Severe Anemia Caused by Babesiosis in a Maned Wolf (*Chrysocyon brachyurus*)

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SEVERE ANEMIA CAUSED BY BABESIOSIS IN A MANED WOLF (*CHRYSOCYON BRACHYURUS*)

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Abstract: An 8-yr-old, captive, spayed, female maned wolf (*Chrysocyon brachyurus*) developed progressive lethargy and weakness over a 24-hr period. Clinical signs included vomiting, recumbency, horizontal nystagmus, possible blindness, pale icteric mucus membranes, and port-wine colored urine. A complete blood cell count revealed severe anemia (packed cell volume [PCV], 6%) and intraerythrocytic piroplasms consistent with a *Babesia* species. Polymerase chain reaction testing later confirmed babesiosis. The wolf was treated with imidocarb dipropionate, antibiotics, and fluid therapy. A whole-blood transfusion from a sibling maned wolf also was performed. Despite aggressive treatment, the wolf failed to improve and was euthanized. To the authors' knowledge, this is the first documented case of babesiosis in a captive maned wolf in North America. Surveillance of infectious diseases in captive and wild maned wolf populations should be expanded to include screening for *Babesia* species. Tick control also should be implemented to prevent and decrease transmission of the disease to this endangered species.

Key words: Anemia, *Babesia, Chrysocyon brachyurus,* maned wolf.

BRIEF COMMUNICATION

The maned wolf (*Chrysocyon brachyurus*), an endangered species, is susceptible to many of the same canine infectious diseases reported in domestic dogs. Exposure to canine distemper virus, rabies virus, canine parvovirus, canine adenovirus, *Dirofilaria immitis, Ehrlichia canis, Rickettsia rickettsii, Leptospira interrogans* spp., and *Toxoplasma gondii* has been documented in South American domestic dogs and the wild populations of maned wolves living close to them.[2,6] Captive populations of maned wolves are at risk for acquisition of the same infectious diseases from other canid species within zoo collections and from domestic dogs that may live in proximity to zoologic institutions. Canine babesiosis caused by various species of the genus *Babesia* has been on the rise in North America within the past 2 decades[1] and should be considered a disease of potential concern in captive maned wolves.

An 8-yr-old, spayed, female maned wolf developed progressive lethargy and weakness, with concurrent reduced appetite and episodes of vomiting over a 24-hr period. Other than chronic bilateral hind limb orthopedic problems, the wolf's medical history had been unremarkable. Preventative care included annual rabies and canine distemper vaccinations, and monthly heartworm (ivermectin; Heartgard®, Merial Limited, Duluth, Georgia 30096, USA) and flea (lufenuron; Program®, Novartis, Greensboro, North Carolina 27408, USA) prevention. There had been no known toxin exposure or dietary changes. The wolf shared an enclosure with a male sibling that was showing no abnormalities.

On visual observation, the wolf was sternally recumbent within its crate and unable to rise. The wolf made exaggerated head and neck excursions when attempting to lift its head and had a mild right head tilt and significant horizontal nystagmus. The wolf's startled responses to sound and inability to focus on surrounding activity suggested that it might be nonvisual. The wolf was transported to the Kansas State University Veterinary Medical Teaching Hospital for further evaluation. The wolf was immobilized by injection with ketamine (1 mg/kg i.m.), medetomidine (0.015 mg/kg i.m.), and midazolam (0.5 mg/kg i.m.). Once induced, the wolf was intubated with an 11-mm endotracheal tube, and anesthesia was maintained with isoflurane gas (0.5–3.0%) and oxygen (2 L/min).

On physical examination, the wolf weighed 24 kg and was in good body condition. Heart rate (85 beats/min), respiratory rate (26 breaths/min), and temperature (38°C) were all within established reference ranges. Mucus membranes were icteric and tacky, with a capillary refill time (CRT) < 2 sec. The sclera also were icteric. The abdomen felt

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Table 1. Serial complete blood cell count values for a maned wolf with babesiosis.

