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LEPTOSPIRA INFECTION IN TWO BLACK RHINOCEROSES (*DICEROS BICORNIS MICHAELI*)

Donald L. Neiffer, V.M.D., Edwin C. Klein, V.M.D., Dipl. A.C.V.P., and Carolee Wallace-Switalski, D.V.M.

Abstract: Two black rhinoceroses (*Diceros bicornis michaeli*) developed clinical leptospirosis without hemolytic crises. The first rhinoceros presented with peracute depression, anorexia, rear leg trembling, dysuria, glucosuria, gastrointestinal discomfort, and decreased fecal output and died within 12 hr. Necropsy and histopathology revealed lesions within multiple organs. Leptospirosis was diagnosed postmortem based on positive fluorescent antibody staining of liver. The second rhinoceros presented 2 mo later with similar signs. It survived with treatment and was diagnosed with leptospirosis based on serology using microscopic agglutination testing and detection of urinary antigen using a fluorescent antibody technique. *Leptospira kirschneri* serovar grippotyphosa was postulated as the etiologic agent, with transmission probably occurring through wallow contamination by wild raccoons (*Procyon lotor*).

Key words: Black rhinoceros, *Diceros bicornis michaeli*, leptospirosis, *Leptospira kirschneri* serovar grippotyphosa.

INTRODUCTION

Infections with the spirochete bacterium *Leptospira* sp. have been reported in captive and wild black rhinoceroses (*Diceros bicornis*)[13,24,33,35,42] and a fetal greater Asian one-horned rhinoceros (*Rhinoceros unicornis*).[31] Serologic surveys have identified exposure in wild and captive black and white (*Ceratotherium simum*) rhinoceroses and a captive greater Asian one-horned rhinoceros.[13,16,24,34,35,48] In the black rhinoceros, *Leptospira* infection has been associated with hemolytic anemia.[13,17,24,33–35] In this report, we describe two cases of leptospirosis in black rhinoceroses in the absence of a hemolytic crisis.

CASE REPORTS

Case 1

A 27-mo-old female eastern black rhinoceros (*Diceros bicornis michaeli*) presented for abnormal behavior. The rhinoceros, which usually stood when the keepers arrived each morning, remained recumbent when approached. Once it did stand, muscle tremors were noted, particularly in the rear legs. The rhino failed to defecate on rising, as it normally did, and no feces were present in the regular overnight latrine area. Inspection of the exhibit yard revealed decreased fecal output on the day before presentation. The animal was anorexic, although normal appetite and behavior had been noted the day before.

From the Pittsburgh Zoo, One Wild Place, Pittsburgh, Pennsylvania 15206, USA (Neiffer, Wallace-Switalski); and the Department of Laboratory Animal Resources, University of Pittsburgh, Pittsburgh, Pennsylvania 15261, USA (Klein). Present address (Neiffer): Walt Disney World, Animal Programs, P.O. Box 10000, Lake Buena Vista, Florida 32830, USA.

Over the next 3 hr, the animal remained standing with normal vital signs (rectal temperature 38.9°C, respiratory rate 44 breaths/min, and heart rate 64–67 beats/min). Dysuria was noted, with urine produced in a large stream rather than multiple sprays. Following urination, the animal appeared to be more comfortable. A free catch sample had a specific gravity of 1.025, marked glucosuria (>2,000 mg/dl), occasional fine granular casts, 0–3 white blood cells (WBC)/high-power field, and occasional red blood cells (RBC).

Blood was collected from the ear vein. Based on normal ranges for the species,[23] there was normoglycemia (64 mg/dl), hypoalbuminemia (1.7 g/dl), hyperglobulinemia (6.7 g/dl), and elevated serum aspartate aminotransferase (AST) (199 IU/L), alanine aminotransferase (ALT) (37 IU/L), and creatine kinase (CK) (2,569 IU/L). Hematology revealed a leukocytosis (15.20×10^3 cells/μl) with absolute neutrophilia (10.49×10^3 cells/μl) and monocytosis (1.37×10^3 cells/μl). There were elevations of packed cell volume (PCV; 50%), hemoglobin (Hgb; 17 gm/dl), and total RBC count (6.60×10^6 cells/μl), suggesting dehydration. Differential diagnoses included gastrointestinal torsion or sand impaction, acute renal failure, and clostridial enterotoxemia.[9]

At 7 hr postpresentation, staff attempted unsuccessfully to administer analgesics and mineral oil orally. At 9 hr, when the rhinoceros began lying down more frequently, 2 L of mineral oil was administered p.o. with the animal in sternal recumbency. Further attempts to administer medications were unsuccessful. All staff left the building at 10 hr postpresentation because the animal was becoming increasingly agitated. Although treatment with flunixin meglumine via remote injection, monitoring the animal overnight while attempting oral min-

