SALMONELLOSIS (*Salmonella enterica*) IN A GROUP OF CAPTIVE BLACK RHINOCEROS (*Diceros bicornis*)

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Abstract

Salmonellosis caused by *Salmonella enterica subsp. arizonae serotype 44:Z4,Z32* (formerly known as *Salmonella arizonae*) was diagnosed in three of five captive black rhinoceroses maintained at the Denver Zoological Gardens. Two animals died despite supportive treatment. Another two rhinoceroses remained asymptomatic and were negative for *Salmonella* spp. after serial fecal cultures. The source for the salmonellosis was never definitely established.

Introduction

Three of five black rhinoceroses (*Diceros bicornis*) at the Denver Zoological Gardens (DZG) contracted *Salmonella enterica subsp. arizonae serotype 44:Z4,Z32*. The signal case was a female that presented with lethargy and partial anorexia over a 1-mo period. *Salmonella enterica subsp. arizonae serotype 44:Z4,Z32* was isolated from a nasal swab. Over the next several weeks two male rhinoceroses developed diarrhea and had positive fecal cultures for *Salmonella enterica subsp. arizonae serotype 44:Z4,Z32*.

Black rhinoceroses at the DZG are housed in the pachyderm building, which has two indoor rhinoceros exhibits, three off-exhibit holding stalls and an outside exhibit yard. The holding stalls are positioned side-by-side and sloped from west to east. The slope of the floor allows fecal material hosed from the west stall to drain through the other two stalls prior to reaching a drain during cleaning. In addition to black rhinoceroses, the building also houses hippopotamuses (*Hippopotamus amphibius*), rock hyraxes (*Procavia capensis*), Malayan tapirs (*Tapirus indicus*), and Asian elephants (*Elephas maximus*). This report will discuss the diagnosis, clinical features, antibiotic therapy, and outcome of salmonellosis in three captive black rhinoceroses.

Case Reports

Case 1: A 24-yr-old female black rhinoceros, weighing approximately 900 kg, developed lethargy, partial anorexia and decreased water consumption which continued intermittently for 1 mo. After epistaxis was noted in the right naris, the rhinoceros was immobilized with 2.5 mg etorphine (M99, Lemmon Co., Sellersville, PA 18960 USA) administered i.m. via pole syringe. A 1-cm-diameter ulcer was found just inside the right nostril and swabbed for bacterial culture. Blood was obtained for hemogram and serum biochemistry. Treatment consisted of 20.1×10^6 units of penicillin G benzathine and penicillin G procaine, 9000 International Units (IU) vitamin E (Vital E TM-300, Schering-Plough Animal Health, Kenilworth, NJ 07033 USA), and 20 mg dexamethasone (Vedco

Inc., St. Joseph, MO 64504 USA), all administered i.m. Two days later 100 mg vitamin K_1 (Veda-K_1, Vedco Inc.) and 10 ml Multi B complex (Phoenix Pharmaceutical Inc., St. Joseph, MO 64506 USA) were administered i.m. Trimethoprim and sulfamethoxazole (TMS) 26880 mg (Geneva Pharmaceuticals, Inc., Broomfield, CO 80020 USA) were administered p.o. s.i.d. for 2 mo.

Two weeks later, the rhinoceros became weaker, ataxic, and developed a purulent vaginal discharge. It was immobilized again to obtain blood for hemogram, serum biochemistry and culture. The vaginal discharge was also cultured. In addition, 5 L of lactated Ringer's and 5% dextrose (LRS & 5% dex) were administered i.v. Three hundred and sixty milliliters of 0.2% nitrofurazone and 4 L of saline were infused into the uterus through a sterile catheter. Fifty-seven milliliters of trimethoprim sulfadiazine (80 mg trimethoprim and 400 mg sulfadiazine/ml [48%], Mortar & Pestle Pharmacy, Des Moines, IA 50310 USA) diluted in an equal volume of sterile water, 9750 mg amikacin (Amiglyde-V®, Fort Dodge Laboratories Inc., Fort Dodge, IA 50501 USA), 20 ml Multi B complex, 100 mg vitamin K_1, 9000 IU vitamin E, 100 units oxytocin (Phoenix) and 25 mg prostaglandin F_2 alpha (Lutalyse,® The Upjohn Co., Kalamazoo, MI 49001 USA) were administered i.m.

