Salmonella spp. Survey of Captive Rhinoceroses in U.S. Zoological Institutions and Private Ranches
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**SALMONELLA SPP. SURVEY OF CAPTIVE RHINOCEROSES IN U.S. ZOOLOGICAL INSTITUTIONS AND PRIVATE RANCHES**

David E. Kenny, V.M.D.

*Abstract:* A survey was mailed to 72 institutions in the USA requesting information on Salmonella spp. cultures in black rhinoceroses (*Diceros bicornis*), white rhinoceroses (*Ceratotherium simum*), and Indian rhinoceroses (*Rhinoceros unicornis*). Sixty-one institutions responded (85% return rate), with seven reporting positive cultures (11% prevalence rate; 10% if survey nonresponders had negative cultures). There were 17 positive cultures from 16 different animals, with nine different serotypes of *Salmonella* and 2 additional cultures identified to the group level.

**Key words:** *Salmonella* spp., black rhinoceroses, *Diceros bicornis*, white rhinoceroses, *Ceratotherium simum*, Indian rhinoceroses, *Rhinoceros unicornis*.

**INTRODUCTION**

*Salmonella* spp. are gram-negative, facultatively intracellular, often motile (flagellated), nonencapsulated and nonsporulating, aerobic and facultatively anaerobic rods that are members of the family Enterobacteriaceae. They can remain viable for extended periods of time in a variety of environments, including freezing conditions, >300 days in soil, >30 mo in dry fecal material, and >9 mo in contaminated aquatic environments. They are deactivated by heat and direct sunlight. Transmission typically occurs via the fecal–oral route. Multiple fecal cultures (three to five) may be necessary to recover *Salmonella*, especially in asymptomatic shedders.

There have been >2,200 *Salmonella* serotypes described. Serotypes are determined by the Kauffman-White scheme according to cell wall or somatic (O) antigens and flagellar (H) antigens. There continues to be much confusion over the appropriate nomenclature for *Salmonella* species and subspecies. The formal species nomenclature for subspecies I serotypes currently utilized by the Centers for Disease Control and Prevention (CDC) is *Salmonella enterica* followed by the subspecies and serotype. An example of the nomenclature for the serotype Typhimurium would be *Salmonella enterica* subspecies enterica serotype Typhimurium. Serotypes for the genus (subspecies IIIa) formerly known as “Arizona” are classified in a similar fashion except that serotypes utilize an antigenic formula instead of a name, e.g., *S. enterica* subsp. *arizona* ser. 44: Z4, Z2. For simplicity in this paper, *Salmonella* spp. will be reported by the genus designation *Salmonella* followed by the serotype, e.g., *S. typhimurium*, or in the case of “Arizona” by the subspecies and antigenic formula, e.g., *S. arizona* 44: Z4, Z2. This is the same format utilized in reports by CDC and the National Veterinary Services Laboratory. Serotyping is critical when conducting epizootiologic investigations.

**MATERIALS AND METHODS**

Following an epizootic of salmonellosis involving three of five captive black rhinoceroses (*Diceros bicornis*) at the Denver Zoological Gardens, a survey was formulated to estimate a prevalence rate for *Salmonella* spp. infection in rhinoceroses within the USA. For the purposes of this report, *Salmonella* prevalence is defined as a positive culture for *Salmonella* spp. from any of the 72 institutions surveyed cultured at any point in time. Seventy-two surveys were mailed with stamped, self-addressed envelopes. Names and addresses for institutions were obtained from the March 1997 Rhinoceros Studbook. According to the Studbook, there were 108 black rhinoceroses from two subspecies, 117 southern white rhinoceroses (*Ceratotherium simum*), and 43 Indian rhinoceroses (*Rhinoceros unicornis*) in institutions in the USA at that time. Sixty-one institutions returned the survey (85% return rate). The initial survey was designed to identify institutions that had positive cultures for *Salmonella* spp. from captive black rhinoceroses, white rhinoceroses, and Indian rhinoceroses. Institutions that responded affirmatively were recontacted for additional details. Positive cultures encompassed the years 1990 through 1997.

