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CASE SERIES: CLINICAL SALMONELLOSIS IN FOUR BLACK RHINOCEROS (*DICEROS BICORNIS*) CALVES

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Abstract: Although *Salmonella* spp. infection has been identified in captive and free-ranging rhinoceros, clinical cases in black rhinoceros (*Diceros bicornis*) calves have not been described. This case series describes clinical salmonellosis in four black rhinoceros calves. Two calves developed self-limiting diarrhea, recovering after treatment. The other two cases were fatal. One of the fatal cases had a short clinical course, whereas the other case was protracted, with signs reflecting multiple organ system involvement. In all cases, diagnosis was by fecal culture and/or quantitative polymerase chain reaction. A variable clinical presentation, which is typical for salmonellosis in domestic hoofstock, was a feature of these rhinoceros cases. Similarly, postmortem pathology in black rhinoceros calves was consistent with domestic neonatal ungulates with salmonellosis. Potential predisposing factors for infection were considered to be primiparity of the dam and failure of passive transfer in the calf. The case investigation included attempts to identify the source of infection, which was aided by organism serotyping. In one case, the patient's dam and another conspecific in the facility were shown to be asymptomatic shedders of the organism strain responsible for disease in the calf. Further surveillance of captive rhinoceros *Salmonella* spp. carrier status is needed to inform screening recommendations for this taxa.

Key words: Black rhinoceros, Calf diarrhea, Clinical salmonellosis, *Diceros bicornis*, *Salmonella*, Septicemia.

INTRODUCTION

Salmonella spp. infections have been reported in nearly all vertebrate taxa. The pathogen is ubiquitous within the environment and remains viable and infectious in soil for almost a year and in fecal matter for almost 3 yr.[12,13,22,26,30,33] *Salmonella* spp. are gram-negative, facultative intracellular, motile, aerobic, rod bacterial members of the family *Enterobacteriaceae*, with over 2,500 serotypes described.[12,19,22]

Salmonellosis is an infection from a bacteria of the genus *Salmonella*. Consequences of infection are variable and range from mild disease and elimination of infection through self-limiting enteritis to fulminating fatal infection.[13] Clinical salmonellosis confirmed by fecal culture has been reported in multiple species of captive and wild rhinoceros of varying ages.[3,14,15,24,38,39] Seventeen of the 27 published salmonellosis cases in the family Rhinocerotidae were in black rhinoceros (*Diceros bicornis*). Ten of these were clinically affected, with diarrhea as the main clinical sign. Only two of the clinically affected cases were in black rhinoceros under the age of 13 mo.[3,14–16,20,39] There are currently no published cases describing clinical salmonellosis in black rhinoceros calves.

The purpose of this case series is to describe the epidemiology, diagnosis, and management of clinical salmonellosis in captive black rhinoceros calves under 3 mo of age.

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CASE REPORTS

Case 1

A 2-mo, 85-kg male black rhinoceros from a multiparous dam at a zoological institution was evaluated for white-yellow, pasty, malodorous diarrhea 10 days after the dam had developed diarrhea. The calf was treated with pureed apple product orally for 3 days for nutritional support. Bovine *Escherichia coli* monoclonal antibody product (Pro-Immune99-ProVentra, GalaGen, Inc., Arden Hills, Minnesota 55112, USA; 40 ml p.o., s.i.d. for 2 days) was administered with poor compliance. Both the dam and calf remained bright and alert with normal appetite throughout the course of diarrhea. Over the following month, stools from both individuals were intermittently loose, ranging from pasty to formed.

Diagnostic results on the dam's feces included positive *Coronavirus* culture/isolation and negative serial cultures for *Salmonella* spp. (Texas Veterinary Medical Diagnostics Laboratory [TVMDL], College Station, Texas 77841, USA). *Salmonella* spp. culture procedure at this laboratory consists of direct inoculation of tergitol agar with 24-hr incubation at 37°C, enrichment in tetrathionate broth with 12- to 24-hr incubation at 37°C, inoculation of brilliant green agar with 24-hr incubation at 37°C, enrichment in Rappaport-Vassiliadis (RV) R10 broth for 12- to 24-hr incubation at 37°C, and finally inoculation of xylose-lysine-tergitol (XLT-4) agar with 24-hr incubation at 37°C before final examination (bacteriologist at TVMDL, pers. comm.). Diagnostic testing of the calf's feces at TVMDL were negative on *Cryptosporidium* direct immunofluorescent antibody assay (DFA, detecting *Cryptosporidium* spp. oocysts), giardia DFA (detecting *Giardia lamblia* cysts), and virus visualization by electron microscopy, but *Salmonella* spp. fecal culture was positive, serotyped as *Salmonella enterica* serovar *javiana* at the National Veterinary Services Laboratory (NVSL, Ames, Iowa 50010, USA).[7] Both dam and calf were placed in isolation, and the calf was prescribed flunixin meglumine (Banamine Paste, Merck Animal Health, Baton Rouge, Louisiana 70814, USA; 1.1 mg/kg p.o., s.i.d. for 2 days) and trimethoprim/sulfamethoxazole (Tribrissen 400, Merck Animal Health; 33 mg/kg p.o., s.i.d. for 7 days), chosen based on antimicrobial sensitivity results. Due to the inability to separate calf from dam, medications were not completely administered. Over the following 2 wk, the calf's feces improved in consistency and he continued to nurse.