Complete blood cell count	Reference interval[10]	Admission	12 hr	24 hr	72 hr	6 days	8 days
Leukocytes, cells/μl	6,580–14,360	6,200			48,800	44,600	38,400
Segmented neutrophils, cells/μL	3,669–10,481	5,200			42,000	40,600	33,400
Band neutrophils, cells/μl	0–395	100			0	400	0
Lymphocytes, cells/μl	1,029–3,343	400			2,900	1,300	1,500
Monocytes, cells/μl	0–334	400			3,400	2,200	3,500
Eosinophils, cells/μl	0–617	0			500	0	0
Basophils, cells/μl	0–79	100			0	0	0
Hematocrit, %	34.3–47.5	6	7	16	25	23	9
Hemoglobin, g/dl	11.3–15.9	2.7			8.4	7.6	3.1
Mean cell volume, fl	68.4–83.2	76			74	76	78
Mean cell hemoglobin, pg	23.2–28.2	44			26	25	26
Mean cell hemoglobin concentration, g/dl	31.1–36.1	59			34	33	34
Platelets, 1,000/μl	145–285	<10			Mildly decreased	Adequate	Adequate
Total protein, g/dl	5.4–7.0	6.9		6.6	8.1	7.8	5.7
Gross plasma appearance		Marked hemolysis			Icterus	Icterus	Icterus

slightly distended, and, on firm palpation, a large amount of port-wine–colored urine was expressed. Distinct organomegaly was not identified on abdominal palpation. Ectoparasites were not found.

A complete blood cell count (CBC) revealed severe regenerative anemia (packed cell volume [PCV], 6%; reference range, 34.3–47.5%), a normal total protein (6.9 g/dl; reference interval, 5.4–7.0 g/dl), and marked hemolysis.[10] A slide agglutination test was negative. There was mild leukopenia with mild-moderate lymphopenia (Table 1). Thrombocytopenia was present, with occasional large forms. Frequently noted within the erythrocytes were 1–2 μm, circular-to-oval intraerythrocytic piroplasms consistent with a small *Babesia* species. No paired piroplasms were observed. There also were rare, small, chain-like structures on erythrocytes, suggestive of possible *Mycoplasma hemocanis* infection. PCR was recommended to confirm *Babesia* infection and to test for possible *M. hemocanis* infection. Subsequent RT-PCR of the blood revealed a strongly positive result (>500 copies/ml blood) for *Babesia canis canis* and/or *Babesia canis vogeli* (Fluorescence Resonance Energy Transfer [FRET] real-time PCR targeting the canine *Babesia* species 18s rNA gene, Auburn University, Auburn, Alabama, USA).[12] RT-PCR testing for *M. hemocanis* was negative. Later in the course of the disease, a cerebrospinal fluid tap also was performed. Microscopic analysis of the fluid was considered normal. The cerebrospinal fluid was negative for Rocky Mountain Spotted Fever (immunofluorescent assay [IFA] screen) and *Cryptococcus* (latex agglutination screen).

A serum chemistry panel revealed an increased urea nitrogen with a normal creatinine, moderate hypocalcemia, moderate hypokalemia, and hyperbilirubinemia (Table 2). The hypocalcemia was likely due to an acidotic state. A SNAP 4Dx Test (Idexx Laboratories, Inc., Westbrook, Maine 04092, USA) was negative for Lyme disease, ehrlichiosis, heartworm disease, and anaplasmosis. Thoracic and abdominal radiographs were taken but did not indicate any clinically significant abnormalities.

Intravenous fluid administration was started (lactated Ringer's solution, at 20 ml/kg/hr). An immunosuppressive dose of dexamethasone SP (0.28 mg/kg i.v.) was given. Enrofloxacin (5 mg/kg i.v.; Baytril®, Bayer Healthcare, Shawnee Mission, Kansas 66216, USA) and famotidine (0.5 mg/kg s.c.; Baxter International Inc., Deerfield, Illinois 60015-4634, USA) also were administered to provide antimicrobial effects and gastrointestinal protection, respectively. Treatment with imidocarb dipropionate (6.6 mg/kg, i.m., q. 14 d; Imizol®, Intervet/Schering-Plough Animal Health, Worthington, Minnesota 56187, USA) also was initiated in light of the possible diagnosis of babesiosis. Once treatments were completed, atipamezole (2 mg i.m.; Antisedan®, Pfizer Animal Health, New York City, New York 10017, USA) was given as a reversal agent.