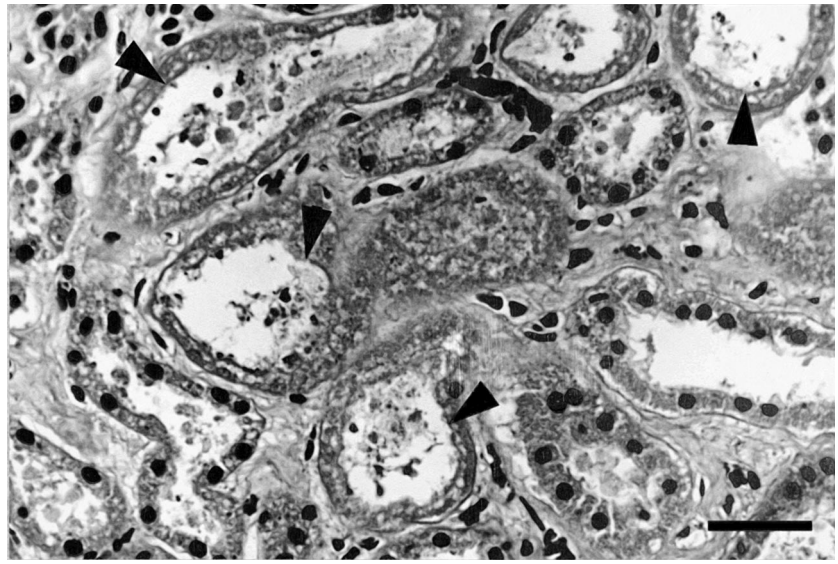

Figure 1. Kidney from a black rhinoceros that died of leptospirosis. Acute tubular necrosis is present (arrowheads). H&E. Bar = approximately 46 μm.

eral oil administration, and subsequent immobilization of the animal were planned, it was found dead 12 hr postpresentation.

At necropsy 14 hr later, the 617-kg animal was in good body condition. Epicardial ecchymoses and multifocal hyperemia of the omentum were noted. The stomach and entire large intestine were full of hay and browse, but the small intestine contained only gray-green fluid. The jejunum and proximal ileum were slate gray with multifocal areas of hyperemia. Hemorrhages were present in the rectal mucosa. The kidneys were swollen and pale. Enteritis with ileus and enterotoxemia were diagnosed preliminarily.

Representative tissue samples were fixed in 10% neutral buffered formalin, trimmed, routinely processed, sectioned at 6 μm, and stained with hematoxylin and eosin (H&E). Anaerobic and aerobic cultures of the endocardium and feces from the rectum, ileum, and colon were submitted. *Escherichia coli*, *Acinetobacter baumanii*, and beta hemolytic *Streptococcus* sp. were isolated from the endocardium. Feces were submitted to Cornell Diagnostic Laboratory (CU; College of Veterinary Medicine, Cornell University, Ithaca, New York 14852, USA) for *Clostridium perfringens* enterotoxin and *Clostridium difficile* toxin screening. Feces tested positive for *C. difficile* toxin.

Histologic examination of the colon revealed multifocal perivascular and perineural inflammatory infiltrates within the submucosa composed primarily of neutrophils and lymphoplasmacytic cells. Severe autolysis of the small intestine precluded morphologic evaluation. The degree of autolysis was greater than expected for the postmortem interval, suggesting a preexisting enteritis or enhancement of autolysis by an antemortem ischemia due to multiorgan failure and cardiovascular collapse. In either event, ileus and loss of gut integrity may have resulted in transmural migration of organisms,[28] leading to bacteremia. Other significant histologic lesions included severe acute multifocal myocarditis with a moderate to marked granulocytic infiltrate with interstitial edema. Cardiac myofibers had irregular staining with contraction band formation, consistent with degeneration and necrosis. Severe acute to peracute renal tubular necrosis of the proximal tubular epithelium was present, characterized by increased cytoplasmic eosinophilia, loss of cellular margin definition, and frequent nuclear pyknosis, karyorrhexis, and some karyolysis (Fig. 1). Some tubular mineralization was noted. No inflammation was evident, and tubular ectasia was rarely seen. The liver had extensive, periportal inflammatory infiltrates with occasional extension through the limiting plate. The infiltrates were comprised of neutrophils and mononuclear cells, including lymphocytes, occasional plasma cells, and histiocytes (Fig. 2). Individual hepatocyte degeneration was seen in areas of inflammation. Some acute congestion of centrilobular areas and mild hepatocellular vacuolar fatty change were also noted.