Repeated fecal cultures (TVMDL) performed 1 mo later were *Salmonella* spp. negative. Surveillance fecal cultures (TVMDL) of other rhinoceros in the zoo were *Salmonella* spp. negative. The calf showed no further clinical signs of salmonellosis and recovered uneventfully. The origin of the causative *Salmonella* spp. agent was unknown.

Case 2

A 2-mo, 90-kg female black rhinoceros from a multiparous dam at a zoological institution was presented with loose stool progressing to diarrhea over the previous 48 hr while maintaining her appetite and remaining bright. She was treated empirically with florfenicol (Nuflor, Merck Animal Health; 20 mg/kg i.m., q4d twice), flunixin meglumine (Banamine, Merck Animal Health; 0.17 mg/kg i.m. once), and bismuth subsalicylate (Pepto-Bismol, Proctor & Gamble, Cincinnati, Ohio 45202, USA; 30 ml p.o., q24h for 18 days). Serial diagnostics including in-house fecal floatation and microscopy, in-house direct fecal smears and microscopy, and fecal viral culture (in rhesus monkey kidney [MA104] cells, for presence of *Rotavirus* or *Coronavirus*) at TVMDL were negative. Fecal bacterial cultures (TVMDL) were *Salmonella* spp. positive, serotyped as *Salmonella enterica* serovar *newport* by the NVSL.[7]

Based on the antimicrobial susceptibility pattern, amikacin (RX Veterinary Products, Westlake, Texas 76262, USA; 8 mg/kg i.m., q24h for 12 days) was initiated with continued oral bismuth subsalicylate. Despite antimicrobial administration and improvement in fecal consistency, fecal cultures remained *Salmonella* spp. positive.

Due to improvement in fecal consistency, all medications were discontinued; 11 days later, normal feces were noted. Fecal cultures (TVMDL) 1 yr later were *Salmonella* spp. negative. The dam maintained normal fecal consistency. The source of exposure to *Salmonella* spp. is unknown.

Case 3

A 2-month, 101.5-kg male black rhinoceros from a multiparous dam at a zoological institution was presented moribund, with malodorous hemorrhagic feces; hemorrhagic mucoid feces had been noted 24 hr prior. Produce offered to both dam and calf had recently changed, but no other diarrhea cases were noted in the vicinity. Emergency measures including cardiopulmonary resuscitation, intubation with mechanical ventilation, and epinephrine administration (Vedco, Inc., St. Joseph, Missouri 64507, USA; 5 ml i.c., 3×; 5 ml i.t., 2×) were unsuccessful, and the calf died.

A complete blood count (CBC) revealed severe anemia (hematocrit [HCT], 9.4%; reference interval [RI] = 23.8%–46.6%), mild inflammatory leukogram (13,500/μl; RI = 4,350–12,790/μl); band cell count (2,430/μl; RI = 20–120/μl), and thrombocytopenia (decreased platelet estimate; RI = 6,400–490,000/μl).[34] Serum biochemistry revealed moderate azotemia (blood urea nitrogen [BUN], 157 mg/dl; RI = 7–22 mg/dl; creatinine, 5.4 mg/dl; RI = 0.6–1.6 mg/dl), severe hyponatremia (107 mmol/L; RI = 124–140 mmol/L), hypoproteinemia (3.3 g/dl; 60-day foal RI = 5.2–6.5 g/dl), and hypoalbuminemia (1.7 g/dl; 60-day foal RI = 2.7–3.5 g/dl).[2,34] *Campylobacter* spp., *Clostridium difficile,* and *C. perfringens* were not isolated in fecal cultures performed at IDEXX

(Irvine, California 92614, USA). Fecal enzyme-linked immunosorbent assay (ELISA) for *C. difficile* toxins (IDEXX) was negative. Fecal culture (IDEXX) was positive for *Salmonella enterica* serovar *lomalinda*, serotyped by the NVSL.[7]

Necropsy revealed severe multifocal mucosal ulcerations with adhered fibrin and clotted blood within the stomach, cecum, and colon. There was diffuse pallor of thoracic and abdominal viscera. Histopathology revealed severe, subacute to chronic ulcerative enteritis, typhlocolitis, and proctitis. Acute ulcerative gastric lesions were attributed to stress. Acute necrotizing inflammation was noted in the liver, heart, and kidneys.

A final diagnosis of ulcerative enteritis, typhylocolitis, and *Salmonella* spp. septicemia was made. Septicemia is the presumed cause of the severe multiorgan inflammation and the serum biochemical derangements. The source of the *Salmonella* spp. infection was unknown, as no surveillance testing was performed. The dam never showed signs of diarrhea or systemic illness before or after the calf's death, and her feces were not screened for *Salmonella* spp.

Case 4

A 1-mo, 53-kg female black rhinoceros from a primiparous dam at a private ranch was presented to a referral institution with a 24-hr history of lethargy, loose stools, and inappetence. The calf was placed in isolation at presentation. Physical examination parameters, mentation, and feces were within normal limits. A neurologic examination revealed an abnormal ambulatory pattern and stance with forelimb extension and a ventral head position due to cervical muscle dystonia. A central neurologic lesion in C6 to T2 was suspected.

