라서 환자는 α-toxin을 합성하는 *C. perfingens*에 의한 장염으로 진단하였으며, amoxicillin/clavulanate를 투여하였다. 치료 1주 후 설사는 소실되었으며, 분변 검사 결과 아포 형성 간균은 소실되었다. 이 증례는 빠르고 정확한 진단으로 type A *C. perfringens*에 의한 장염이 효과적으로 치료됨을 보여준다.

주요어 : 개, 장염, *Clostridium perfringens*, α-toxin, amoxicillin/clavulanate.