Sections of frozen liver were sent to the National Animal Disease Center (NADC, Ames, Iowa

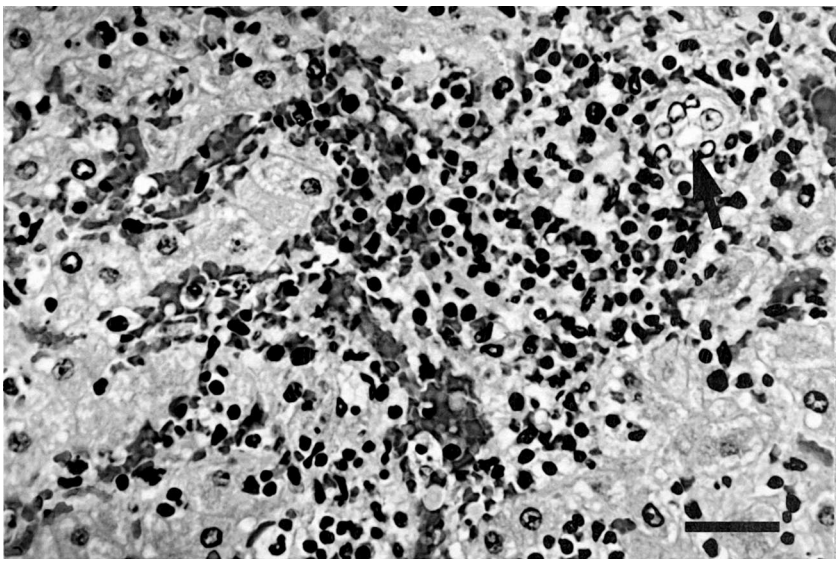

Figure 2. Liver from a black rhinoceros that died of leptospirosis. Portal region of hepatic lobule has a prominent mixed inflammatory infiltrate extending across the limiting plate. Bile duct within portal area is marked by arrow. H&E. Bar = approximately 46 μm.

50010, USA) for leptospiral antigen fluorescent antibody (FA) testing. Leptospires were detected in the liver tissue, indicating an acute leptospiral infection. Warthin–Starry silver stain was applied to sections of liver and kidney, but no spirochetes were observed.

Banked serum samples collected 15 and 6 mo prior to presentation were submitted to CU for microscopic agglutination testing (MAT) for antibodies against five pathogenic *Leptospira* serovars: four *L. interrogans* serovars (pomona, hardjo, icterohaemorrhagiae, and canicola) and *L. kirschneri* serovar grippotyphosa. Positive antibody titers were detected in the sample collected 6 mo prior to presentation (hardjo 1:100, icterohaemorrhagiae 1: 3,200, canicola 1:800).

Case 2

A 2.5-yr-old male eastern black rhinoceros weighing approximately 600 kg presented 2 mo later with depression, reluctance to stand, rolling, and anorexia. This animal had been previously housed with rhinoceros 1. A free-catch urine sample was collected and submitted to CU for *Leptospira* FA testing; an in-house urinalysis also performed revealed no abnormalities. Fresh stool was submitted for enteric pathogen culture and *C. perfringens* enterotoxin and *C. difficile* toxin screening. Blood collected from the ear vein was submitted for complete blood count (CBC), serum biochemical analysis, and *Leptospira* MAT. Banked serum collected 27 days, 40 days, and 7 mo prior to the acute onset of disease was submitted for *Leptospira* MAT as for rhinoceros 1. Treatment was initiated within 1 hr and included flunixin meglumine (Banamine, Schering-Plough Animal Health, Omaha, Nebraska 68103, USA; 1.25 mg/kg i.m.), ceftiofur sodium (Naxcel, SmithKline Beecham, Philadelphia, Pennsylvania 19101, USA; 5 mg/kg i.m.), and *C. perfringens* C and D antitoxin (Clostratox-BCD, Grand Laboratories, Larchwood, Iowa 51241, USA; 130 ml administered as divided i.m. and s.c. injections). Within 50 min of treatment with flunixin meglumine, the rhinoceros exhibited reduced discomfort and an increased appetite. An oral regimen was initiated with sulfamethoxazole and trimethoprim (SMZ-TMP; Mutal Pharmaceutical Co., Philadelphia, Pennsylvania 19124, USA; 30 mg/kg p.o). Throughout the night, the rhinoceros remained standing or moved without evidence of colic. It appeared depressed, and its conjunctivae were hyperemic. Twitching of the skin over the shoulders was also noted. The rhinoceros consumed hay, fruit, and vegetables but refused concentrate pellets. It was offered warm bottles of water and tubs of water with dextrose (100 ml 50% dextrose/3 L water); consumption was noted. The rhinoceros passed small to normal amounts of feces and urinated normally seven times during the night. Four urine samples were evaluated; glucosuria was not detected. Urine dipstick evaluation revealed specific gravity of 1.005–1.010 and pH of 7.5–8.5.