Supportive treatment was initiated consisting of flunixin meglumine (Prevail, Vet One, Boise, Idaho 83705, USA; 1.1 mg/kg p.o., q12h for 21 days), omeprazole (Gastrogard, Merial LLC, Duluth, Georgia 30096, USA; 4 mg/kg p.o., q24h for 34 days), and *Saccharomyces booulardii* (FullBucket Probiotic, Animal Stewards International LLC, Dennis, Texas 76439, USA; 50 billion CFUs p.o., q12h for 34 days). She was maintained on San Diego Zoo (SDZ) rhinoceros milk formula (15% body weight at six feedings per day).[35] Medial saphenous venipuncture with butorphanol (Torbugesic, Zoetis Inc., Kalamazoo, Michigan 49007, USA; 0.17 mg/kg i.m./i.v.) or midazolam (BD Rx Inc., Franklin Lakes, New Jersey 07417, USA; 0.1 mg/kg i.m./i.v.) sedation was performed every other day. Significant hemogram and biochemistry parameters compared with case 3 are summarized in Figures 1 and 2.

Initial serum biochemistry revealed hyperglobulinemia (4.7 g/dl; 30-day foal RI = 1.8–3.7 g/dl).[2] A CBC revealed a mild inflammatory leukogram (white blood cell [WBC] count, 16,500/μl) with hyperfibrinogenemia (1,400 mg/dl; RI = 0–785 mg/dl), which normalized over the next few days.[34] Plasma ammonia (<15 mmol/L; lab equine RI = <15 mmol/L; TVMDL), serum copper (2.71 μg/ml; RI = 1.18–5.64 μg/ml; TVMDL), serum selenium (228 μg/L; RI = 139.3–251.5 μg/L; TVMDL), and serum zinc (1.01 μg/ml; RI = 1.0–1.38 μg/ml; TVMDL) levels were normal, with slightly increased serum vitamin E levels (1.12 μg/ml; RI = 0.72–0.82 μg/ml; TVMDL).[5,6] Whole blood polymerase chain reaction (PCR) testing (TVMDL) for *Anaplasma* spp., *Babesia* spp., *Bartonella* spp., *Borelia burgdorferi*, *Rickettsia* spp., *Ehrlichia* spp., and *Francisella tularensis* were negative. Plaque reduction neutralization tests (Cornell University Animal Health Diagnostic Center, Ithaca, New York 14853, USA) showed negative titers for eastern/Venezuelan equine encephalitis (1:10) and an exposure titer for western equine encephalitis (1:100). Serial blood cultures were negative. Urinalysis revealed a moderate proteinuria (300 mg/dl) and severe leukocyturia (>100 WBCs), consistent with a urinary cystitis, which resolved.

Serial fecal *Clostridium difficile* A and B toxin ELISA and *Clostridium perfringens* virulence gene/enterotoxin PCR tests (TVMDL) were negative. In-house fecal floatation and microscopy were negative. *Salmonella* spp. quantitative PCR (qPCR) fecal assay and culture (TVMDL) were positive, serotyped as *Salmonella enterica* serovar *typhimurium* var 5 by the Pennsylvania Animal Diagnostic Laboratory System (PADLS, University Park, Pennsylvania 16802, USA).[7] The clinical diagnosis was enteric salmonellosis with progressive systemic sequelae, including a suspected focal spinal cord lesion. The animal was hospitalized for intensive management and further clinical investigation. Serial fecal *Salmonella* spp. cultures and qPCR assays (TVMDL) remained positive while the animal was hospitalized.

Intravenous catheters were rotated between auricular, medial saphenous, and medial radial veins. Meropenem (Novation LLC, Irving, Texas 75038, USA; 20 mg/kg i.v., q6h for 12 days) was chosen based on antimicrobial susceptibility results, as well as the drug's large volume of

