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HEMORRHAGIC ENTEROCOLITIS AND DEATH IN TWO FELINES (PANTHERA TIGRIS ALTAICA AND PANTHERA LEO) ASSOCIATED WITH CLOSTRIDIUM PERFRINGENS TYPE A

Yanlong Zhang, Ph.D., Zhijun Hou, Ph.D., and Jianzhang Ma

Abstract: Severe hemorrhagic enterocolitis was observed in a Siberian tiger (Panthera tigris altaica) and a lion (Panthera leo). Both animals developed acute depression, anorexia, and bloody diarrhea several days before death. Small and large intestines were diffusely congested, edematous, necrotic, and filled with hemorrhagic fluid, and mesenteric lymph nodes were enlarged and congested. Pure and abundant growth of gram-positive bacilli was obtained in culture under anaerobic conditions from the livers of both felines. Identification of highly virulent Clostridium perfringens Type A was based on pathologic lesions, hemolytic patterns, morphologic structure, and polymerase chain reaction. Animal inoculation assays indicated that C. perfringens Type A played an important role in the pathogenesis of both felines.

Key words: Alpha-toxin, z-toxin, Clostridium perfringens, hemorrhagic enterocolitis, lion, Panthera leo, Siberian tiger, Panthera tigris altaica.

BRIEF COMMUNICATION

Clostridium perfringens is a gram-positive, spore-forming, anaerobic bacillus bacterium responsible for a variety of diseases in humans, including gas gangrene, food poisoning, and diarrhea, as well as enterotoxemia and hemorrhagic gastroenteritis in many domestic animals and wildlife. Clostridium perfringens is considered to be the most important cause of clostridial enteric disease and is classified into five types (A, B, C, D, and E) based on the production of four major toxins: alpha, beta, epsilon, and iota (α, β, ε, and ι).

Two adult felines (a Siberian tiger [Panthera tigris altaica] and a lion [Panthera leo]) among a population of more than 100 felines in a Harbin Zoo in northeastern China died several days after developing acute depression, anorexia, and bloody diarrhea. Necropsy of both animals was performed following routine procedures. Portions of liver, heart, and intestines were collected from each animal and fixed in 10% formalin (10% formaldehyde neutral buffer solution, Kermel Chemical Reagent Co., Ltd., Tianjin, 300350, China) for 24 h. Tissues were then embedded in paraffin (paraffin, Kermel Chemical Reagent Co.) and sectioned at a thickness of 4 μm. Hematoxylin and eosin (1% Eosin Y Eosin ethanol solution and Mayer’s hematoxylin solution, Tianhe Microorganism Reagent Co. Ltd., Hangzhou, 310008, China) staining showed very similar lesions for both animals. Macroscopic lesions in the small and large intestines were diffusely congested, edematous, necrotic, and filled with hemorrhagic fluid. The mesenteric lymph nodes were enlarged and congested. Severe multifocal necrosis of the superficial epithelium was observed in both the large and small intestines, and the lamina propria had a diffuse infiltration of lymphocytes, plasma cells, and neutrophils and a few eosinophils.

Liver samples from each animal were also collected aseptically and cultured on sheep blood agar (nutrient agar, Tianhe Microorganism Reagent Co., with sheep blood) under aerobic and anaerobic conditions. No bacteria were isolated under aerobic conditions. However, anaerobic liquid culture (ordinary broth medium, Tianhe Microorganism Reagent Co.) from both animals had a dense growth of short bacilli when grown for 24 hr at 37°C. Many bubbles were observed under liquid paraffin (liquid paraffin, Kermel Chemical Reagent Co.), which were attributed to bacterial growth. Gram staining (Gram staining solution, Tianhe Microorganism Reagent Co.) revealed short, gram-positive bacilli. A presumptive diagnosis of enterotoxemia caused by C. perfringens was made, based on the pathologic lesions, hemolytic patterns, and bacterial morphology.

Ten anaerobic isolates of C. perfringens from liver samples (five isolates from each animal) were randomly selected and tested for the presence of genes for major toxins, including α (cpa), β (cpb), ε (etx), ι (itx), β2 (cpb2), and C. perfringens enterotoxin (cpe), by multiplex polymerase chain reaction (PCR), as described previously. Sterile
conditions were used, and negative (no DNA) controls were included in every trial. Reactions were run in a TaKaRa thermocycler (TaKaRa PCR Thermal Cycler Dice™ [Gradient model], TaKaRa Bio Inc., Shiga 520-2193, Japan) using a step-cycle program. Cycling conditions comprised an initial DNA denaturation at 94°C for 5 min; followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min; and a final extension at 72°C for 10 min. PCR products were visualized on agarose gels (Biowest® Regular Agaros G-10, Gene Co. Ltd., Hong Kong 202100, China) stained with EB (ethidium bromide, TaKaRa Biotechnology Co., Ltd., Dalian 116600, China). PCR results demonstrated that the strains isolated from the liver were positive for the gene encoding α-toxin, indicating that the isolates were \textit{C. perfringens} type A. Furthermore, the strains lacked the genes coding for other \textit{C. perfringens} major toxins (\textit{cph}, \textit{etx}, and \textit{itx}), as well as the \textit{cph2} and \textit{cpa} genes.