The following morning, the rhinoceros was more active and alert. Conjunctival hyperemia was still present. Flunixin meglumine i.m. injection was repeated. Treatment with ceftiofur i.m. b.i.d. and SMZ-TMP p.o. b.i.d. was continued. A CBC from blood collected at presentation revealed a normal leukocyte count (8.00×10^3 cells/μl) with mild absolute monocytosis (0.88×10^3 cells/μl) and lymphopenia (0.80×10^3 cells/μl) with 50% reactive lymphocytes. No evidence of anemia was present (PCV = 40%; total RBC = 5.60×10^6 cells/μl; Hgb = 14 g/dl). Serum biochemical analysis revealed moderately elevated AST (157 IU/L) and ALT (35 IU/L) (Table 1). Over the course of the day, the rhinoceros showed improvement in attitude, appetite, and water consumption. Defecation and urination were normal. Dipstick tests on five free-catch urine samples collected throughout the day were normal.

On day 2 postpresentation, the rhinoceros showed continued clinical improvement. Conjunctival reddening was limited to the nictitans, but photophobia characterized by forced blinking was observed for the first time. The neck was swollen and sensitive to palpation at injection sites. Flunixin meglumine treatment was changed to 500 mg p.o. s.i.d. for 4 days. Water mixed with powdered artificial fruit drink mix or apple-flavored electrolyte/dextrose supplement was offered throughout the day. There was a slight leukopenia (4.95×10^3 cells/μl) with mild absolute lymphopenia (1.34×10^3 cells/μl). Packed cell volume, total RBC, and Hgb were slightly lower than those from the day before but were still within normal ranges. Serum total protein was mildly decreased (5.0 g/dl) (Table 1). Analyses of two free-catch urine samples were unremarkable.

On day 6 postpresentation, positive results of leptospiral FA testing on urine collected at the time of presentation confirmed clinical leptospirosis with shedding of organisms. Serum MAT revealed elevated titers for all five pathogenic serovars, most notably grippotyphosa (1:12,800) and canicola (1:12,800), on the day of presentation and 27 days prior (Table 1). Because of the possibility of continued urine shedding of leptospiral organisms, access to the rhinoceros was limited and a strict quarantine protocol was instituted. Feces were positive for *C. difficile* toxin and negative for *C. perfringens* enterotoxin. Fecal culture for enteric pathogens was negative.

With treatment, appetite and behavior returned to normal by day 6. Because of increased neck swelling and resistance to injection, ceftiofur treatment was discontinued (11 treatments total) and replaced with amoxicillin treatment (15 mg/kg p.o. b.i.d. for 10 days). Oral SMZ-TMP was also continued for a total of 14 days. Treatment with oral flunixin meglumine was discontinued after day 5. Follow-up diagnostics were performed over the next 3 mo. Feces were submitted for clostridial enterotoxin/toxin tests on days 7, 34, and 41 postpresentation with isolation of *C. difficile* toxin on days 34 and 41. Because of repeated isolation of the toxin and the documented association between antibiotic use and clostridial enteritis in horses,[25,29] an active *Lactobacillus* sp. and *Enterococcus* sp. probiotic (Probios, CHR Hansen Biosystems, Milwaukee, Wisconsin 53214, USA; 15 g p.o. s.i.d. for 7 days) was prescribed on day 44 postpresentation.

Evaluation of blood drawn on day 8 postpresentation revealed a moderate leukopenia (3.40×10^3 cells/μl) with moderate absolute neutropenia (1.53×10^3 cells/μl) and mild lymphopenia (1.73×10^3 cells/μl). The RBC parameters remained within the normal range. There were slightly low levels of calcium, sodium, and chloride and hypoalbuminemia (1.5 g/dl) and hypoglobulinemia (4.2 g/dl). By day 21, the CBC was returning to normal with a slight leukopenia (6.10×10^3 cells/μl), normal differential, and normal RBC parameters. Serum biochemical analysis was normal with the exception of ongoing hypoalbuminemia (Table 1).

Urine was submitted for *Leptospira* FA testing on days 7, 34, 41, 70, and 89 postpresentation. All tests were negative, indicating that shedding of organisms had ceased shortly after initiation of antibiotic therapy (Table 1). Follow-up MAT on day 21 showed that titers to serovars grippotyphosa and canicola had dropped to 1:3,200, with a concurrent decline in titers for the other three serovars. This reduction was followed by a rebound in titers on day 81, with grippotyphosa and canicola titers again at 1:12,800. Evaluation of sera drawn on day 93 revealed titers to be dropping once more, with grippotyphosa and canicola titers of 1:6,400 (Table 1).

Eleven months after initial presentation, a positive result was obtained with a urine *Leptospira* FA test conducted during routine screening of the clinically normal rhinoceros. Urine submitted to NADC for leptospire culture was negative. Microscopic agglutination testing revealed increased titers to all five pathogenic serovars, with most marked increases to serovar grippotyphosa (1:12,800) and canicola (1:12,800). Amoxicillin therapy (15 mg/kg p.o. b.i.d. for 7 days) was initiated, but the animal became mildly depressed and partially anorexic 5 days into the treatment. At this time, probiotic (15 g p.o. s.i.d. for 7 days) and flunixin meglumine

Table 1. Selected laboratory results for a black rhinoceros (*Diceros bicomis michaeli*) prior to, during, and following an episode of clinical leptospirosis.

