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SALMONELLOSIS IN CAPTIVE BLACK RHINOCEROSES (*DICEROS BICORNIS*)

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Abstract: Salmonellosis caused by *Salmonella enterica* subspecies *arizonae* (formerly known as *Salmonella arizonae*) was diagnosed in three of five captive black rhinoceroses (*Diceros bicornis*) at the Denver Zoological Gardens. Two of the three animals died despite supportive treatment. The other two rhinoceroses remained asymptomatic and were negative for *Salmonella* spp. after serial fecal cultures. The source for the salmonellosis was never identified.

Key words: Black rhinoceros, *Diceros bicornis*, salmonellosis, *Salmonella enterica* subspecies *arizonae*.

INTRODUCTION

Salmonella spp. have been isolated and reported in the literature from captive pachyderms including African elephants (*Loxodonta africana*) and Asian elephants (*Elephas maximus*), Indian rhinoceros (*Rhinoceros unicornis*), white rhinoceros (*Ceratotherium simum simum*), and black rhinoceros (*Diceros bicornis*).[5,6,14,20,22,26] 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 that continued intermittently for 1 mo. After epistaxis was noted from the right nostril, the rhinoceros was immobilized with 2.5 mg etorphine (M99, Lemmon Co., Sellersville, Pennsylvania 18960, USA) administered i.m. via pole syringe. A 1-cm-diameter ulcer was found just inside the right nostril and was swabbed for bacterial culture. Blood was obtained for hemogram and serum chemistry. Treatment consisted of 20.1×10^6 units of penicillin G benzathine and penicillin G procaine, 9,000 IU vitamin E (Vital E™-300, Schering-Plough Animal Health, Kenilworth, New Jersey 07033, USA), and 20 mg dexamethasone (Vedco Inc., St. Joseph, Missouri 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, Missouri 64506, USA) were administered i.m. Trimethoprim and sulfamethoxazole (TMS, Geneva Pharmaceuticals, Inc., Broomfield, Colorado 80020, USA), 26,880 mg, was administered p.o. s.i.d. for 2 mo.

This rhinoceros became weaker and ataxic and developed a purulent vaginal discharge 2 wk later. It was again immobilized with 1.75 mg etorphine, and blood samples were taken for culture, hemogram, and serum chemistry. The vaginal discharge was also cultured. Lactated Ringer's (LRS) and 5% dextrose (5% dex) were administered (5 L i.v.), and 180 ml of 0.2% nitrofurazone, 4 L sterile saline, followed by another 180 ml nitrofurazone were administered through a sterile catheter into the uterus. Trimethoprim sulfadiazine (80 mg trimethoprim, 400 mg sulfadiazine/ml, 48%; Mortar & Pestle Pharmacy, Des Moines, Iowa 50310, USA), 57 ml diluted in an equal volume of sterile water, 9,750 mg amikacin (Amiglyde-V®, Fort Dodge Laboratories Inc., Fort Dodge, Iowa 50501, USA), 20 ml Multi B complex, 100 mg vitamin K_1, 9,000 IU vitamin E, 100 Units oxytocin (Phoenix Pharmaceutical Inc.), and 25 mg lutalyse® (The Upjohn Co., Kalamazoo, Michigan 49001, USA) were administered i.m.

The rhinoceros's clinical condition continued to deteriorate. Ulcers developed on the back, neck, face, and extremities, and the animal seemed to be in pain on its feet, groaning when it rose. Banamine (Schering-Plough Animal Health Corp.), 500 mg, was administered p.o. s.i.d. for 5 days, but the rhinoceros's condition did not improve. The caudal surface of the right hock became ulcerated and started to drain an odiferous purulent exudate. The rhinoceros was found dead 4.5 mo after clinical signs began.

