Case Report

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Bacterial Sepsis Associated with a Captive State Caused by *Edwardsiella tarda* in a Eurasian Brown Bear (*Ursus arctos arctos*)

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Abstract *Edwardsiella (E) tarda* belongs to the Enterobacteriaceae family and is a motile, gram-negative, rod-shaped, facultative anaerobe regarded as an opportunistic and food-borne pathogen in animals and humans. A 21-year-old male Eurasian brown bear (*Ursus arctos arctos*) died suddenly without any preliminary signs. Necropsy performed according to standard protocol revealed swollen abdomen with hemorrhagic congestions of the gastroenteric organs, ascites, and hemorrhagic exudates around the mouth. The liver showed discoloration, along with a severely swollen and multiple hemorrhages of the spleen, an elongated gallbladder, and a congested cortex and medullar lesion of kidneys. The stomach contained semi-liquid exudates and undigested chicken exuding a decayed odor. The stomach membranes were dark-gray in color with several cysts in the fundus lesions. Rod-shaped bacteria were found in the major organs by Giemsa staining, identified as *E. tarda* using a biochemical rapid diagnostic identification kit.

Key words bear, *Edwardsiella tarda*, septicemia, *Ursus arctos arctos*, zoo.

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Introduction

Edwardsiella (E) tarda is a gram-negative, facultatively anaerobic bacterium that belongs to the family Enterobacteriaceae (2,22). *E. tarda* has been reported worldwide in diseases of snakes, toads, lizards, turtles, monkeys, cattle, pigs, opossums, fish, penguins, marine mammals, birds, and wildlife (4,6,7,15,17,18). *E. tarda* is a normal flora of reptiles and amphibians and is found in diverse aquatic environments (12,18). It has been implicated in various animal diseases, such as septicemia, enteritis, subcutaneous abscesses, and wound infections (6,18,22). However, most *E. tarda* can be an asymptomatic carrier in animals, therefore concomitant factors including stress conditions, immune deficiency, suffering diseases were seem connected to developed infection (7,8,10). *E. tarda* infections in humans usually present as uncomplicated cases of gastroenteritis; however, cases of wound infections, tubo-ovarian abscesses, sepsis, meningitis, and osteomyelitis have been documented (2,14,16,20,21). Most patients with extraintestinal *E. tarda* infection have underlying hepatic or hematologic diseases (16,21). We herein report a rare case of acute gastrointestinal infection caused by *E. tarda* in a Eurasian brown bear (*Ursus arctos arctos*) at a zoo.

Case Report

A 21-year-old male Eurasian brown bear died suddenly without any clinical signs. The animal was housed in an enclosed facility at the Daejeon Park Zoo located in central Korea (36°17′19.00″ N, 127°23′52.04″ E) and was fed a diet of chicken, mackerel, Pacific saury, and commercial pellets (Mazuri® Omnivore Diet #5635, St. Louis, MO, USA). The chicken was sanitized, commercial, uncooked meat with whole bone, and the mackerel, the Pacific saury were frozen and thawed out. Necropsy was performed according to a standard protocol; the abdomen was found to be severely swollen, and hemorrhagic exudates were found around the mouth. In the abdomen, swollen and hemorrhagic congestion of the gastroenteric organs and greater omentum was observed, with a decaying odor and hemorrhagic ascites (Fig. 1A). The stomach resembled a balloon with a dark red color (Fig. 1B), found mixed with semi-liquid exudates and undigested chicken with a decaying odor. The stomach membranes were found to be half-dark gray and half-red in color, with erosions and ulcers in the fundus lesions (Fig. 1C). Inflammation was observed in the esophagus, and the intestines were swollen. The spleen was congested (Fig. 1D) along with congested kidneys in the cortex-medulla region. The lung lobes and inner face of the trachea were congested. Giemsa staining revealed rod-shaped organisms tinted violet in the major organs, including the liver, spleen, kidney, and heart; microscopic examination revealed bacteria in ascites (Fig. 2).