Because recheck PCV 12 hr later remained unchanged and the wolf was recumbent and

Table 2. Serial serum chemistry values from a maned wolf with babesiosis.

Serum chemistry	Reference interval[a]	Admission	72 hr	8 days
Glucose, mg/dl	90–140	112	149	285
Urea nitrogen, mg/dl	14–32	64	14	68
Creatinine, mg/dl	0.9–1.7	1.4	0.8	3.7
Total protein, g/dl	5.4–7.0	6.8	6.8	4.7
Albumin, g/dl	2.7–3.5	2.4	2.6	2.1
Calculated globulin, g/dl	2.4–3.8	4.4	4.2	2.6
Total calcium, mg/dl	9.1–10.5	7.9	8.1	8.3
Phosphorus, mg/dl	3.3–8.5	5.7	4.4	7.1
Sodium, mmol/L	141–149	144	145	145
Potassium, mmol/L	4.3–5.3	2.5	2.8	4.9
Chloride, mmol/L	110–118	112	114	109
Bicarbonate, mmol/L	18–29[b]	14	16	11
Alanine transaminase, U/L	28–171[b]	73	676	1,047
Alkaline phosphatase, U/L	1–142[b]	NV[c]	465	635
Creatine kinase, U/L	128–328[b]	548	7,386	1,373
Total bilirubin, mg/dl	0.1–0.3[b]	5.5	6.1	3.0
Cholesterol, mg/dl	203–571	228	220	84
Serum appearance		Marked hemolysis	Marked icterus	Icterus

[a] Reference intervals from Kennedy-Stoskopf,[10] unless otherwise indicated.
[b] Domestic dog reference intervals from the Kansas State University Veterinary Diagnostic Laboratory.
[c] No value due to marked hemolysis.

obtunded, a whole-blood transfusion was administered. The male sibling weighed 26 kg and was immobilized by using ketamine (1 mg/kg i.m.), medetomidine (0.02 mg/kg i.m.), and midazolam (0.3 mg/kg i.m.), intubated, and maintained on isoflurane and oxygen. A unit of whole blood (450 ml) was collected from the jugular vein by using a Teruflex® closed single bag collection system that contained CDPA-1 anticoagulant (Terumo Medical Corporation, Elkton, Maryland, 21921-5330, USA). The maned wolf was reversed with atipamezole (2.6 mg i.m.) and recovered without complications. A CBC of the donor blood later showed a normal PCV (39%; reference interval, 34.3–47.5%) and no evidence of parasitemia on blood film evaluation.

To perform the blood transfusion, the female wolf was sedated with midazolam (0.45 mg/kg i.m.) and masked with isoflurane gas and oxygen. The whole-blood transfusion was given via an 18-gauge catheter placed in the lateral saphenous vein over a period of approximately 2 hr. Heart rate, respiratory rate, temperature, blood pressure, and electrocardiography were monitored every 5 min during the transfusion, and all parameters remained stable. After the transfusion, fluids were administered (lactated Ringer's solution 700 ml s.c.). At 4 hr after the transfusion, the wolf's PCV and total solids were 16% and 6.6 g/dl, respectively (Table 1).

At 24 hr after transfusion, the wolf's attitude had not improved appreciably, although the horizontal nystagmus had resolved, and, subjectively, it was thought that the wolf could see. Dark-red urine was seen in the cage. Treatments consisted of dexamethasone SP (0.28 mg/kg i.m., q. 24 hr), famotidine (0.5 mg/kg s.c., q. 24 hr), and fluids (lactated Ringer's solution, 500 ml s.c., q. 12 hr). Oxytetracycline (13.3 mg/kg i.m., q. 24 hr) administration was started to address the possible *Mycoplasma* infection. Syringe feeding with wet food, and water was attempted but was unsuccessful.