**RESULTS**

Seven of the responding institutions reported cultures of *Salmonella* spp. from rhinoceroses, which is a 11% prevalence rate for responders, or a 10% prevalence rate if it is assumed that the 11 nonresponding institutions were also negative. There were 17 *Salmonella*-positive cultures from 16 different rhinoceroses (Table 1). Nine different serotypes of *Salmonella* were isolated. Two *Salmonella*...
positive cultures were not identified past the group level. Five serotypes were cultured more than once; *S. arizonae* (*n* = 3), 11 *S. typhimurium* (*n* = 2; this is the pathogenic strain found in 60–70% of *Salmonella*-positive domestic equids), 16 *S. dublin* (*n* = 2, pathogenic strain in cattle), 16 *S. hadar* (*n* = 2), and *S. oranienburg* (*n* = 2). Four institutions had the same serotype cultured more than once. Ten isolates were from black rhinoceroses (six male and four female, \( \bar{x} \pm SD \text{ age} = 13.45 \pm 11.2 \text{ yr, range} = 1.5 \text{ mo to 30 yr} \)), four were from white rhinoceroses (one male and three female, \( \bar{x} \pm SD \text{ age} = 24.8 \pm 8 \text{ yr, range} = 13–30 \text{ yr} \)), and two were from Indian rhinoceroses (one male and one female, \( \bar{x} \pm SD \text{ age} = 12.5 \pm 0.7 \text{ days, range} = 12–13 \text{ days} \)). All positive cultures but one were obtained from feces or feces plus another source. Five animals had positive cultures from sources in addition to feces, including blood, lung and pleural fluid, vaginal and nasal discharge, liver, and stomach aspirate. One sample was obtained only from urine during post-mortem evaluation.

Six of the rhinoceroses were asymptomatic (three black and three white rhinoceroses). Eight of the rhinoceroses (six black, one white, and one Indian rhinoceroses) had diarrhea; in two of these animals the diarrhea was malodorous. Two of the animals were being quarantined when the *Salmonella* was recovered. One male had recently been separated from a female. Four of the black rhinoceroses presented with a reluctance to stand. Two of these animals at necropsy had histologic lesions compatible with laminitis. One of the Indian rhinoceroses calves presented with signs compatible with septic arthritis (joint il), with a left rear leg lameness at 10 days of age, but no diarrhea. Four of the black rhinoceroses had skin ulcerations grossly similar to the mucocutaneous lesions that have been previously described for this species. 16

Blood was obtained from five rhinoceroses (two black, one white, and two Indian rhinoceroses) that were presumed to have clinical salmonellosis (Table 2). A 24-yr-old female black rhinoceros presented with an initial hyperproteinemia, a persistent leukopenia (two samples, two wk apart) with neutropenia and lymphopenia, a persistent hyperglycemia, and an initial hypophosphatemia. 8 A 23-yr-old male black rhinoceros presented with persistent hemocencentration (two samples, two wk apart), an initial hyperproteinemia, initial leukopenia with neutropenia and lymphopenia followed by a marked leukocytosis with neutrophilia and lymphopenia, persistent hyperglycemia, and an initial profound hypophosphatemia. A 30-yr-old male white rhinoceros presented with hyperproteinemia and a low normal to borderline leukopenia and hyperglycemia. A 13-day-old Indian rhinoceros had an initial leukocytosis characterized by a neutrophilia and

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**Table 1.** Survey results from U.S. zoological institutions and ranches holding rhinoceroses with positive culture results for *Salmonella* spp.