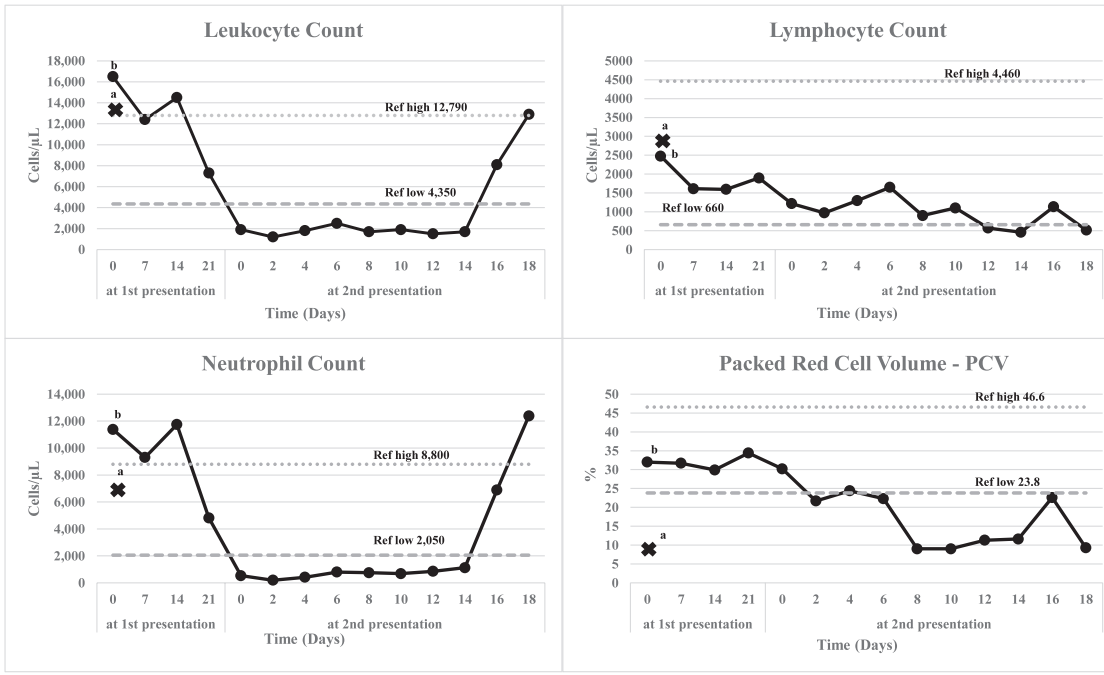

Figure 1. Significant hemogram values from two black rhinoceros (*D. bicornis*) calves[a,b] with clinical *Salmonella* spp. septicemia compared with reference intervals.[33] [a]Case 3, single measurement taken at presentation, denoted by bold "X." [b]Case 4, serial measurements over two hospitalizations, denoted by bold "O" and lines.

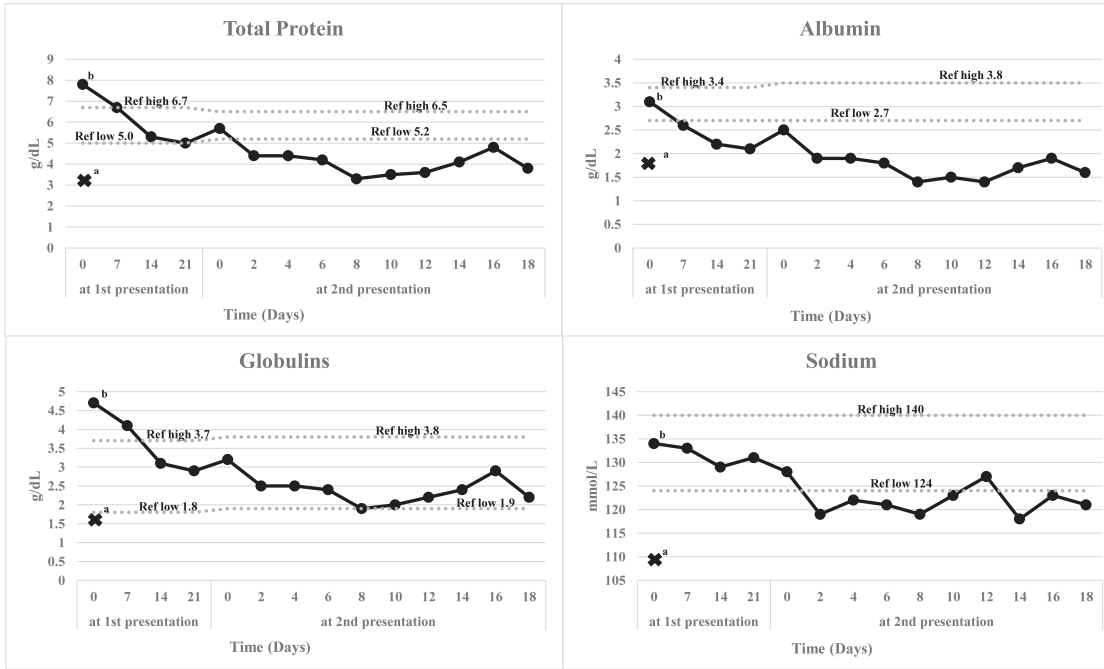

Figure 2. Significant serum biochemistry values from two black rhinoceros (*D. bicornis*) calves[a,b] with clinical *Salmonella* spp. septicemia compared with reference intervals.[2,33] [a]Case 3, single measurement taken at presentation, denoted by bold "X." [b]Case 4, serial measurements over two hospitalizations, denoted by bold "O" and lines.

distribution and high lipophilicity allowing blood–brain barrier penetration. Vitamin E (Elevate Vitamin-E, KPP LLC, Versailles, Kentucky 40383, USA; 20 IU/kg p.o., q24h for 34 days), and docosahexaenoic acid/eicosapentaenoic acid (DHA/EPA; Welactin Canine, Nutramax Laboratories Inc., Edgewood, Maryland 21040, USA; 66 mg/kg p.o., q24h for 34 days) were added to her treatment. Serial abdominal ultrasonography was unremarkable, as were thoracic, abdominal, and cervical radiographs. After 48 hr of supportive treatment, advanced imaging was pursued due to the persistence of neurologic abnormalities. The calf was premedicated with butorphanol and midazolam and induced/maintained on sevoflurane inhalant (Piramal Critical Care Inc., Bethlehem, Pennsylvania 18017, USA; 1%–5% to effect in 100% oxygen). A contrast-enhanced computed tomography (CT) scan using iohexol (Omnipaque, GE Healthcare Inc., Princeton, New Jersey 08540, USA; 115 ml i.v.), magnetic resonance imaging (MRI) using gadolinium (Magnevist, Bayer HealthCare Pharmaceuticals Inc., Whippany, New Jersey 07981, USA; 0.1 mmol/kg i.v.), and an atlanto-occipital ultrasound-guided cerebrospinal fluid (CSF) tap were performed.