At 48 hr after transfusion, a CBC and chemistry panel were repeated. The CBC revealed a PCV of 25%, a marked leukocytosis, with a mature neutrophilia likely due to a combination of chronic inflammation and the effects of steroids and mildly decreased platelets (Table 1). On blood film examination, very rare ring-shaped organisms were seen on the erythrocytes. The serum chemistry panel indicated continued hyperbilirubinemia and elevated liver enzymes (Table 2). Ionized calcium was mildly low (4.4 mg/dl; reference interval, 4.55–5.77 mg/dl). Six days after presentation, the wolf ate a small quantity on its own. The wolf stood but exhibited marked ataxia and weakness. Urination and defecation were noted. A CBC was rechecked, and the abnormalities were similar to the previous assessment (Table 1). Eight days after presentation, the

wolf's condition deteriorated. Its PCV decreased to 9%, and it was completely anorexic and hypothermic (37°C), and exhibited severe obtundation. Given the wolf's very poor response to treatment and visible suffering, euthanasia was elected. Blood for a CBC and chemistry panel was collected just before euthanasia (Tables 1, 2).

On postmortem examination, the wolf was in good body condition, with adequate internal fat stores. The mucus membranes and skin were pale and yellow. The liver was diffusely yellow to green in color, and the serosa of the gallbladder had multiple hemorrhages. The spleen appeared grossly normal. Bilaterally, there were dark-red linear tracts that extended from the cortex to the medulla in the kidneys. The renal cortices were dark red to brown. The urinary bladder was contracted, and the urine was dark brown. The left coxofemoral joint was luxated, and the left femoral head had undergone extensive remodeling, with flattening of the articular surface and abundant proliferative bone at the base of the head and neck. The acetabulum was shallow, with circumferential proliferative bone.

On histopathologic examination, the centrilobular and midzonal hepatocytes had markedly vacuolated cytoplasm. The gallbladder had a moderate amount of hemorrhage within the adventitial layer. Within the left ventricle of the heart, there was a focal area of degenerate and necrotic cardiomyocytes, with infiltration by small numbers of macrophages and increased numbers of fibroblasts. Within the kidneys, approximately 50% of the cortical tubules were dilated, and there was occasional tubular epithelial regeneration. Approximately 20% of the cortical and medullary tubules contained bile. Depressed areas of the cortex corresponded to areas of tubular atrophy, with intratubular deposition of red granular material (hemoglobin).These histopathologic findings were consistent with marked hepatocyte degeneration and bile stasis; moderate diffuse serosal hemorrhage of the gallbladder; focal cardiomyocyte degeneration and necrosis; multifocal renal tubular epithelial degeneration, loss, and regeneration, with multifocal areas of tubular loss and hemoglobin casts; chronic luxation and bony remodeling of the left coxofemoral joint; and severe icterus.

Canine babesiosis is a tick-borne infectious disease caused by the protozoal genus *Babesia*. There currently are 4 species of *Babesia* that are known to infect canids within North America: *B. canis, Babesia gibsoni, Babesia conradae,* and a yet unnamed North Carolina *Babesia* species.[1,8] To the authors' knowledge, this is the first documented case of canine babesiosis in a captive maned wolf in North America. Babesiosis has previously been found in captive maned wolves in South America[5] and in other canid species, such as the red fox (*Vulpes vulpes*), and has been experimentally induced in coyotes (*Canis latrans*).[3,7,11]

Transmission of *Babesia* species is typically through the bite of a tick vector; however, other forms of hematogenous horizontal and vertical transmission may be possible.[8] Once inside a new host, the protozoa enters the red blood cell. The intraerythrocytic protozoa leads to erythrocyte destruction by two mechanisms: erythrocyte fragility is increased, predisposing the cell to lysis, and antibody deposition on the surface of the infected erythrocytes targets the cells for destruction by the immune system.[1]