Case 2

A 23-yr-old male rhinoceros, weighing approximately 1,140 kg, which occupied an adjacent off-exhibit stall to the rhinoceros in Case 1, developed diarrhea 2 mo after the female started showing signs of disease. Four fecal cultures were obtained over a 1-wk period. The rhinoceros became an-

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orexic 2 days later and refused to take oral TMS (34,200 mg). Daily injections of 1 g of ceftiofur sodium (Naxcel®, The Upjohn Co.) were administered i.m. s.i.d. for 17 days by pole syringe. Continued deterioration of the rhinoceros's condition prompted an immobilization with 1.5 mg etorphine administered i.m 2 wk after the onset of clinical signs. Blood was obtained for culture, hemogram, and serum chemistry. Six liters of LRS and 3 L of LRS and 5% dex were administered i.v. Two grams of ceftiofur sodium, 70 ml TMS 48% (Mortar & Pestle) diluted in equal volume of saline, 10 ml Multi B complex, and 6,000 IU vitamin E were administered i.m., and 1,000 mg banamine was given i.v.. Recovery from this immobilization was extremely violent, with the rhinoceros slamming its head on the concrete floor as it struggled to rise.

The rhinoceros was found moribund in lateral recumbency 4 wk after the onset of diarrhea. Blood was taken for hemogram and serum chemistry prior to euthanasia with 30 ml i.v. succinylcholine (Abbott Laboratories, North Chicago, Illinois 60064, USA) and 20 ml i.v. pentobarbital (Anpro Pharmaceutical, Arcadia, California 91006, USA).

Case 3

A 13-mo-old male rhinoceros, weighing approximately 410 kg, shared a back holding stall adjacent to the rhinoceros in Case 2. A week after the rhinoceros in Case 2 became ill, this rhinoceros developed diarrhea, and a fecal culture was performed. The rhinoceros was placed on 12,480 mg TMS p.o. s.i.d. for 3 wk. The stool began to form within 24 hr, and the rhinoceros showed no additional signs.

Bacteriology

Bacterial culture samples were transported to the Denver Zoo Hospital's Diagnostic Laboratory (DZHL) in transport medium (Culturettes®, BBL, Becton Dickinson Microbiology Systems, Cockeysville, Maryland 21030, USA). The swabs were streaked on blood agar (TSA with 5% sheep blood), MacConkey agar, and hektoen plates (Remel Microbiology Products, Lenexa, Kansas 66215, USA) and incubated at 35°C for 24 hr. To improve recovery rates, samples were also placed in gram-negative enrichment broth (Remel Microbiology Products), 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 Microbiology Systems) and incubated at 35°C for 1 wk. One blood culture 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 Diagnostic Laboratory (CSU).

For bacteria identification, the DZHL used the BBL Crystal enteric/NL ID panels (Becton Dickinson Microbiology Systems). Samples positive for *Salmonella* were sent to CSU in Port-A-Cul® transport tubes (Becton Dickinson Microbiology Systems) for confirmation. Samples positive for *Salmonella* spp. were forwarded to the National Veterinary Services Laboratories (NVSL, Ames, Iowa 50010, USA) by CSU for additional confirmation and serotyping.[7,8] The NVSL uses the Kauffman-White scheme for serotype identification.[16] The genus formerly known as "Arizona" has been reclassified to *Salmonella enterica* subspecies *arizonae* followed by the antigenic formula, which includes the somatic O antigen and the flagellar H antigens, respectively.[16] Somatic O antigens that are untypeable are reported as rough O.[16] Finally, isolates are differentiated biochemically.[7] Serotypic level identification is critical in determining the source of the infection and planning control strategies.

The nasal, vaginal, and blood cultures taken in Case 1 were positive for *S. enterica* subspecies *arizonae* serotype 44:Z_4, Z_{32}. A culture of the thoracic fluid in Case 1 was positive for *S. enterica* subspecies *arizonae* serotype 44:Z_4, Z_{32} at postmortem examination. Fecal cultures in Cases 2 and 3 were positive for *S. enterica* subspecies *arizonae* serotype untypeable rough O: Z_4, Z_{32}. Blood culture in Case 2 was negative. Postmortem cultures of intestinal contents in Case 2 were negative for *Salmonella.* Approximately 6 wk after fecal cultures for *Salmonella* in Cases 2 and 3 were positive, a male rock hyrax (*Procavia capensis*) from a glass-enclosed exhibit in the lobby of the Pachyderm Building developed a facial abscess. *Salmonella enterica* subspecies *arizonae* serotype 44:Z_4, Z_{23}, Z_{32} was recovered from the abscess, and the hyrax died 2 wk later. Feces were cultured on all animals in the Pachyderm Building twice a month for 4 mo and then monthly for an additional 4 mo. None was positive for *Salmonella* spp.