<table>
<thead>
<tr>
<th>Rhinoceros species</th>
<th>Age</th>
<th>Sex</th>
<th>Identification</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black</td>
<td>24 yr</td>
<td>F</td>
<td><em>S. arizonae</em> 44: Z&lt;sub&gt;4&lt;/sub&gt;, Z&lt;sub&gt;12&lt;/sub&gt;</td>
<td>feces, vaginal discharge, blood, thoracic cavity</td>
</tr>
<tr>
<td>Black</td>
<td>23 yr</td>
<td>M</td>
<td><em>S. arizonae</em> Rough O: Z&lt;sub&gt;4&lt;/sub&gt;, Z&lt;sub&gt;12&lt;/sub&gt;</td>
<td>feces</td>
</tr>
<tr>
<td>Black</td>
<td>13 mo</td>
<td>M</td>
<td><em>S. arizonae</em> Rough O: Z&lt;sub&gt;4&lt;/sub&gt;, Z&lt;sub&gt;12&lt;/sub&gt;</td>
<td>feces</td>
</tr>
<tr>
<td>Black</td>
<td>2.25 mo</td>
<td>F</td>
<td><em>S. derby</em></td>
<td>stomach aspirate, feces</td>
</tr>
<tr>
<td>Black</td>
<td>14 yr</td>
<td>F</td>
<td><em>S. mbandaka</em></td>
<td>feces</td>
</tr>
<tr>
<td>Black</td>
<td>1.5 mo</td>
<td>M</td>
<td><em>S. javiana</em></td>
<td>feces</td>
</tr>
<tr>
<td>Black</td>
<td>13.5 yr</td>
<td>M</td>
<td>group B, no serotype</td>
<td>feces, liver, lung</td>
</tr>
<tr>
<td>Black</td>
<td>1.5 yr</td>
<td>M</td>
<td><em>S. typhimurium</em></td>
<td>feces</td>
</tr>
<tr>
<td>Black</td>
<td>12 yr</td>
<td>M</td>
<td><em>S. dublin</em></td>
<td>feces</td>
</tr>
<tr>
<td>Black</td>
<td>1.5 yr</td>
<td>M</td>
<td><em>S. dublin</em></td>
<td>feces</td>
</tr>
<tr>
<td>White</td>
<td>30 yr</td>
<td>M</td>
<td><em>S. gaminara</em></td>
<td>feces</td>
</tr>
<tr>
<td>White</td>
<td>30 yr</td>
<td>F</td>
<td><em>S. hadar</em></td>
<td>feces</td>
</tr>
<tr>
<td>White</td>
<td>13 yr</td>
<td>F</td>
<td><em>S. hadar</em></td>
<td>feces</td>
</tr>
<tr>
<td>White</td>
<td>23 yr</td>
<td>F</td>
<td>group E, no serotype</td>
<td>feces</td>
</tr>
<tr>
<td>Indian</td>
<td>12 days</td>
<td>M</td>
<td><em>S. oranienberg</em></td>
<td>feces, blood</td>
</tr>
<tr>
<td>Indian</td>
<td>17 days</td>
<td>F</td>
<td><em>S. oranienberg</em></td>
<td>feces, peritoneal and pericardial fluid</td>
</tr>
</tbody>
</table>

* F = female, M = male.
lymphopenia and hyperglycemia. Following 2 wk of antibiotic therapy, this picture had improved to a moderate leukocytosis with a marginally elevated neutrophilia and a lymphocytosis. A 16-day-old, Indian rhinoceros presented moribund, hemocoagulatred, hypoproteinemic, and leukopenic with neutropenia and lymphopenia.

Five rhinoceroses (four black and one white rhinoceroses) were given a trimethoprim–sulfadiazine combination (TMS, either the sulfadiazine/trimethoprim or sulfamethoxazole/trimethoprim combination) p.o. by tablet or paste at 30 mg/kg q12–24 hr. A black rhinoceros became anorectic and refused oral medication, so it was given 1 g of the cephalosporin ceftiofur (Naxcel®. Merck, Rahway, New Jersey, USA) by i.m. injection q24 hr. A 12-day-old Indian rhinoceros calf was treated with 300 mg of ceftiofur by i.m. injection q12 hr for 7 days.

Disease duration for eight animals with suspected clinical salmonellosis ranged from 3 days to 3.5 mo. Six of the 16 rhinoceroses did not survive. Four of the six deaths were attributed to salmonellosis, and three of those appeared to have involved septicemia. The first, a 24-yr-old female black rhinoceros, had positive blood cultures (S. arizonae 44: Z4, Z5) and positive cultures of antemortem nasal and vaginal discharge. The same Salmonella serotype was cultured from a gray/black flocculent odiferous material in the thoracic cavity at postmortem. In addition, the rhinoceros had multifocal fibrinous pleuritis with adhesions, gastric ulcerations, multifocal skin ulcerations, laminitis, and no epiplioic, perirenal or pericardial fat. In the second animal, a 13.5-yr-old male black rhinoceros, there was marked edema and hemorrhage of the small bowel, multifocal lymphoplasmocytic enterocolitis with mucosal ulcerations and fibrinous plaques, 3 L of ascitic fluid, focal suppurrative pneumonia, enlarged and hemorrhagic mesenteric lymph nodes, and multifocal skin ulcerations. Positive cultures were obtained for this animal from liver and lung for Salmonella group B (not identified past the group level). The third animal with lesions compatible with septicemia was a 17-day-old Indian rhinoceros calf that had a 4-day history of diarrhea. Necropsy revealed a suppurrative omphalitis with intra-arterial gram-negative rods, moderate chronic-active multifocal suppurrative interstitial pneumonia, mild multifocal granulomatosus hepatitis, moderately diffuse chronic-active fibrinosuppurative epicarditis with rare intraleisonal rods, a moderate to severe diffuse chronic-active fibrinosuppurative polyserositis of the pleural, pericardial, and peritoneal cavities and the right carpus, and mild subacute to chronic-active multifocal suppurrative colitis with severe focal ulcerations and submucosal abscessation. The fourth animal was a 30-yr-old male black rhinoceros that at necropsy had mild, multifocal, lymphoplasmocytic gastritis and mild catarrhal en-
teritis.11 This animal also had multifocal skin ulcerations, laminitis, and no epiploic, perirenal, or pericardial fat. The three black rhinoceroses also had various degrees of hemosiderosis. The same three black rhinoceroses had a substantial quantity of sand/gravel in the cecum at postmortem examination.