Babesia infections may be subclinical, acute, or chronic in nature.[8] Clinical signs of babesiosis are variable, depending on the particular *Babesia* species and the nature of the infection. Clinical signs can include depression, weakness, anorexia, pallor, anemia, thrombocytopenia, and vomiting. Splenomegaly may be identified on physical examination and necropsy. Uncomplicated babesiosis cases may have any of the previous abnormalities, whereas, complicated babesiosis may result in secondary systemic effects, such as cardiovascular, respiratory, hepatic, renal, gastrointestinal, neurologic, and coagulopathic dysfunction.[1] One study found that central nervous system involvement in canine *Babesia canis rossi* cases in South Africa increased the risk of death by 57 times.[9] In this particular case, we suspect that the maned wolf had a complicated form of babesiosis as evidenced by her neurologic signs, hepatopathy, and renal abnormalities. The characteristic splenomegaly of babesiosis was not identified on physical examination or necropsy.

Diagnosis can be made by visualizing characteristic intraerythrocytic organisms on blood smears. However, this method does not allow definitive species identification. The current diagnostic method of choice for both diagnosis and speciation of *Babesia* is PCR testing.[1,8] In the present case, morphologic appearance on the blood film and PCR seemed contradictory. Based upon the small size and signet-ring shape of the intraerythrocytic parasite seen on blood smear, it was thought that a small *Babesia* species (such as *B. gibsoni*) was the causative agent. The paired piroplasms characteristic of *B. canis* were not seen. However, FRET-PCR testing identified *B.*

canis within the blood sample, which is a large *Babesia* species. Interestingly, in addition to the unambiguous melting curve of *Babesia canis canis* (or *B. canis vogeli*), with a peak at a melting temperature (Tm) of about 60°C,[12] there also was a small secondary peak identified with a Tm of about 67°C, higher than the Tm of 64°C that would be expected for *B. gibsoni*. The higher Tm of this second peak could indicate that the probe had a perfect match with the target sequence, which could further indicate an as yet undescribed *Babesia* species (Kaltenboeck, pers. comm.). Unfortunately, it was determined that sequencing would not help with identification because of the mixture of amplification products. Only cloning of the products and sequencing many of them would give the precise sequence and the relative proportions of this sequence among the *B. canis* sequences. Cloning was not performed in this cased due to a lack of an appropriate sample. Whereas *B. gibsoni* is the most common cause of canine babesiosis in the United States[1,12] and was thought to be the most likely *Babesia* species in this case based on blood smear evaluation, FRET-PCR testing identified *B. canis canis and/or B. canis vogeli* and a possible novel *Babesia* species as the causative agent(s).

Treatment of babesiosis typically consists of two intramuscular injections of imidocarb dipropionate, separated by 2 wk. Other drug options exist, such as diminazene aceturate or a combination of atovaquone and azithromycin, but these are not currently approved in the United States. Other important aspects of therapy include fluid support, blood transfusions if severe anemia is present, and immunosuppressants if a component of immune-mediated hemolytic anemia is suspected. The maned wolf in this case received a whole-blood transfusion from her male sibling. The transfusion approach taken differed from an ideal method because a blood type and cross match were not performed before transfusion. Due to time constraints, lack of other readily feasible treatment options, and the presence of a sibling wolf for blood donation, the decision was made to proceed with the transfusion despite the risks (including the possibility that the donor also could be positive for *Babesia* or other pathogens). Immunosuppressant administration also was started because there was a high degree of suspicion that an immune-mediated process was contributing to the wolf's critically low hematocrit. However, there also was substantial concern that an immunosuppressant would weaken the wolf's ability to clear the hemoprotozoa.

Prognosis for canine babesiosis is largely based upon degree of complication and response to treatment. Unfortunately, the maned wolf in this case failed to make substantial improvement during the course of treatment. The lack of noticeable clinical improvement after the blood transfusion and the increased hematocrit were most concerning and may have been indicators of other systemic complications. Prevention of babesiosis is best accomplished by preventing tick attachment to the host. The tick vector has to be attached for 2 to 3 days for transmission of *Babesia* to occur, so, frequent screening for ticks and rapid removal may help prevent the disease.[1] In domestic animals, topical anti-tick medications and tick collars can be used to keep ticks away. However, in zoo and wildlife species, both these prevention methods are problematic and not necessarily practical.