Because reptiles are a common reservoir host for *S. enterica* subspecies *arizonae,* Tokay geckos (*Gekko gecko*) released in the Pachyderm Building years earlier became suspects.[19,30] Two geckos were eventually captured and euthanatized, and their intestinal tracts were cultured for *Salmonella. Salmonella eastbourne* was cultured from a gecko found above the indoor hippopotamus exhibit pool adjacent to the rhinoceroses, but we did not recover *S. enterica* subspecies *arizonae.*

Table 1. Selected results from hemograms and serum chemistries of black rhinoceroses.

	Total WBC[a] ($10.2 \times 10^6/\mu l$; SD, ± 3.0)[b]	Neutrophils ($6.3 \times 10^6/\mu l$; SD, ± 2.4)	Lymphocytes ($3.1 \times 10^6/\mu l$; SD, ± 1.4)	Glucose (75.1 mg/dl; SD, ±26.3)	Phosphorus (4.5 mg/dl; SD, ±1.2)
Case 1					
Sample 1[c]	$4.9 \times 10^6/\mu l$	$2.6 \times 10^6/\mu l$	$1.4 \times 10^6/\mu l$	146 mg/dl	2.7 mg/dl
Sample 2	$4.8 \times 10^6/\mu l$	$3.8 \times 10^6/\mu l$	$0.5 \times 10^6/\mu l$	132 mg/dl	5.2 mg/dl
Case 2					
Sample 1	$4.3 \times 10^6/\mu l$	$3.2 \times 10^6/\mu l$	$0.8 \times 10^6/\mu l$	188 mg/dl	0.73 mg/dl
Sample 2	$16.3 \times 10^6/\mu l$	$15.6 \times 10^6/\mu l$	$0.3 \times 10^6/\mu l$	243 mg/dl	3.14 mg/dl

[a] WBC = white blood cells.
[b] Normal reference ranges are given in parentheses.[11]
[c] Samples 1 and 2 were taken 2 wk apart.

Clinical pathology

Two blood samples for hemograms and serum chemistries were taken 2 wk apart from the animals in Cases 1 and 2 (Table 1). The samples taken in Case 1 demonstrated a persistent leukopenia with neutropenia and lymphopenia over the 2-wk period when compared with normal reference values for this species.[11] Abnormal serum chemistry findings were a persistent hyperglycemia and an initial hypophosphatemia. The samples in Case 2 initially demonstrated a leukopenia with neutropenia and lymphopenia that changed 2 wk later to a neutrophilic leukocytosis with a lymphopenia and mild degenerative left shift (bands $0.35 \times 10^6/\mu l$). Persistent hyperglycemia and initial hypophosphatemia was also demonstrated in Case 2. The hypophosphatemia in both rhinoceroses was treated with 550 g steamed bone meal p.o. s.i.d. The second blood sample from Case 2 also demonstrated severe urinary dysfunction with a BUN of 282 mg/dl and creatinine 3.33 mg/dl. No blood sample was obtained in Case 3.

Gross necropsy and histopathology

The animal in Case 1 had multiple areas of healing ulcers along the back 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 a result of pressure necrosis. The thoracic cavity contained a large volume of gray/black flocculent odiferous material. Multifocal fibrinous pleuritis with adhesions between the lungs and pleura was present. The right dorsal caudal lung lobe contained a 25×35-cm abscess. Three gastric ulcers were present. No abdominal, pericardial, or perirenal fat was noted. The third digits of both hind feet were bluntly dissected, revealing 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 lung, liver, and gastrointestinal tract.

