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Authors: Chou, Shih-Jen, Shieh, Yao-Ching, and Yu, Chang-You

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Hematologic and Biochemistry Values for Black-faced Spoonbills (*Platalea minor*) with and Recovering from Botulism

Shih-Jen Chou,[1] Yao-Ching Shieh,[2] and Chang-You Yu[1,3] [1]Department of Veterinary Medicine, National Chiayi University, Chiayi 600, Taiwan; [2]Tainan Hsien Livestock Disease Control Center, Tainan 730, Taiwan; [3]Corresponding author (email: cyoyu@mail.ncyu.edu.tw)

ABSTRACT: Type C1 botulism outbreaks in Black-faced Spoonbills (*Platalea minor*) occurred in Taiwan from 2002 to 2003, and hematologic and biochemistry parameters from botulism-paralyzed birds and recovered birds were compared. Values for creatinine and uric acid were higher ($P<0.0025$) in birds with botulism than in recovered birds. Lower white blood cell counts ($P<0.005$) and values for alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, and triglycerides ($P<0.025$) were observed in recovered birds. Based on these observations, we suggest that hematologic and biochemistry analyses should be performed to assess the health condition of birds recovering from botulism.

Key words: Biochemistry, Black-faced Spoonbill, botulism, hematology, *Platalea minor*, wading bird.

The Black-faced Spoonbill (*Platalea minor*) is a migratory wading bird that inhabits wetlands and forages in shallow, fresh, brackish, or saltwater (Liou, 2005b). Black-faced Spoonbills winter in Southeast Asia, particularly Taiwan; about half of the world's population of Black-faced Spoonbills arrive in Taiwan in October, and they leave in April or May of the following year. The worldwide population of Black-faced Spoonbills in 1988 was estimated at 288 and increased to 1,069 in 2003; the species was listed on the World Conservation Union Red List as Critically Endangered from 1994 to 2000 (Liou, 2005a). In Taiwan, the Council of Agriculture preserved the north bank of the Tainan County Zengwen River Estuary as a Black-faced Spoonbill refuge in November 2002. This area is one of the world's most important winter habitats for this species (Liou, 2005a).

Black-faced Spoonbills in Taiwan were found to be intoxicated with botulism C1 toxin from December 2002 to March 2003. Clinical signs included respiratory distress; open-mouth breathing; paralysis in the legs, wings, and neck; coma; and death. Tissues from dead birds were evaluated by histologic and microbiologic methods. *Clostridium botulinum* was detected in their gastrointestinal tract, and *C. botulinum* type C1 toxin was identified in the gastric supernatants and sera (Chuang et al., 2005). Botulism-affected birds were captured by hand, put in cloth bags to maintain body temperature (one bag/bird), and transferred to spoonbill nursing pens in Tainan Hsien Livestock Disease Control Center (Tainan, Taiwan). Seventeen of 90 (19%) birds recovered from botulism, and they were released. Seven of 17 (42%) released birds returned to Tseng-wen Estuary (Tainan, Taiwan) the following year (Fang, 2005). In this study, blood and serum from 11 of 17 birds recovering from botulism were analyzed.

Serum samples were collected from sick birds 1 day after capture. Birds were treated by intramuscular injection with antitoxin to botulism C1 toxin (National Wildlife Health Center, US Geological Survey, Madison, Wisconsin, USA), and birds were forced fed with fluid, nutrients, and live fish. Blood samples from recovered birds were collected 17–30 days after treatment, and paired blood parameters were compared with pretreatment samples. Blood samples were collected at the same time each day (10:00 AM–12:00 PM) to minimize variations owing to circadian rhythms (García-Rodríguez et al., 1987b). Blood (4 ml) was drawn from the brachial vein with a 25-gauge butterfly needle using a 5–6-ml syringe. The blood was placed into Vacutainers (BD Biosciences,

TABLE 1. Hematologic values of 11 Black-faced Spoonbills (*Platalea minor*) with botulism.