Measure[a]	Date										Normal range
	17 Jun 97	12 Dec 97[b]	24 Dec 97[c]	20 Jan 98[d]	22 Jan 98	28 Jan 98	06 Feb 98	12 Feb 98	11 Mar 98	16 Apr 98	25 Apr 98	
Leptospira interrogans MAT												
serovar grippotyphosa	1:100	1:3,200	1:12,800	1:12,800	np[e]	np	np	1:3,200	np	1:12,800	1:6,400	<1:100[f]
serovar canicola	1:100	1:3,200	1:12,800	1:12,800	np	np	np	1:3,200	np	1:12,800	1:6,400	<1:100[f]
serovar icterohaemorrhagiae	1:100	1:800	1:6,400	1:6,400	np	np	np	1:800	np	1:3,200	1:1,600	<1:100[f]
serovar hardjo	1:100	1:200	1:200	1:200	np	np	np	1:100	np	1:800	1:100	<1:100[f]
serovar pomona	1:100	1:100	1:800	1:800	np	np	np	1:100	np	1:800	1:800	<1:100[f]
Urine *Leptospira* FA testing	np	np	np	positive	np	negative	negative	negative	negative	np	np	negative[f]
Hematology												
White blood cell count (10^3 cells/ul)	6.40	7.60	np	6.00	4.95	3.40	4.25	6.10	np	np	8.50	9.84 ± 3.08[g]
Packed cell volume (%)	42	40	np	40	34	32	41	38	np	np	40	35.7 ± 7.8[g]
Red blood cell count (10^3 cells/ul)	5.20	5.08	np	5.60	4.50	np	np	5.21	np	np	4.70	4.17 ± 1.14[g]
Hemoglobin (g/dl)	15	13.7	np	14	12	12.5	np	14.8	np	np	15	12.6 ± 2.8[g]
Serum biochemical analysis												
Aspartate aminotranferase (IU/L)	55	np	np	157	np	106	np	84	np	np	95	60 ± 37[g]
Alanine aminotranferase (IU/L)	13	np	np	35	np	12	np	12	np	np	18	13 ± 7[g]
Total protein (g/dl)	7.1	np	np	7.9	5	5.7	np	7.4	np	np	7.1	7.9 ± 1.0[g]

[a] MAT = microscopic agglutination testing; FA = fluorescent antibody.
[b] Sample drawn 18 wk following vaccination with 1 ml of bacterin against all five *Leptospira interrogans* serovars and 2 wk after death of another rhinoceros from leptospirosis.
[c] Sample drawn 13 days following vaccination with 2 ml of bacterin against all five *Leptospira interrogans* serovars.
[d] Sample drawn at time of presentation for clinical leptospirosis.
[e] np = not performed.
[f] College of Veterinary Medicine, Diagnostic Laboratory, Cornell University.
[g] International Species Inventory System.

(0.83 mg/kg p.o. s.i.d. for 3 days) therapy was initiated, and the rhinoceros's appetite returned to normal within a few days. Urine *Leptospira* FA testing was negative 4 days after initiation of therapy and has remained negative to date. Microscopic agglutination testing 3 wk following initiation of treatment revealed lower titers for all five serovars. To date, MAT titers have fluctuated between 1:1,600 and 1:6,400 for serovars grippotyphosa and canicola. The rhinoceros has been clinically normal with no detected shedding of organisms for >2 yr.

Epizootiology

Medical records from other animals in the zoo were examined, and selected serum samples were tested for leptospiral antibodies to determine the incidence of leptospirosis in the collection and the source of infection for these two rhinoceroses. Numerous animals had been tested for leptospiral antibodies since 1980, but positive titers were detected in only three elk (*Cervus elaphus canadensis*) in 1997 (approximately 6 mo prior to onset of disease in the first rhinoceros). Two 20-yr-old unvaccinated female elk had positive titers (1:100) to serovar grippotyphosa by MAT. A male elk vaccinated with a five-way bacterin at age 15 mo had no titers to the five serovars 2.75 yr later, at which time the elk received a booster vaccination. Titers to serovars icterohaemorrhagiae (1:100), hardjo (1:100), and grippotyphosa (1:200) were positive in this animal at 5 yr of age.

Banked frozen sera from selected wild specimens, fresh samples from newly trapped feral and wild specimens, and fresh samples from selected collection specimens were surveyed. Initially, sera that had been collected from five wild raccoons (*Procyon lotor*) 4–6 mo before the presentation of these rhinoceroses were submitted for MAT and were negative. Following the illness in rhinoceros 2, sera for *Leptospira* MAT were obtained from two house mice (*Mus musculus*), two rats (*Rattus norvegicus*), one cat (*Felis cattus*), and two raccoons livetrapped on zoo grounds and from five African elephants (*Loxodonta africana*) housed close to the rhinoceroses. Titers to serovar grippotyphosa (1:200) were positive in two raccoons. One raccoon was trapped from the exhibit that had contained the elk and the other was from an enclosure adjacent to the rhinoceros exhibit. Screening of potentially exposed workers was performed, but no individuals tested positive for the five serovars.