Brain and cervical region MRI scans revealed a discrete area of enlargement and intramedullary cervical spinal cord (C6 to T2) T2 hyperintensity, a left forebrain focal T2 hyperintense area, mild contrast enhanced meningeal thickening, and relative CSF T2 heterogeneity with hypointense strands (Fig. 3). Thoracic and abdominal CT scans revealed a fluid-filled small intestine. The enlargement and hypersensitivity in the cervical spinal cord and meninges were consistent with an infectious etiology (using domestic equine foal parameters), most likely due to *Salmonella* spp. infection and subsequent inflammation based on previous diagnostics. Atlanto-occipital CSF tap using a 22-gauge spinal needle showed a colorless to white, hazy fluid with marked septic neutrophilic pleocytosis (WBC count, 5,590/μl; 91% neutrophils containing bacilli). CSF culture (TVMDL) grew the same previously cultured enteric strain of *Salmonella* spp. (serotyped at PADLS).[7] Diagnosis of a cervical spinal cord lesion with underlying meningoencephalomyelitis caused by *Salmonella* spp. infection was made.

Normal ambulation, improved head posture, and increased coordination were noted after 7 days of treatment. Appetite and weight gain (2 kg/day) remained normal. After 12 days of meropenem therapy, vascular phlebitis limited intravenous catheter patency and replacement; she was transitioned to cefpodoxime proxetil (Putney Inc., Portland, Maine 04101, USA; 10 mg/kg p.o., q12h for 21 days) based on antimicrobial susceptibility results and penetration of drug into the central nervous system (CNS). Oral transfaunation using enteric pathogen-negative black rhinoceros feces was added to the treatments.

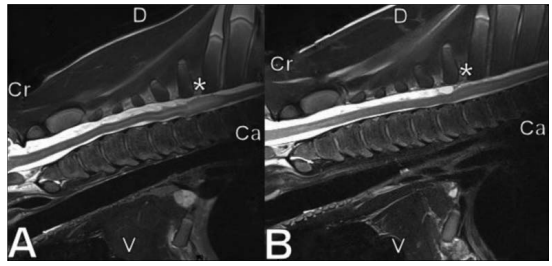

Figure 3. Serial magnetic resonance imaging (MRI) scans of *Salmonella* spp.-induced cervical spinal lesion in a black rhinoceros (*D. bicornis*) calf before and after treatment (case 4). (**A**) T2 sagittal contrast-enhanced MRI image showing discrete area of cervical spinal cord (C6 to T2) enlargement and intramedullary hyperintensity before treatment. (**B**) T2 sagittal contrast-enhanced MRI image showing improvement in the enlargement and hyperintensity of the intramedullary cervical spinal cord lesion 18 days after treatment. Cervical spinal lesion denoted by asterisk (*). Orientation of the sagittal images is denoted by Cr = cranial, Ca = caudal, D = dorsal, and V = ventral.

An MRI scan under general anesthesia (as previously described) was performed 18 days after the initial scans. Improvement in the hyperintensity and enlargement of the cervical spinal cord was seen, suggesting decreased severity of the cervical lesion and underlying meningoencephalomyelitis (Fig. 3). The cervical–dorsal subarachnoid space was widened, suggestive of subarachnoid cyst formation or spinal cord atrophy, and contained T2 hypointense strands, suggestive of fibrinous material. Thoracic and abdominal CT scans were normal. The calf was discharged after a 27-day hospitalization due to the absence of ascertainable neurologic deficits and normal bloodwork; fecal *Salmonella* spp. qPCRs and cultures, however, remained positive. One week of cefpodoxime proxetil, *Saccromyces booulardii*, omeprazole, vitamin E, and DHA/EPA fatty acid supplement was prescribed.

The calf was presented 13 days after initial discharge with a 24-hr history of hemorrhagic diarrhea. Cardiorespiratory parameters and mentation were normal. Frequent malodorous diarrhea containing blood clots and sloughed mucosa was seen. *Giardia lamblia* and *Cryptosporidium*

fecal DFA, fecal floatations/microscopy, *C. difficile* toxin ELISA, and PCR for *C. perfringens* virulence gene/enterotoxins were negative at TVMDL. Fecal *Salmonella* spp. culture and qPCR (TVMDL) were positive, serotyped at PADLS as the previously cultured strain. CBC revealed a severe leukopenia (1,200/μl) and neutropenia (192/μl; RI = 2,050–8,800/μl).[34] Serum biochemistry was within normal limits. Intravenous catheters were rotated between the auricular, medial radial, and medial saphenous veins as needed. Ampicillin (Polyflex, Boehringer Ingelheim Vetmedica Inc.; 20 mg/kg i.v., q6h for 18 days), amikacin (Amiglyde-V, Zoetis Inc.; 25 mg/kg i.v., q24h for 18 days), metronidazole (Baxter Healthcare Corporation, Deerfield, Illinois 60015, USA; 10 mg/kg i.v., q12h for 16 days), omeprazole, flunixin meglumine (0.25 mg/kg i.v., q6h for 18 days), and *Saccharomyces booulardii* were administered for enterocolitis. Oral water (50% requirements) and electrolytes/dextrose (Pedialyte, Abbott Laboratories, Lake Bluff, Illinois 60044, USA; 50% requirements) providing 90 ml/kg fluids were offered. After 48 hr, introduction (5% body weight) and gradual increase (15% body weight) of SDZ milk formula was initiated.