For histopathological analysis, the spleen, lungs, and kidneys were collected and fixed in 10% neutral buffered formalin for two weeks; subsequently, they were embedded in paraffin, sectioned at 4 μm including lung, spleen, kidney, and stained with hematoxylin and eosin for microscopic examination. Histopathological examination revealed severe congestion of the red

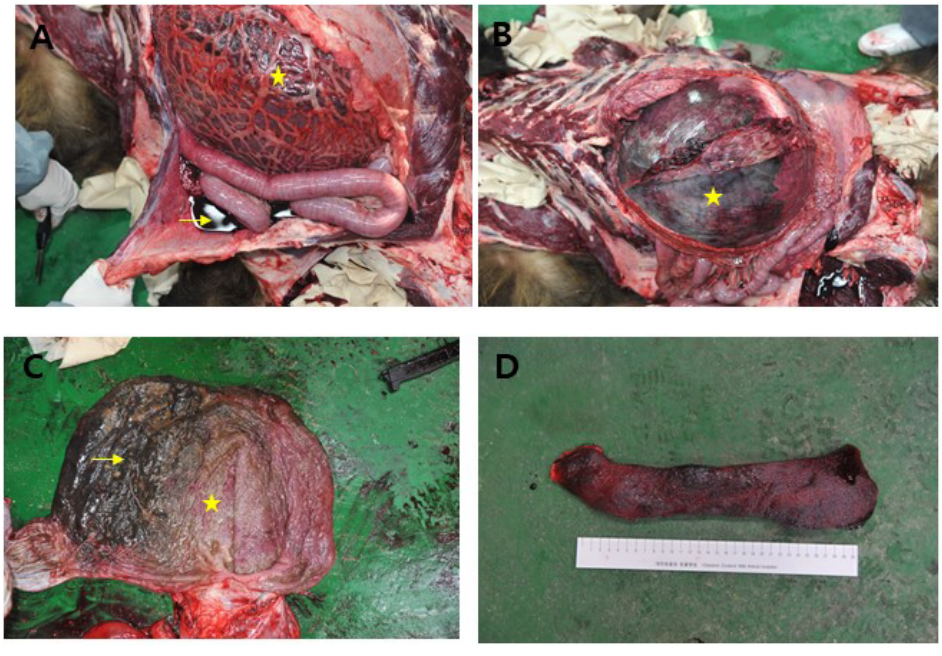

Fig. 1. Macroscopic examination of systemic infection caused by *Edwardsiella tarda* in a 21-year-old male Eurasian brown bear. (A) The greater omentum has severe haemorrhage (yellow star) and haemorrhagic ascites (yellow arrow). (B) Swollen and haemorrhagic congestion of the stomach (yellow star) with a decayed odour. (C) Redness and several ulcerous lesions (yellow star) along with regurgitated bile acid (yellow arrow) in the stomach membranes. (D) Congested spleen.

pulp, infiltration of connective tissue surrounding the trabecula, infiltration of inflammatory cells, and thickening of the venous sinus wall (Fig. 3A). Severe congestion in the air sacs of the lung parenchyma and mild interstitial pneumonia were observed. Tubular interstitial congestion and necrotic changes, including exfoliation and nuclear condensation of tubular epithelial cells, were observed in the medullary region of the kidneys (Fig. 3B). Samples such as the liver, spleen, heart, kidney, stomach, and ascites were submitted to the laboratory housed at the theme park for bacterial culture. Samples were cultured on blood agar (Asan Pharmacy, Seoul, Korea) at 37°C for 18 hr. A pure culture of hemolytic, dew-drop colonies was isolated on agar after the incubation period. The colonies were composed of gram-negative, coccobacillic, and rod-shaped bacteria. These microorganisms were identified as *E. tarda* using a biochemical API 20NE identification kit (BioMérieux, Craponne, France). The susceptibility of *E. tarda* to antibiotics was determined using the disc diffusion method (3). The bacteria were cultivated in a Mueller Hinton (MH; BD Biosciences, San Jose, CA, USA) broth, and the turbidity of the suspension was adjusted to a 0.5 McFarland standard (1.5×10^8/mL). Bacterial cultures were subsequently inoculated onto MH agar plates. The antibiotic concentrations on the discs (BBL Sensi-Disc; Becton and Dickinson, Franklin Lakes, NJ, USA) were as follows: ampicillin (10 μg), amikacin (30 μg), bacitracin (10 μg), cephalothin (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), cefazolin (30 μg), colistin (10 μg), erythromycin (15 μg), gentamicin (10 μg), kanamycin (30 μg), novobiocin (30 μg), enrofloxacin (5 μg), norfloxacin (10 μg), penicillin (10 μg), streptomycin (10 μg), trimethoprim-sulfamethoxazole (25 μg), oxytetracycline (30 μg), and vancomycin (30 μg). Resistance breakpoints were defined according to the performance standards for antimicrobial susceptibility testing of Clinical and Laboratory standards Institute (3). The results of the antimicrobial susceptibility test revealed amikacin, chloramphenicol, ciprofloxacin, cefazolin, gentamicin, enrofloxacin, norfloxacin, and trimethoprim-sulfamethoxazole as the sensitive drugs, whereas the resistant drugs were ampicillin, bacitracin, kanamycin, novobiocin, penicillin, streptomycin, oxytetracycline, and vancomycin. But, vancomycin resistance cannot be determined by the disc diffusion method, and an additional minimum inhibitory concentration test is needed. The intermediate drugs used were cephalothin, colistin, and erythromycin.