**DISCUSSION**

There are two reports of salmonellosis in free-ranging black rhinoceroses during translocation operations in East Africa.34 In the first report, the rhinoceros started exhibiting strange behavior 4 days after capture and died 2 days later.34 A pure culture of *S. typhimurium* was isolated from the liver. In the second report, the rhinoceros developed pneumonia and died 10 days following capture and transport.3 Salmonella weltevreden was recovered from heart blood, spleen, and intestine. Both cases were associated with capture and transport. It is unclear whether the animals contracted *Salmonella* at capture or were carriers that developed clinical disease.

*Salmonella* infections are typically mild, self-limiting, and of short duration.4,7 In domestic animals, clinical salmonellosis is most common in stressed, old, young, or debilitated animals.10,21,32 Four of the clinically affected animals in this report were young (12 days, 13 days, 2.25 mo, and 13 mo), three were older animals (23, 24, and 30 yr), and one was a young adult (13.5 yr). Some factors that can stress domestic animals are transportation, prior antibiotic therapy, diet changes, crowding, pregnancy, weaning, and anesthesia and surgery.10,18,20,25,31

Sources of *Salmonella* spp. infection include asymptomatic carrier animals that may begin to shed, contaminated soil, stagnant water, feed (fish, meat, bone, and feather meal), milk, rodents, birds, and insects.10,20,27,31 More than 40% of these meal products in the USA may be contaminated with *Salmonella*.27 Many of these conditions and products are present at most zoological parks.

*Salmonella* epizootics occur in close confinement because of the enormous number of organisms that can be shed in feces from infected animals in crowded environments with poor sanitation.10,32 Two of the zoological institutions surveyed experienced *Salmonella* epizootics. Three black rhinoceroses managed in adjacent holding stalls experienced salmonellosis, resulting in two deaths.11 At the second institution, pregnant Indian rhinoceroses (*n* = 4) were moved, as they neared parturition, from a 70-acre exhibit into a smaller holding complex located within the exhibit (boma). All the births occurred over a 12-wk period. Each female and its baby were kept in separate areas of the boma but were shifted for cleaning and had contact with feces from the other females and calves. Two of the calves became infected with the same serotype of *Salmonella*, and one calf died. Fecal cultures from seven additional Indian rhinoceroses that were either in the boma or in the surrounding exhibit yard were negative for *Salmonella*. Suspected sources included fecal shedding from a stressed dam or infected rodents or birds that frequent the boma, but the actual source was never identified.

Clinical syndromes observed in the affected rhinoceroses included asymptomatic shedders, acute and chronic enterocolitis, and septicemia. Six (three black and three white rhinoceroses) of the surveyed animals were asymptomatic. One of the asymptomatic animals was in quarantine, and the other five rhinoceroses were cultured because there was a positive animal in the building. One animal that did not appear to have clinical salmonellosis did intermittently shed the organism over a 13-mo period prior to euthanasia for leukoencephalomalacia.32 Eight infected rhinoceroses presented with diarrhea. One rhinoceros had just been shipped and developed diarrhea while in quarantine. A male had diarrhea after being separated from a female. A calf developed diarrhea after its mother had diarrhea, but the dam never had a positive *Salmonella* culture.

Reported hemogram changes in domestic large animals with salmonellosis include elevated fibrinogen and an initial leukopenia with neutropenia and lymphopenia. In severe cases, there may be a degenerative left shift and an initial elevation in hematocrit and total protein from dehydration followed by lowered total protein from a protein losing enteropathies due to mucosal damage. Leukopenia, hemoconcentration, and hyperproteinemia were seen in some hemograms (Table 2).18,20,26,27,29,31 Three of the rhinoceroses had a leukopenia with neutropenia and lymphopenia. A fourth rhinoceros had a low normal to borderline leukopenia. Two rhinoceroses had degenerative left shifts on the day they were discovered moribund (bands: 0.35 and 0.16 × 109/μL, respectively). These individuals were also hemoconcentrated. Two adult rhinoceroses were hyperproteinemic, and two Indian rhinoceroses calves were hypoproteinemic.