The calf continued to have malodorous, intermittently hemorrhagic diarrhea that cultured *Salmonella* spp. positive for the remainder of her hospitalization. Phlebotomy every other day was facilitated by midazolam or butorphanol sedation. Serial CBC and serum biochemistries showed continued leukopenia and the development of worsening hypoproteinemia (3.6 g/dl; 60-day foal RI = 5.2–6.5 g/dl), hypoalbuminemia (1.4 g/dl; RI = 2.7–3.6 g/dl), and hyponatremia (119 mmol/L).[2,34] Coagulation parameters were clinically unremarkable, revealing slightly increased prothrombin time (12.4 sec; foal RI = 9.0–9.8 sec), decreased partial thromboplastin time (13.6 sec; RI = 34.8–46.8 sec), decreased antithrombin-III activity (102%; RI = 125.9%–207.1%), and decreased D-dimer (0.153 μg/ml; RI = 0.9–6.1 μg/ml).[1] Serial abdominal ultrasonography revealed a fluid-filled colon. Colloidal oncotic support was initiated (6% Hetastarch, Novation LLC; 5 ml/kg i.v. as needed) and equine plasma containing *Salmonella* spp. antibodies (Immunoglo-7000, Mg Biologics, Ames, Iowa 50014, USA; 1 L i.v. q24h for 2 days) was administered due to clinical decline; no transfusion reactions were noted. Gastrointestinal support using sucralfate (Teva Pharmaceuticals USA, Sellersville, Pennsylvania 18960, USA; 1g/100 lbs p.o., q4h for 9 days), ditri-octahedral smectite (Equine Biosponge, Platinum Performance, Buellton, California 93427, USA; 27.6g p.o. q4h for 5 days), and misoprostol (Cytotec, Pfizer Inc, New York City, New York 10017, USA; 2 mcg/kg p.o. q8h for 9 days) was initiated. Antiendotoxic dosages of polymyxin-B (Fresenius Kabi USA LLC, Lake Zurich, Illinois 60047 USA; 3000 IU/kg in 500 ml 0.9% NaCl, i.v. q24h for 5 days) and fluid support (Lactated Ringer's, Zoetis Inc.; 500 ml i.v. q2h for 5 days; 5% dextrose, Zoetis Inc.; 1.5% in fluids) were administered.

The calf showed signs of intermittent colic 12 days after admittance, but maintained normal mentation. Serial bloodwork showed progression of anemia, with normal serum iron (174 μg/dl; RI = 148–316 μg/dl; Kansas State Veterinary Diagnostic Laboratory [KSVDL], Manhattan, Kansas 66506, USA), low ferritin on sandwich ELISA (117 ng/ml; KSVDL), and low transferrin saturation (52%; KSVDL).[5,25,34] Due to severe anemia (packed red-cell volume [PCV], 9%), black rhinoceros whole blood transfusions (aseptically collected at origin ranch; 1 L, i.v. twice) were administered, with minimal improvement.[34] Equine plasma containing *Salmonella* spp. antibodies (Immunoglo-Enteric, Mg Biologics; 500 ml; p.o. q24h for 4 days) was offered.

Due to poor venous access and worsening condition, a central venous catheter (Arrow-Howes Quad-Lumen Central Venous Catheter, Teleflex Inc., Wayne, Pennsylvania 19080, USA; 8.5 F) was surgically placed into the right jugular vein under general anesthesia 14 days after presentation, with no complications. A CBC showed leukocytosis (12,900/μl) with neutrophilia (12,384/μl) and lymphopenia (516/μl; RI = 660–4460/μl).[34] All oral nutrition was discontinued, and total parenteral nutrition (Clinimix 5% amino acids in 25% dextrose, mixed with Intralipid 20% lipid emulsion, Baxter Healthcare Corporation; i.v.) was initiated along with intravenous fluid therapy using serial blood glucose monitoring for a slow increase. Low-molecular-weight heparin (Fragmin, Pfizer Inc.; 40 IU/kg s.c. q24h for 2 days) was initiated due to oral, suggesting disseminated intravascular coagulation (DIC).

Abdominal ultrasonography revealed hyperechoic gas shadows within the small intestinal wall consistent with pneumatosis intestinalis 17 days after presentation. Feces remained *Salmonella* spp. positive on culture (TVMDL) with the same strain noted previously. Despite treatments, the patient made a severe clinical decline, and hu-

mane euthanasia was elected 18 days after the second presentation.

Necropsy revealed diffuse abdominal petechiation, mild interstitial pneumonia, and an edematous small intestine. Mucosal ulcers were present throughout the gastrointestinal tract, with blood clots in the distal colon and rectum. Histopathology confirmed the ulcerative enteritis and revealed widespread septic emboli and infarctions in multiple organs. Marked granulation tissue thickening of the cervical dura and diffuse spinal cord inflammation were consistent with the previous diagnosis. Trigeminal, enteric, and bladder ganglia had lymphoplasmacytic inflammation. The final diagnosis was enterocolitis with terminal septicemia and nonsuppurative meningoencephalomyelitis.