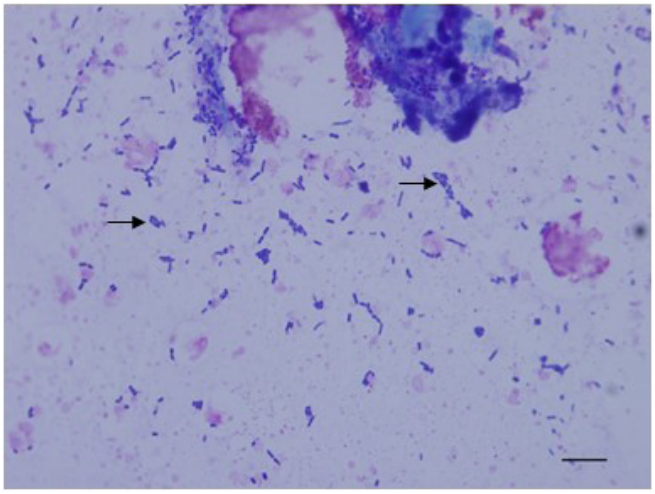

Fig. 2. A 21-year-old male Eurasian brown bear; direct smear from stomach contents by Giemsa staining showing coccobacillic bacterial colony (arrow; ×1,000). Bar = 10 μm.

Discussion

E. tarda has a worldwide distribution and can be found in pond water, mud, and the bacteria known to the normal flora of fish and other marine animals and hence is spread by carrier animal feces (11,18). It is an important zoonotic pathogen that can infect various animals, including fish, amphibians, reptiles, mammals, and human beings (12,17). These bacteria produce a wide range of infections in animals and are recognized as causative agents, in septicemia, emphysematous putrefactive diseases, wound infection, abscesses, and enteritis (14,17,18). *E. tarda* infections in humans can be classified into two types: gastrointestinal disease, including

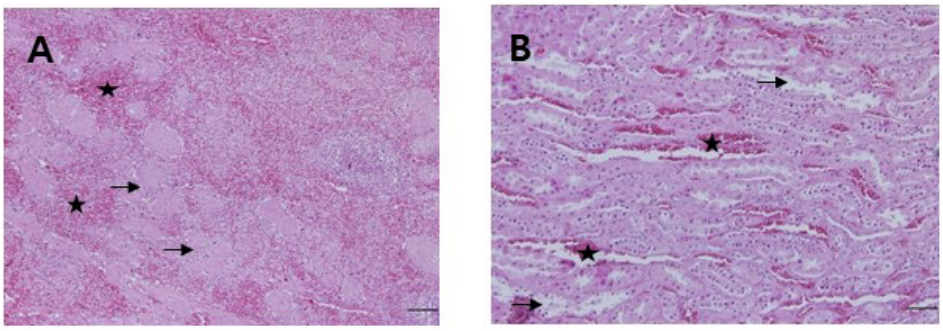

Fig. 3. Microscopic examination of systemic infection caused by *Edwardsiella tarda* in a 21-year-old male Eurasian brown bear. (A) The spleen has severe congestion of red pulp (star) and infiltration of connective tissue surround ing the trabecula by inflammatory cells by hematoxylin and eosin (H&E) staining (arrow, ×100). Bar = 100 μm. (B) The kidneys show tubular interstitial congestion (star) with exfoliated epithelial cells and necrosis of tubular lesions by H&E staining (arrow, ×200). Bars = 50 μm.