The rhinoceros' clinical condition continued to deteriorate. Cutaneous ulcers developed on the back, neck, face, and extremities. The animal appeared to be painful and groaned when it rose. Flunixin meglumine (Banamine,® Schering-Plough Animal Health Corp.) was administered 1 g, p.o., daily for 5 days. The caudal surface of the right hock became ulcerated and exuded an odoriferous, purulent material. Four and one-half months after the initial clinical signs, the rhinoceros was found dead.

Case 2: A 23-yr-old male rhinoceros, weighing approximately 1140 kg, developed diarrhea. It had been housed in the stall adjacent to Case 1. This animal developed diarrhea 2 mo after Case 1. Four fecal cultures were obtained over a 1-wk period. *Salmonella enterica subsp. arizonae serotype 44:Z4,Z32* was isolated. The flagellar (H) antigens were identical to those found in Case 1, but the somatic (O) antigen was untypeable. Treatment consisted of oral TMS (34200 mg) s.i.d. Two days later the rhinoceros became totally anorexic. Daily injections of 1.0 g ceftiofur (Naxcel,® SmithKline Beecham Corp., Philadelphia, PA 19101 USA) were administered for 17 days i.m. via pole syringe. Two weeks after onset of clinical signs, it was immobilized with 1.5 mg etorphine i.m. for hemogram, serum biochemistry and blood culture. Six liters of LRS and 3 L of LRS & 5% dex were administered i.v. Two grams of ceftiofur, 70 ml TMS 48% diluted in an equal volume of sterile water, 10 ml Multi B complex, 6000 IU vitamin E were administered i.m., and 1 g banamine was given i.v. Recovery was violent, with the rhinoceros slamming its head on the concrete floor as it struggled to rise.

Four weeks after the onset of diarrhea, the rhinoceros was found moribund. Blood was taken prior to euthanasia with 600 mg succinylcholine (Abbott Laboratories, North Chicago, IL 60064 USA) and 20 ml pentobarbital (6 g/ml) (Anpro Pharmaceutical, Arcadia, CA 91006 USA) administered i.v.

Case 3: A 13-mo-old male rhinoceros, weighing approximately 410 kg, and housed adjacent to Case

2, developed diarrhea. Fecal culture was positive for *Salmonella enterica subsp. arizonae serotype 44:Z4,Z32*, with the same flagellar and somatic antigen pattern as found in Case 2. It was given 12480 mg TMS p.o. s.i.d. for 3 wk. The stool began to become firm within 24 hr. *Salmonella enterica subsp. arizonae serotype 44:Z4,Z32* has not been isolated on further fecal cultures. The animal has remained healthy since that time.

Bacteriology

Bacterial culture samples were transported to the Denver Zoo Hospital's diagnostic laboratory on transport swabs (Culturette®, BBL, Becton Dickinson Microbiology Systems, Cockeysville, MD 21030 USA). The swabs were streaked on blood agar (TSA w/5% sheep blood), MacConkey agar and hektoen plates (Remel Microbiology Products, Lenexa, KS 66215 USA) and incubated at 35°C for 24 hr. To improve recovery rates, the samples were also placed in gram-negative enrichment broth (GN Broth, Remel), incubated for 24 hr and subcultured onto the three aforementioned types of agar. Blood for culture was placed in brain heart infusion media (Becton Dickinson) and incubated at 35 °C for 1 wk. One sample was maintained under anaerobic conditions with the top screwed closed and the second under aerobic conditions with the top vented. A duplicate set of blood cultures was sent to the Colorado State University (CSU) Diagnostic Laboratory.

Bacterial identification was made utilizing the BBL Crystal Enteric/NLF ID panels (Becton Dickinson). Isolates identified as a *Salmonella* spp. were sent to CSU in Port-A-Cul® transport tubes (Becton Dickinson) for confirmation. Confirmed samples were forwarded to the National Veterinary Services Laboratories (NVSL) for additional confirmation and serotyping.[2,3] NVSL utilized the Kauffman-White scheme for serotype identification.[6]

The nasal, vaginal and blood cultures from Case 1 contained isolates of *Salmonella enterica subsp. arizonae serotype 44:Z4,Z32*. A culture of thoracic fluid obtained from Case 1 contained isolates of *Salmonella enterica subsp. arizonae serotype 44:Z4,Z32*. Fecal cultures obtained from Case 2 and Case 3 contained isolates of *Salmonella enterica subsp. arizonae serotype 44:Z4,Z32*. Antemortem blood culture and postmortem culture of intestinal contents obtained from Case 2 contained no isolates of *Salmonella*.