Raccoon paw prints were regularly found surrounding the wallows in the rhinoceros exhibit. *Leptospira kirschneri* serovar grippotyphosa, perhaps with a raccoon reservoir, may therefore have been responsible for the clinical disease in these rhinoceroses.

DISCUSSION

Leptospirosis is a worldwide zoonosis, affecting humans in addition to wild, domestic, zoo, and laboratory animals.[2,3,6,11,15,20–22,26,31,36,37,43,46–48] Although disease has been attributed to pathogenic serovars of *L. interrogans*, reclassification of the genus based on genetic homology now places some pathogenic serovars in different species. Serovar grippotyphosa is now classified as *L. kirschneri* (serovars pomona, canicola, and icterohaemorrhagiae are still members of *L. interrogans*). In addition, serovar hardjo has been split into two serologically indistinquishable types, *Leptospira borgpetersenii* serovar hardjo and *L. interrogans* serovar hardjo.[7,30,38,41,51] Although the diagnostic laboratory (CU) reports *L. interrogans* serovar hardjo in MAT testing, only *L. borgpetersenii* serovar hardjo has been reported to infect animals in the United States.[51]

Each pathogenic serovar is adapted to and may cause disease in particular maintenance host species. Maintenance species serve as reservoirs of infection for less susceptible incidental hosts.[2,6,8,18–20,22,30,38,44,47] For the rhinoceroses described here, raccoons may have been the source of infection. Raccoons are maintenance hosts for several serovars and are a major reservoir for leptospirosis, with reported infection rates of up to 23%.[4,47,49] Of the raccoon *Leptospira* serovars, grippotyphosa appears to be the most common.[26,32,49] Serologic assessments of rhinoceros 2 and the raccoons trapped on zoo grounds suggested that serovar grippotyphosa was responsible for the clinical disease. Elevated titers for other serovars, particularly canicola, were also found in rhinoceros 2 but probably represented cross-reactivity.[3,19,46] However, inconclusive antibody titers in rhinoceros 2 and the lack of serologic evidence of grippotyphosa infection in rhinoceros 1 at presentation allows for speculation regarding the infecting serovar. Microscopic agglutination testing of rhinoceros 1 serum drawn 6 mo prior to presentation supported recent exposure to serovars icterohaemorrhagiae, hardjo, and canicola but was negative for grippotyphosa. Immunity to one serovar provides no cross protection against infection with other serovars, so it is possible that rhinoceros 1 died as a result of novel exposure to serovar grippotyphosa.[3,27,45]

Previous cases of leptospirosis in black rhinoceros have involved hemolytic crises. Antibody titers to serovars canicola and icterohaemorrhagiae have implicated leptospirosis as a cause of hemolytic anemia in two animals that died,[13] and elevated convalescent titers for serovars icterohaemorrhag-

iae and grippotyphosa were detected in two rhinoceroses surviving hemolytic episodes.[33] In addition to clinical disease, serologic surveys have documented titers to serovars grippotyphosa, pomona, icterohaemmorrhagiae, javanica, ballum, canicola, bratislava, tarssovi, and copenhageni in rhinoceroses.[13,16,24,34,35,48]

Leptospirosis is generally acquired by contact with urine or tissue from an infected animal or through contaminated water, food, or soil. Skin abrasions or mucous membranes are the usual portals of entry.[2,15,19,26,27,45] Transmission to humans via contact with raccoon urine has been documented.[15] Both rhinoceroses described here had access to possibly contaminated wallows, which are used for skin health, temperature regulation, and behavioral enrichment.[17] Rhinoceros 1 spent more time in the wallows than did rhinoceros 2, and rhinoceros 2 may have contracted the disease from either the wallows or rhinoceros 1, assuming leptospires were being shed. Raccoon control and wallow maintenance protocols were subsequently developed. The wallows were emptied and relocated regularly, allowing complete drying of the soil.

Following penetration of mucous membranes or abraded skin, leptospires enter the blood and multiply rapidly. They spread and further replicate in many tissues, including the kidney, liver, spleen, central nervous system, eyes, and genital tract.[19,38] Following increases in serum antibody levels, spirochetes are cleared from most organs but may persist in the kidney or reproductive organs and thus may be shed in urine for months. In surviving maintenance hosts, renal colonization may be long term, with shedding in urine for years, occasionally in large numbers.[15,19,45] Because of the severe acute nature of the disease in these and other rhinoceroses,[13,17,24,33–35] rhinoceroses are considered incidental rather than maintenance hosts for *Leptospira* serovars.[30] However, the possibility of shedding continues to be a concern in rhinoceros 2, based on a positive urine FA test and the resurgence of titers, particularly for serovars grippotyphosa and canicola, that occurred following treatment.