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diarrhea, gastroenteritis, typhoid fever-like illness, bacteremia, and sometimes an asymptomatic carrier state (21), and extraintestinal infection, a rare form of infection with various types of reported diseases, including bacteremia, wound infection, necrotizing fasciitis, meningitis, osteomyelitis, endocarditis, hepatobiliary infection, turbo-ovarian abscess, salpingitis, brain abscess, and empyema (16,20). Several studies have indicated that *E. tarda* can survive and adapt to various host environmental conditions, including host hormonal changes, temperature, pH, salinity, and variations in several important nutritional elements, such as iron, phosphate, and magnesium (18). *E. tarda* can also replicate in phagocytes, leading to systemic infection (18,19). Moreover, it can cause systemic immunosuppression by inducing lymphocyte apoptosis, which suppresses systemic immune responses during the initial stages of septicemia (13,19). Stressed, weakened, and debilitated animals in particular may have insufficient protection due to a compromised immune system, which helps the initiation of the bacterial infection (11). Although an immunocompromised animal may already be at higher risk for developing sepsis, *E. tarda* may also cause sepsis because it has various virulence factors for adhering to and invading host cells, thereby infecting the whole body (6). In the present case, the Eurasian brown bear was housed in a captive state at a zoo for most of its life. The observed *E. tarda* associated sepsis could be attributed to the consumption of contaminated food, although other bears that ate the same diet did not show any clinical symptoms. Among the factors presented in the case history, old age, an indifferent habit, and stress in captivity may be important parts of the infection. Therefore, it was presumed that the number of bacteria increased by a diet contaminated with *E. tarda*, eventually causing death by bacterial sepsis. This assumption was supported by the fact that *E. tarda* was isolated from major organs such as the heart, liver, kidney, spleen, lung, and blood ascites, and by the fact that no other bacteria were observed. The death was caused by sepsis and multiple organ failure. In zoo animals, increased corticosterone levels have been shown to decrease immunocompetence. These captive states, including immunosuppression, could be the underlying mechanism of infection with this opportunistic pathogen (11,17). *E. tarda* has been isolated from 72% of eel farms, 23.3% of fecal samples from zoo animals, and 15.1% of wildlife samples (8,18). In contrast, it was found in 0.0073% of fecal samples from healthy humans, despite its absence in the human gut flora (21). Thus, *E. tarda* may be transmitted to humans by contaminated seafood, which is an asymptomatic carrier state. Underlying disease conditions in humans infected with *E. tarda* include hepatobiliary diseases, malignancy, iron overload, old age, and immunosuppression, which lead to increased mortality (15,20). In the present study, the Eurasian brown bear was housed for 18 years in captivity at a zoo which is an enclosed facility. Stomach swelling and congestion were typical of gastric dilation and volvulus, yet gastrointestinal organ torsion was not observed. According to the history of this case, the deceased Eurasian brown bear typically consumed chicken and fish, including mackerel and Pacific saury, which are important sources of infection. Additionally, old age, stress due to small enclosures, and lack of exercise caused by the exhibition and captivity states may have decreased the bear's immune system and, consequently, may have contributed to the *E. tarda* infection (16,17,21). Furthermore, bears are known as intelligent animals, in this case, the deceased bear suffers excessive stress through exhibitions associated with confined spaces, it is estimated that the bear had experienced stress for a long time, lived in captivity, and was always exposed to zoo visitors (5,14). Although *E. tarda* is found as normal flora in reptiles, amphibians, and diverse aquatic environments, previous attempts to isolate it from free-living reptiles have failed (7). Therefore, it is very likely that the captive brown bear was fed with contaminated fish, which are known reservoirs for *E. tarda*. We, thus, assume that food-borne infection occurred in this study. In addition, a captivity-induced stressful environment may increase bacterial growth and promote infection (8,9). Therefore, alleviating captivity-induced stress in zoo animals by providing a more relaxed environment and minimizing exposure to stressors can be effective in reducing bacterial infections (1).

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Conflicts of Interest

The authors have no conflicting interests.

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