Approximately 3.5 mo after the identification of salmonellosis in Case 1, a male rock hyrax housed in the pachyderm building developed a facial abscess. Culture samples from this abscess contained isolates of *Salmonella enterica subsp. arizonae serotype 44:Z4,Z32*. The hyrax subsequently died 2 wk later. Fecal cultures were obtained on the remaining animals housed in the pachyderm building every 2 wk for the next 4 mo. No additional isolates of *Salmonella* were recovered.

Gross Necropsy and Histopathology

Case 1 had multiple areas of healing cutaneous ulcers along the backbone and on all four extremities. A 1.5-cm-diameter ulcer was present on the caudal surface of the right hock, exposing the extensor tendons. A 10 × 15 cm ulcer was noted on the caudal surface of the left elbow. Both lesions were

thought to be due to pressure necrosis. The thoracic cavity contained a large volume of gray/black flocculent, odoriferous material. Generalized pleuritis was present and multifocal adhesions were present between the lungs and thoracic wall. The right dorsal caudal lung lobe contained a 25 × 35 cm abscess. Three gastric ulcers were present. No pericardial, perirenal or abdominal fat was present. The third digits of the left and right hind feet contained hemorrhage in the soft tissues adjacent to the distal phalanges.

Microscopically, neutrophils and histiocytes were aggregated throughout the lung tissue associated with numerous bacterial microcolonies. A distal phalanx submitted for histopathology had mild superficial bony resorption and mild epithelial hyperplasia of the derminal laminae. Moderate to marked hemosiderosis was present in the lung, liver and gastrointestinal tract.

Case 2 had multifocal areas of traumatically-induced excoriation on the face, elbows and hocks. The skin over the vertebrae was peeling. A superficial ulcer was also found on the caudal aspect of the left hock. Ulcers of the soles of both hind feet with necrosis on the lateral aspect of the second digits were also found. The coronary bands of these digits were also erythematous. As in Case 1, no abdominal, pericardial perirenal fat was noted. The mucosa of the stomach was hemorrhagic, ulcerated, and contained foul-smelling red liquid. The small and large intestines also contained blood.
Microscopically, multifocal areas of mild lymphoplasmacytic gastritis and mild catarrhal enteritis were noted. Mild hemosiderin deposition was present in lung, kidney, liver, spleen, heart, colon, and pancreas. Marked accumulations of hemosiderin deposition were noted in a visceral lymph node. The kidneys contained multifocal areas of interstitial fibrosis and tubular atrophy.

Discussion

Possible sources of *Salmonella* include contaminated water and nutritional supplements of animal origin, such as bone, fish or feather meal.[5,10] It has been estimated that 40% of feed products of animal origin are contaminated with *Salmonella*.[10] Infected animals are often a source of infection for other animals through fecal shedding. Other sources of *Salmonella* that have been reported and would need to be considered for this outbreak are rodents, birds, insects and reptiles.[1,5,9]

Reptiles are a common reservoir for *Salmonella* species.[4,8] Tokay geckos (*Gecko gecko*) living in the building were suspected as being a possible source for infection but a direct causal relationship could not be proven. Tokay geckos had previously been released into the pachyderm building for insect control. Two geckos were subsequently captured, euthanatized and had culture samples obtained from the intestinal tracts. *Salmonella eastbourne* was isolated, but *Salmonella enterica subsp. arizonae serotype 44:Z4,Z32* was not isolated.

Case 1 was probably shedding large numbers of *Salmonella* and contaminated the adjacent stalls, exposing the two males. To prevent exposure of the other animals, the building was quarantined and movement of animals and keepers within the building restricted. Foot baths were installed and coveralls, boots, and cleaning tools were dedicated to the area. After control measures were

implemented, with the exception of the rock hyrax, no additional cases of *Salmonella enterica subsp. arizonae serotype 44:Z4,Z32* occurred. Although it did not lead to identification of a causal relationship in this case, serotypic level identification of S*almonella* isolates is necessary in determining the source of the infection and planning control strategies.

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