This case demonstrates that captive maned wolf populations in North America are at risk for babesiosis and provides a readily accessible documentation of this disease process in the maned wolf. Canine babesiosis has been documented in domestic dog populations and in captive maned wolves in Brazil.[4,5,11] Wild and captive populations of maned wolves that live in proximity to domestic dogs may be at risk for *Babesia* infection. Surveillance of infectious diseases in captive and wild maned wolf populations should be expanded to include screening for *Babesia* species. Tick control also should be considered and implemented to prevent and decrease transmission of the disease to this endangered species.

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LITERATURE CITED

1. Ayoob, A. L., S. G. Hackner, and J. Prittie. 2010. Clinical management of canine babesiosis. J. Vet. Emerg. Crit. Care 20: 77–89.

2. Bronson, E., L. H. Emmons, S. Murray, E. J. Dubovi, and S. L. Deem. 2008. Serosurvey of pathogens in domestic dogs on the border of Noel Kempff Mercado National Park, Bolivia. J. Zoo Wildl. Med. 39: 28–36.

3. Clancey, N., B. Horney, S. Burton, A. Birkenheuer, S. McBurney, and K. Tefft. 2010. *Babesia (Theileria) annae* in a red fox (*Vulpes vulpes*) from Prince Edward Island, Canada. J. Wildl. Dis. 46: 615–621.

4. Costa-Junior, L. M., M. F. Ribeiro, K. Rembeck, E. M. Rabelo, M. Zahler-Rinder, J. Hirzmann, K. Pfister, and L. M. Passos. 2009. Canine babesiosis caused by *Babesia canis vogeli* in rural areas of the State of Minas Gerais, Brazil and factors associated with its seroprevalence. Res. Vet. Sci. 86: 257–260.

5. Cubas, Z. S. 1996. Special challenges of maintaining wild animals in captivity in South America. Rev. Sci. Tech. 15:267–287.

6. Deem, S. L., and L. H. Emmons. 2005. Exposure of free-ranging maned wolves (*Chrysocyon brachyurus*) to infectious and parasitic disease agents in the Noel Kempff Mercado National Park, Bolivia. J. Zoo Wildl. Med. 36: 192–197.

7. Evers, H. V., A. A. Kocan, M. V. Reichard, and J. H. Meinkot. 2003. Experimental *Babesia gibsoni* infection in coyotes (*Canis latrans*). J. Wildl. Dis. 39: 904–908.

8. Holman, P. J., and K. F. Snowden. 2009. Canine hepatozoonosis and babesiosis, and feline cytauxzoonosis. Vet. Clin. Small Anim. 39: 1035–1053.

9. Jacobson, L. S. 2006. The South African form of severe and complicated canine babesiosis: clinical advances 1994–2004. Vet. Parasitol. 138: 126–139.

10. Kennedy-Stoskopf, S. 2003. Canidae. *In:* Fowler, M. E., and R. E. Miller (eds.). Zoo and Wild Animal Medicine, 5th ed. W. B. Saunders Co., Philadelphia, Pennsylvania. Pp. 482–491.

11. O'Dwyer, L. H., V. V. Lopes, A. S. Rubini, S. Paduan Kdos, and P. E. Ribolla. 2009. *Babesia* spp. infection in dogs from rural areas of São Paulo State, Brazil. Rev. Bras. Parsitol. Vet. 18: 23–26.

12. Wang, C., S. K. Ahluwalia, Y. Li, D. Gao, A. Poudel, E. Chowdhury, M. K. Boudreaux, and B. Kaltenboeck. 2010. Frequency and therapy monitoring of canine *Babesia* spp. infection by high-resolution melting curve quantitative FRET-PCR. Vet. Parasitol. 168:11–18.

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