The animal in Case 2 had multifocal, traumatically induced excoriations 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, and coronary bands were erythematous. As in Case 1, no abdominal, pericardial, or perirenal fat was noted. The stomach mucosa was hemorrhagic and ulcerated and contained foul-smelling red liquid. The small and large intestines also contained blood.

Microscopically, mild, multifocal, lymphoplasmocytic gastritis and mild catarrhal enteritis were diagnosed. Mild hemosiderin deposition was noted in lung, kidney, liver, spleen, heart, colon, and pancreas, and marked accumulations were found in a visceral lymph node. Kidneys contained multifocal areas of interstitial fibrosis and tubular atrophy.

DISCUSSION

Salmonella spp. are gram-negative, motile, flagellated, nonencapsulated aerobic and facultatively anaerobic, rod-shaped bacteria that are members of the family Enterobacteriaceae.[24,25] Over 2,200 serotypes of *Salmonella* spp. have been described.[27]

The primary mode of *Salmonella* infection in domestic animals is ingestion of contaminated feces.[10,27] Cattle can shed 10^{10} *Salmonella* organisms

per gram of feces.[17] Some animals may be carriers, with the bacteria surviving intracellularly and subsequently recrudescing during stress.[27] Contaminated water and some nutritional supplements of animal origin, including bone, fish, and feather meal, are additional sources of *Salmonella*.[10,27] Forty percent of feed products of animal origin have been estimated to be contaminated with *Salmonella*.[27] Other sources of salmonellosis that have been reported and were considered for this outbreak are rodents, birds, insects, and reptiles.[1,10,21]

Salmonella enterica subspecies *arizonae* was first recovered in 1939 from reptiles collected near Tucson, Arizona, and was originally recommended to be classified as *Salmonella* sp. (*Dar-es-salaam* variety from Arizona).[3,16] The NVSL currently reports results using the description *Salmonella* followed by the antigenic formula and then (ARIZONA). This group of bacteria were formerly reported as *S. arizonae* or *Arizona* spp., so the (ARIZONA) helps establish the linkage to the previous nomenclature. Although the NVSL was unable to serotype the somatic O antigen in Cases 2 and 3, the probability is that the organism was the same as in Case 1. Antibiotic therapy can cause a change in the organism to untypeable rough O (NVSL, pers. comm.). The different flagellar antigen formula from that of the hyrax probably represents a different serotype (NVSL, pers. comm.).

Several studies have demonstrated that reptiles, including lizards, can be carriers of *Salmonella* spp. and that reptiles serve as reservoirs for *S. enterica* subspecies *arizonae*.[9,12,13,23,24,29] In two studies, 33% and 36% of captive lizards sampled were found to be positive for *Salmonella*.[4,15] In a survey of dead Tokay geckos, *Salmonella* spp. were isolated from 5% (5/100).[2] Another report found a 30% prevalence for *Salmonella* spp. in 90 wall geckos (Geckonidae) collected in Nigeria.[18]

Clinical syndromes of salmonellosis include enteritis or septicemia, which can present as an acute or chronic process.[10,17] Young, geriatric, immunosuppressed, or stressed animals are more susceptible to clinical salmonellosis.[10,17,21] Intractable diarrhea with an initial febrile period is common.[10] Meningitis, polyarthritis, or pneumonia may occur in animals that become bacteremic.[10]

Hemosiderin deposition was noted microscopically in Cases 1 and 2 at postmortem. Hemosiderosis has been frequently observed in captive black rhinoceroses but not white rhinoceroses.[28] The etiology for this iron accumulation in captive black rhinoceroses remains unknown.

Although we suspected that the Tokay geckoes living in the building were probably the source for the infection, a direct causal relationship could not be proven. After the female rhinoceros became infected and started shedding, contaminated fecal material could easily have been distributed to the adjacent stalls during cleaning, thus exposing and infecting the two males. To prevent exposure of the other animals, the rhinoceros side of the building was quarantined and movements in the building were restricted. Foot baths were established, and coveralls, boots, and cleaning tools were dedicated to the area. After control measures were implemented, with the exception of a rock hyrax, no additional case of *S. enterica* subspecies *arizonae* occurred.

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