Five cats and two rabbits were obtained from The Institute of Oncology of Harbin (license: SCXK[HEI]:2007–2010) and experimentally inoculated with \textit{C. perfringens} (under guidelines of local animal care committees) using cultures from liver samples sporulated in anaerobic broth. All inoculated animals died between 18 hr and 5 days after inoculation, and all exhibited pathology similar to that seen in the felines.

Although enterotoxemias are commonly observed in some domestic animals and wildlife,\cite{1,7,9,10} description of enterocolitis in big felines caused by \textit{Clostridium} is rare in the literature, except one report of Amur leopards (\textit{Panthera pardus orientalis}) that had been infected by \textit{C. perfringens}.\cite{8} In the present study, \textit{C. perfringens} type A infection was found in a Siberian tiger and a lion. Some authors have reported that sudden access to carbohydrate-rich food stimulates \textit{C. perfringens} growth and subsequent pathogenesis in ruminants.\cite{9} Lubbs et al.\cite{4} had reported that \textit{C. perfringens} populations were elevated ($P < 0.05$) in adult cats fed a high-protein diet when compared with those fed moderate-protein diets. The diet of tigers and lions consists primarily of meat,\cite{4} suggesting that felines may be at a higher risk to \textit{C. perfringens} infection because of their high protein diet.

\textit{Clostridium perfringens} type A is ubiquitous in the environment and is a normal inhabitant of the gastrointestinal tract of most healthy warm-blooded animals. Therefore, controversy exists regarding its pathogenic role in animals.\cite{5,7} Although enterotoxemia in the two felines could not be confirmed to be caused by \textit{C. perfringens}, animal inoculation assays documented that isolates of \textit{C. perfringens} from both felines were highly virulent, indicating that \textit{C. perfringens} played an important role in the pathogenesis of enterotoxemia in both felines.

Some isolates of \textit{C. perfringens} type A can be considered nontoxigenic because they do not produce sufficient α-toxin to kill experimental animals under test conditions.\cite{1} However, animal inoculation data from this study indicated that this was not the case for the \textit{C. perfringens} type A isolates from both felines.

Multiplex PCR revealed the presence of the \textit{cpa} toxin gene, but not other toxin genes, in \textit{C. perfringens} isolates from both felines in this study, indicating that only the α-toxin was produced by the isolates. The α-toxin is a lecithinase that is thought to be responsible for yellow lamb disease in sheep, a condition characterized by massive hemolysis and icterus, as well as red-tinged urine,\cite{7,10} symptoms not observed in either the Siberian tiger or the lion in this study. Studies have reported goats, calves, pigs, bears, Amur leopards, and deer infected with \textit{C. perfringens} type A that did not display these symptoms,\cite{1,7,5,7–10} indicating that the symptoms do not always appear in \textit{C. perfringens} enterotoxemia, or possibly only appear in some species.

Although \textit{C. perfringens} is a normal inhabitant of the intestines of most animal species, it proliferates in large numbers and produces several potent toxins when the intestinal microbial environment is altered by diet or other factors. Furthermore, the toxin can act locally, systemically, or both, with devastating effects on the host.\cite{3,10} Braxy, a clostridial disease caused by \textit{Clostridium septicum}, is most often related to consumption of frozen food.\cite{5} Any injury to the mucosa of the small intestine might also predispose an individual to changes in the bacterial flora, resulting in overgrowth of clostridia. Mild indigestion could also cause the intestines to become static, preventing normal flushing of toxic substances from the system.\cite{1} Neiffer\cite{8} thought that partially thawed meat in the leopards’ diet might have precipitated the production of an endogenous clostridial enterotoxosis by disrupting digestive tract flora with resultant clostridial overgrowth and sporulation.\cite{4} The diet of felines in the zoo involved with this study consists of frozen beef and chicken, which is stored in a freezer at −20°C and thawed before feeding. Incomplete thawing of the meat might, therefore, have contributed to enterotoxemia of the Siberian tiger and lion.
Diagnosis of enterotoxicosis caused by *C. perfringens* type A is difficult because the bacterium is ubiquitous in the environment, and healthy individuals can be carriers. However, evaluation of gross and pathologic lesions, culture morphology, multiplex PCR results, and animal inoculation assays, as well as the absence of other enterotoxigenic pathogens in both the Siberian tiger and lion, make *C. perfringens* type A enterotoxicosis the most likely diagnosis.

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**LITERATURE CITED**


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