Serial fecal *Salmonella* spp. qPCR and cultures of all seven rhinoceros from the origin ranch were performed for surveillance testing at TVMDL. Fecal samples were serially positive on both tests in two adult females, including the calf's dam. *Salmonella enterica* serovar *typhimurium* var 5 was serotyped at PADLS from both individuals, suggesting these individuals were the source of exposure of the calf.[7]

DISCUSSION

Clinical salmonellosis in black rhinoceros calves in these cases varied in presentation, from simple diarrhea to multiorgan infection, septicemia, and acute death. Fecal *Salmonella* spp. culture and PCR testing were performed on other animals in direct and indirect contact with the affected calf in two of the four cases, resulting in the identification of the source of infection for one of two animals. This sort of surveillance testing is an important part of disease investigation and should be targeted at other animals in direct and indirect contact with the case, the local environment, and any potential fomites. Three of the four calves described here contracted *Salmonella* spp. from unknown sources, whereas the fourth likely contracted it from her asymptomatic carrier dam. Epidemiologic tracing using serotypes is important when investigating cases of salmonellosis to help determine the primary source.

Salmonella spp. transmission is through the fecal–oral route with either ingestion of infected feces or contaminated food or water sources.[12,26,33] The infective dose is determined by the strain virulence and host susceptibility, ranging from a few hundred organisms in susceptible individuals to millions in healthy animals.[19,36] *Salmonella* spp. possess virulence factors that allow for mucosal adhesion and invasion, production of enterotoxins stimulating increased intestinal fluid secretion, and up-regulation of host immune response. Infection requires invasion and establishment within the intestinal epithelial cells. *Salmonella* spp. lipopolysaccharides cause inflammatory cell recruitment and cytotoxic mediator release, initiating the systemic response. Toxin release and local inflammation allows bacterial translocation from the gastrointestinal tract to the regional lymph nodes, leading to a disseminated bacteremia and clinical sepsis.[12,19] Intracellular survival in macrophages, hepatocytes, and other cells can cause a persistent infection.[27,36]

The severity of disease ranges from asymptomatic colonization to severe systemic illness.[19] Asymptomatic carriers are most commonly survivors of the septicemic or enteric form of the disease, intermittently shedding bacteria during periods of stress, such as pregnancy, diet changes, transportation, anesthesia, surgery, or antibiotic use.[18] Similar stressors can cause gastrointestinal microbiota disruption, reducing efficiency of the microbial barrier and decreasing colonization resistance, predisposing naïve animals to *Salmonella* spp. infection and potentiating dissemination in active infections.[19]

Young animals are very susceptible to clinical salmonellosis, developing both enteric and septicemic forms of the disease.[22,36] Pathogen exposure occurs most commonly through contaminated colostrum and milk, surface fecal contamination of teats, human/equipment fomites, or environmental contamination.[17,22,27,36] Asymptomatic shedding of *Salmonella* spp. by adult animals is often the primary route of neonatal exposure.[17,22] Failure of passive maternal antibody transfer is one of the largest risk factors in neonatal ungulates, commonly predisposing them infections and subsequent septicemia.[12,22,27,29,36] Clinical signs of neonatal *Salmonella* spp. infection includes loose stools, hemorrhagic diarrhea, septicemia, pneumonia, meningitis, and septic arthritis/physitis.[19,22]

Diagnosis of *Salmonella* spp. infection is performed on fecal samples or infected tissues or body fluids by culture or PCR testing, with increased specificity (nearly 100%) using PCR.[4,28] Culture can be used to confirm PCR-positive cases and to perform serotyping.[4,12,19,28,37] Although comparison of fecal PCR and culture as screening tests in rhinoceros showed no significant difference in detecting prevalence, more studies are needed to determine appropriate *Salmonella* spp. screening recommendations in

rhinoceros.[20] For equids, three to five serial PCR tests confirmed by culture are performed to increase sensitivity and specificity in the diagnosis of *Salmonella* spp. infection in both symptomatic and asymptomatic individuals.[12]

Treatment of clinical salmonellosis in adult horses relies on supportive care; antimicrobial therapy is controversial and may prolong the carrier state, allowing longer periods of environmental contamination without shortening the course or intensity of the disease process.[12,19,26,27,32] Antibiotics alter enteric microflora, disrupting the normal microbiota and favoring growth of invaders such as *Salmonella* spp., which may worsen the disease process.[26,32] In neonates, early aggressive antibiotic therapy (based on antimicrobial susceptibility results, location of infection, and lipophilicity characteristics of the drug) used in combination with intensive supportive care is recommended to limit dissemination and decrease the persistence of *Salmonella* spp. within the host tissues, especially in cases of sepsis.[8,17,22,27,36]

In both nonfatal cases presented here, 2-mo-old calves developed diarrhea lasting from 22 to 30 days, with fecal consistency varying from paste to liquid diarrhea. Previously reported cases of clinical salmonellosis in rhinoceros lasted from 3 days to 3.5 mo.[14] Diarrhea in the described cases occurred in the absence of systemic illness and was treated with antibiotics. Case 1 had resolution of diarrhea despite poor medication compliance, signifying presumed self-limiting enteric salmonellosis. Case 2 was successfully treated with parenteral antibiotics (amikacin) and showed clinical resolution soon after antibiotic discontinuation. In cases of salmonellosis-associated diarrhea in rhinoceros where pathogen antimicrobial sensitivity is not known, trimethoprim/sulfamethoxazole (TMS) is the preferred antibiotic for empirical treatment based on *Salmonella* spp. antimicrobial sensitivity trends, with ceftiofur being the second choice if oral administration was not feasible.[14]