Two of the black rhinoceroses had a hypophosphatemia, which has been previously reported in sick black rhinoceroses (Table 2).8 The significance of hypophosphatemia in sick black rhinoceroses is unknown. Three of the rhinoceroses were hyperglycemic. A black rhinoceros that became moribund had severe urinary dysfunction suggestive of pre-
renal azotemia (blood urea nitrogen = 282 mg/dl, creatinine = 3.35 mg/dl).

The use of antibiotics to treat salmonellosis is controversial. Treatment might prolong the carrier and shedding state, further contaminating the environment, without shortening the course of the disease. Most humans recover without antibiotics, and antibiotic therapy might also encourage the development of resistance.1,2,4,5,18,30 Antibiotics may alter the enteric microflora to favor the development of Salmonella spp., thereby worsening the disease process.2,5,20,24,26 Prior antibiotic therapy may also lower the infective dose of salmonella that is capable of causing symptomatic salmonellosis.4 Drug resistance can develop quickly, particularly if antibiotics are overutilized on the premises, due to the transfer of plasmids between Salmonella spp. and other members of the family Enterobacteriaceae.20,31

In general, Salmonella spp. are sensitive in vitro to TMS compounds, aminoglycosides, nitrofurans, ceftiofur, and anoxicillin.27 Both TMS and ceftiofur were utilized in some of the affected rhinoceroses. Dosing recommendations for TMS in the horse are 30 mg/kg q12–24 hr p.o.22 Black rhinoceroses may be at increased risk of developing intravascular hemolysis from sulfonamides because of a species sensitivity to oxidative stress.19 Ceftiofur has in vitro and in vivo activity against S. choleraesuis in swine and S. typhimurium in mouse disease models (Manufacturer’s package insert, 1996). The recommended dosing schedule for the horse varies considerably from 0.45 to 4.4 mg/kg s.i.d. (Manufacturer’s package insert, 1996).22 Multidrug-resistant Salmonella strains may require treatment with other more expensive cephalosporins and fluoroquinolones.31

Supportive care also includes fluid and electrolyte replacement and the judicious use of small doses of nonsteroidal antiinflammatory agents such as flunixin meglumine (Banamine®, Schering-Plough Animal Health, Kenilworth, New Jersey 07033, USA).20,27 Fluid therapy is critical in combating dehydration and correcting acid–base disturbances and electrolyte abnormalities.20,26,27 Animals with severe dehydration and diarrhea of >10 days have a poor survival rate due to severe intestinal mucosal damage, infarction, laminitis, and septicemia.11 Fluid therapy for a rhinoceros is a daunting proposition, although some individuals have been trained to accept aural venipuncture. An aural i.v. line might be established in a cooperative individual, but maintaining patency and supplying fluids in the volumes required to meet replacement and daily maintenance is probably unrealistic. Two of the black rhinoceroses21 that eventually succumbed to salmonellosis were given fluids (5.5 and 9 L, respectively) through bilateral catheters in the medial radial veins of the forelimbs during an immobilization.15

Six of the animals reported in this survey died, but only four of the deaths were probably directly attributable to salmonellosis. The other two non-survivors probably died from causes other than salmonellosis (euthanasia for leuкоencephalomalacia12 and undetermined death in a chronically ill animal). In equids, the most severely affected tissues are typically the distal small intestine, cecum, and ascending bowel.31 Gross lesions observed are fluid-filled bowel, congestion, ulceration, fibrinonecrosis, sloughing of the intestinal mucosa, and enlarged and hemorrhagic mesenteric lymph nodes.23 Histologically, there is hemorrhage, edema, necrosis, and leukocyte infiltration.9 In septic animals there may be pneumonia, arthritis, and meningitis.9 Many of these lesions were noted at postmortem in the four deaths directly attributable to salmonellosis.

CONCLUSIONS

Rhinoceroses with salmonellosis should be placed in isolation and quarantined if possible, foot traffic minimized to essential personnel, footbaths with a Salmonella spp. effective germicidal agent at entry and exit points and caretaker clothing and tools dedicated to the area. New arrivals should have three to five negative fecal cultures performed as part of the quarantine evaluation. It would be very difficult to rationalize withholding antibiotics from such a valuable endangered species even if there is only the slightest chance that therapy may positively affect the outcome. In the epizootic at the Denver Zoo, the rhinoceros that became septic died despite oral TMS therapy, the animal that became anorectic and was treated with i.m. ceftiofur died despite treatment, but the young animal that had diarrhea but was still eating and was treated promptly with oral TMS survived. It would be best to initiate antibiotic therapy in a rhinoceros with diarrhea and positive culture results for Salmonella spp.

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LITERATURE CITED


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