Leptospiral infection varies from asymptomatic to severe with potential fatal sequelae,[15,45,46] and clinical signs are numerous.[2,3,11,15,22,45,46] Interactions among host adaptation, virulence factors, the host immune status, the species affected, and the serovar involved account for this variation.[22] Clinical illness tends to be more acute and severe in incidental hosts than in maintenance hosts.[19,22,30] Both rhinoceroses in this report presented with gastrointestinal discomfort, depression, weakness, and anorexia. Rhinoceros 1 exhibited abnormal urination and either resisted defecating or did not defecate because of ileus, constipation, or pain. In addition, ocular abnormalities, conjunctivitis and photophobia, were observed in rhinoceros 2.

Clinical pathology findings associated with leptospirosis have been reported. Hemogram abnormalities include leukopenia, leukocytosis, thrombocytopenia, anemia, and elevation of RBC sedimentation rate.[3,15,45,46] Hemogram findings were different in the two rhinoceroses reported here; rhinoceros 1 presented with leukocytosis, but rhinoceros 2 progressed from a normal leukocyte count to leukopenia that persisted for 3 wk. Although slight decreases in RBC parameters were seen in rhinoceros 2, neither animal developed hemolytic anemia as has been seen in other presumptive or proven cases of rhinoceros leptospirosis. Numerous serum biochemical abnormalities have been reported, including elevation of hepatocellular enzymes, hyperglobulinemia, azotemia, and CK elevation.[3,15,46] Both rhinoceroses exhibited AST and ALT elevations but neither had azotemia. Rhinoceros 1 presented with hyperglobulinemia, but both animals were hypoalbuminemic. Rhinoceros 1 had significant elevation of CK.

Common urinary abnormalities reported with leptospirosis include hematuria, hemoglobinuria, proteinuria, and pyuria,[15] but glucosuria appears to have been reported only in dogs.[3,27] Significant glucosuria was exhibited by rhinoceros 1. Paradoxical glucosuria, a component of acquired Fanconi's syndrome, is related to proximal renal tubular dysfunction, which may be caused by a variety of insults but seems to be most commonly associated with toxin exposure.[5] Paradoxical glucosuria may be related to tubular damage with only a basement membrane barrier existing between interstitial and luminal spaces through which diffusion of glucose and other solutes can occur.[10] Clostridial enterotoxemia, reported in sheep, can also lead to glucosuria.[6] Glucosuria of acute tubular necrosis can be differentiated from clostridia-induced glucosuria by the presence of concurrent hyperglycemia in enterotoxemia. The mechanism for the development of renal lesions in leptospirosis is not well understood; renal ischemia, a toxin, bacterial migration, and an immunologic reaction have been suggested.[50] Concurrent clinical conditions in humans leading to acute renal failure in cases of leptospirosis include dehydration, jaundice, and rhabdomyolysis.[1] Based on the severity of renal lesions in rhinoceros 1, the presence of glucosuria in future cases of leptospirosis in rhinoceroses may suggest a poor prognosis.

Pathology findings of leptospirosis vary with the

species infected, the organs affected, and the serovars involved.[27,45] The gross and histopathologic findings in rhinoceros 1 were similar to those reported with leptospirosis in domestic species, humans, squirrel monkeys (*Samiri sciureus*), and California sea lions (*Zalophus californianus*).[11,20,26,37,45] Electrocardiographic abnormalities have been reported in up to 46% of cases of human leptospirosis.[40] These abnormalities are often thought to be of little significance,[46] although one study revealed that 25% of the fatal cases were directly attributable to myocarditis.[14] Based on the degree of myocarditis present and the presumed metabolic/physiologic derangement in association with multisystem involvement, the likely cause of death in rhinoceros 1 was an acute severe arrhythmia. Postmortem tissue vitamin E levels, including those in the heart, were extremely low, which may have contributed to cardiac disease, predisposing the rhinoceros to development of an arrhythmia.[12]

Leptospirosis can only be definitively diagnosed by bacteriologic culture, a technique that is difficult and rarely successful.[3,27,45] Demonstration of leptospires in fluids by dark-field microscopy or in tissue by Warthin–Starry silver staining is sometimes possible, although neither technique is very sensitive or specific.[11,19,30,45] Serology (MAT) is the most common method for diagnosing leptospirosis, although cross-reactivity among serovars may prevent definitive identification.[3,22,27,45] In addition to being laborious and requiring maintenance of potentially dangerous live organisms, the MAT requires subjective interpretation because strict criteria for serologic confirmation of an active leptospiral infection have not been established and laboratory variation makes standardization difficult.[3,22,44,45] The laboratory used in these rhinoceros cases considers a titer of $<$1:100 negative and a titer of $\geq$1:1,600 indicative of recent infection or vaccination. Other diagnostic tests include polymerase chain reaction, genetic probes, restriction endonuclease analysis, enzyme-linked immunosorbent assays, and FA techniques, which can be performed on tissue and/or body fluids.[3,19,30,38,44,45,51] In rhinoceroses, both MAT and FA techniques have been used.[13,24,33–35] In rhinoceros 1, the tissue FA technique was diagnostic. Urine FA screening and MAT were utilized in rhinoceros 2 for diagnosis and monitoring.