The decision to use antibiotics for the treatment of salmonellosis must be made with consideration to the risk of inducing carrier status in the host and the potential for development of antibiotic resistance. Antibiotic therapy is generally reserved for patients with severe or systemic disease or those at high risk, such as neonatal animals, immunocompromised individuals, or adult animals with multiorgan infection.[8,11,32,33] Prophylactic antibiotic treatment to prevent dissemination is provided for most cases of salmonellosis in domestic neonatal ungulates and should be considered for high-risk neonatal rhinoceros.[12,22,27,36]

Case 3 presented with a rapid clinical decline, whereas case 4 had a chronic clinical course; both cases resulted in a fatal outcome. The fatalities were likely due to bacterial dissemination and sepsis leading to severe metabolic derangements with bacterial endotoxemia, systemic inflammatory response syndrome (SIRS) leading to multiple organ dysfunction syndrome (MODS), and subsequent DIC.[10,27,36] Meropenem use in case 4 was initiated following bacterial dissemination into the CNS due to its high safety index and high lipid solubility, allowing excellent penetration into the CNS; cefpodoxime proxetil was chosen for similar reasons. Persistent *Salmonella* spp. septicemia, suspected after the subsequent *Salmonella* spp. enterocolitis diagnosis, likely led to the initiation of an endogenous cascade of inflammatory mediators resulting in SIRS and MODS, causing the decline of the calf. Bacterial encephalomeningitis is seen in 8%–10% of neonatal *Salmonella* spp. septicemia infections of calves and foals requiring early aggressive treatment.[8,21] Failure of passive transfer (FPT) after birth was suspected in this calf, but due to the age at presentation, this could not be confirmed. History of a primiparous rhinoceros cow supports this contributing factor, as primiparous bovids generally produce low quality and/or volume of colostrum, increasing susceptibility of their calves to FPT.[9,23]

Reported clinicopathologic abnormalities in both domestic ungulates and rhinoceros species with salmonellosis included hemoconcentration, initial leukocytosis, leukopenia, neutropenia, and lymphopenia, with a severe degenerative left shift, hypoproteinemia (in calves), and anemia from hemorrhagic diarrhea were noted in advanced disease.[14,15,17,19,22,27] Clinicopathologic findings in cases 3 and 4 were similar to these reported abnormalities. The leukopenia is due to high sequestration of cells within the affected gastrointestinal tract, and the hypoproteinemia, hypoalbuminemia, and hyponatremia result secondarily from the *Salmonella* spp.-induced protein-losing enteropathy.[5,8,9,15,18,21]

Pathologic findings of terminal *Salmonella* spp. cases in adult rhinoceros include ulcerative and hemorrhagic gastroenteritis, lymphoplasmacytic gastritis and enterocolitis, focal pneumonia, submucosal or subserosal petechial hemorrhages, and fibrinous membranes adhered to mucosal surfaces, which are similar to lesions found in adult domestic ungulates.[14,15,19,22] Young domestic

animals may also develop severe gastritis (often attributed to stress), as well as meningitis.[36] Pathologic lesions in the described terminally affected black rhinoceros calves were similar to those reported in both adult and neonatal rhinoceros, as well as neonatal domestic ungulates, with widespread lymphoplasmacytic and histiocytic inflammation, hepatitis with hepatic necrosis suggestive of septicemia, severe gastritis, and one case of meningitis.[14,22,24,31,36] One calf also showed evidence of DIC, a common sequela of terminal septicemia in neonatal ungulates, with widespread petechiation and embolic lesions within numerous abdominal organs.[8,10,14,31,36]

In conclusion, clinical salmonellosis in black rhinoceros calves follows a similar progression to disease as in domestic neonatal ungulates, with variations of presentations including self-limiting diarrhea, disseminated disease, or acute death. Despite appropriate treatment and supportive care, septicemia and death of rhinoceros calves can occur due to the insidious nature of *Salmonella* spp. and the associated inflammatory response. A diagnosis can be made through culture or PCR, the latter of which may be a useful screening tool during outbreaks. Recommended treatment of rhinoceros calves depends on the signs and severity of the disease, but generally includes broad-spectrum antibiotic therapy to decrease dissemination, chosen based on antimicrobial sensitivity results and location of infection (with highly lipophilic drugs chosen in neurologic cases to facilitate CNS penetration). If antimicrobial sensitivity results are not available, empirical treatment with TMS or ceftiofur can be initiated. Supportive care includes gastroprotectants, probiotics, transfaunation, antiendotoxic therapy, correction of acid/base and electrolyte abnormalities, colloidal support, fluid therapy, and parenteral nutrition if indicated. Serial blood values may help guide therapy. Multilumen central venous catheter placement is helpful and can be attempted in severe cases. Potential predisposing factors for infection in black rhinoceros calves include failure of passive transfer and asymptomatic *Salmonella* spp.–positive carrier status of dam. Primiparity of the dam may increase the risk of partial or full failure of passive transfer, leading to increased risk for calf infection with *Salmonella* spp. Knowledge of *Salmonella* spp. carrier status in rhinoceros breeding situations is important for development of precautionary protocols for preventing calf infection. Further surveillance of captive rhinoceros *Salmonella* spp. status is needed to enhance screening recommendations.

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