Treatment aims are to clear the organism and to provide supportive care. Penicillins and tetracyclines are recommended in humans, pinnipeds, dogs, and horses to eliminate leptospires.[15,20,26] Tetracyclines eliminate the renal carrier phase.[3,27,45] Enrofloxacin, alone or in combination with ampicillin or amoxicillin, may be leptospirocidal in dogs,[45] and ciprofloxacin has cleared leptospires from blood and urine of hamsters experimentally.[3] Treatments recommended for rhinoceroses are similar and include injectable penicillins and aminoglycosides for acute leptospirosis and tetracyclines for animals that survive acute infection.[33] In rhinoceros 2, ceftiofur sodium was the primary choice because of its broad spectrum of activity and because it could be reconstituted to a practical volume (15 ml of 200 mg/ml solution) for i.m. injection.[39] Humans and hamsters have been treated successfully with ceftiofur.[15,20] Because of concerns about pain and tissue damage, the rhinoceros was switched to oral amoxicillin after 11 injections of ceftiofur. Sulfamethoxazole-trimethoprim has not been shown to be effective against leptospirosis and was primarily used to increase the coverage against concomitant bacterial infections.

Flunixin meglumine is a potent inhibitor of cyclooxygenase with analgesic, anti-inflammatory, and antipyretic effects.[39] Rapid relief was noted in the rhinoceros following i.m. injection of the drug, which allowed oral medications to be administered and encouraged consumption of large quantities of water and electrolytes. Flunixin meglumine also may have antiendotoxin properties.[39]

Leptospirosis is prevented in domestic species by minimizing contamination of the environment and by vaccination with serovar-specific vaccines.[22,27] In the black rhinoceros, postvaccination immune responses similar to domestic species have been reported.[13,24,34] Reported adverse reactions to vaccination with leptospiral antigens in black rhinoceroses include injection site abscessation in 5–10% of animals and two animals with apparent anaphylactic reaction, with weakness in both animals and significant skin sloughing in one.[17,24] Annual vaccination of black rhinoceroses with a five-way leptospiral bacterin (containing *Leptospira* serovars icterohemorrhagiae, grippotyphosa, pomona, canicola, and hardjo) or six-way bacterin (with the addition of serovar bratislava)[17,24,33] instead of biannual vaccination is now recommended because of vaccine reactions in the population and higher titer levels obtained by annual prophylaxis (R. Eric Miller, pers. comm.). Neither of the rhinoceroses in this report had been vaccinated prior to arrival at the Pittsburgh Zoo. Rhinoceros 2 had received a partial vaccine (1 ml) of a five-way bacterin (LEPTO-5, Rhone Merieux, Athens, Georgia 30601, USA) 15 wk prior to the death of rhinoceros 1. When evaluated 17 wk postvaccination (2 wk after the death of rhinoceros 1), titers to serovars grippotyphosa, canicola, icterrohaemorrhagiae, and hardjo were elevated (Table 1). The rhinoceros received a full 2-

ml vaccine dose 4 wk after the death of rhinoceros 1 (5 wk before presenting with clinical disease). Titers were identical at 2 wk after the full vaccination and at the time of presentation with clinical disease, with marked increase in titers to serovars grippotyphosa, canicola, and icterrohaemorrhagiae and lower but significant titers to pomona and hardjo (Table 1). Because previous MAT titers were negative in rhinoceros 2, the three sets of results indicated an immune response consistent with exposure to pathogenic serovars, either through vaccination or natural infection. The cause of the marked increase in titers in the later two samples could not be determined. Consequently, an anamnestic response cannot be ruled out and vaccination may have contributed to the survival and lack of peracute presentation in rhinoceros 2. In light of the ongoing risk of reinfection, the Pittsburgh Zoo rhinoceros collection is vaccinated biannually.

CONCLUSIONS

Clinical leptospirosis without hemolytic crisis was diagnosed in two black rhinoceroses. In rhinoceros 1, leptospirosis was diagnosed postmortem by FA staining of liver, and in rhinoceros 2 leptospirosis was diagnosed in the living animal using serum MAT titers and urinary FA techniques. Institutions with black rhinoceroses should serologically screen wild and feral animal populations to determine local prevalence and risk. Contact between rhinoceroses and these animals should be minimized, and wallows and other water sources should be maintained to inhibit disease transmission. Vaccination of rhinoceroses against leptospires may prevent disease or lessen the severity of infection.

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