Heamtologic parameter[a,b]	Body condition	*n*	Mean±SD	Median	Range
RBC ($\times 10^6$/μl)[NS]	Sick	10	2.43±0.60	2.095	1.51–3.06
	Recovered	11	2.50±0.20	2.53	2.30–2.8
Hb (g/dl)[NS]	Sick	10	16.56±3.03	16.15	11.0–20.5
	Recovered	11	18.61±1.94	19.1	13.8–20.9
PCV (%)[NS]	Sick	9	45.38±7.13	44	30–52
	Recovered	11	45.13±4.56	46	37–53
MCV (fl)[NS]	Sick	9	187.54±28.69	192.1	153.6–241.7
	Recovered	11	181.28±21.51	177.4	132.1–221.7
MCH (pg)[NS]	Sick	9	69.9±8.72	76.6	55.6–82.7
	Recovered	11	74.69±8.64	78.4	49.3–84.3
MCHC (g/dl)[NS]	Sick	9	37.28±1.66	38.9	34.1–41.0
	Recovered	11	41.26±2.98	40.6	36.7–46.3
WBC ($\times 10^3$/μl)*	Sick	10	24.5±5.68	23	8–33
	Recovered	11	15.0±2.67	14	11–17

[a] RBC = red blood cell; WBC = white blood cell; Hb = hemoglobin; PCV = packed cell volume; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration.

[b] NS = no significant differences detected between sick and recovered birds as determined by Students paired *t*-test; *, differences significant at $P<0.005$.

Franklin Lakes, New Jersey, USA) with and without lithium heparin; blood smears also were made for leukocyte differential counts. Lithium heparin Vacutainers were inverted gently 20 times to mix the blood with the lithium heparin, and samples were stored at 4 C for 4–10 hr before processing. Serum was harvested from the remaining Vacutainer after blood was allowed to stand at room temperature for 30 to 45 min, and the blood was centrifuged at 800 × G for 10 min.

Methods of hematologic analysis were adapted according to the descriptions of Howlett (2000) and Pierson (2000). Packed cell volume (PCV) was measured by the standard method; blood samples were centrifuged in microhematocrit capillary tubes (Oxford, St. Louis, Missouri, USA) for 5 min at 13,000 × G, and results are presented as a percentage. Hemoglobin (Hb) was measured on a Sysmex™ F-800 15-parameter, semiautomated hematology analyzer (TOA Medical Electronics Co., Ltd., Bath, UK). For red blood cell (RBC) and white blood cell (WBC) counts, heparinized blood was mixed at a 1:100 dilution with Natt-Herrick's solution, and a Neubauer hemacytometer (Reichert-Jung, Buffalo, New York, USA) was used for analyses. Counts were performed in duplicate. Red blood cell indices, including mean corpuscular volume (MCV), mean corpuscular Hb (MCH), and mean corpuscular Hb concentration (MCHC), were then calculated using standard formulae (Pierson, 2000).

Kodak Ektachem DTII System (Eastman Kodak, Rochester, New York, USA) and Kodak Ektachem DT Slides (Eastman Kodak) were used for the biochemistry analysis. Sera were analyzed for glucose, blood urea nitrogen (BUN), creatinine, uric acid (UA), total protein, albumin, globulin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), cholesterol, triglycerides, sodium (Na), potassium (K), chloride (Cl), calcium (Ca), phosphorus (P), and cholinesterase.

Student's paired *t*-test was used to compare data from botulism-affected birds with the same birds after they recovered. The WBC count was higher ($P<0.005$) in affected birds (Table 1). No botulism-related differences were found for the RBC count, Hb, PCV, MCV,

TABLE 2. Biochemisty values of 11 Black-faced Spoonbills (*Platalea minor*) with botulism.

Parameter[a]	Body condition	No.	Mean±SD	Median	Range	P[b]
GLU (mg/dl)	Sick	11	231.63±52.18	228	193–278	NS
	Recovered	11	242.73±28.99	228	196–269	
BUN (mg/dl)	Sick	11	—	2	<1–11	NT
	Recovered	11	—	<1	<1–1	
Creat. (mg/dl)	Sick	11	1.64±0.5	1	0.2–2	<0.0025
	Recovered	11	0.2±0.04	0.2	0.1–0.3	
Uric acid (mg/dl)	Sick	11	9.39±5.92	13.1	4.2–16	<0.0025
	Recovered	11	4.81±1.13	4.8	2.7–7	
TP (g/dl)	Sick	11	2.68±0.38	2.6	2.4–3.3	NS
	Recovered	11	2.74±0.46	2.7	2.6–3.7	
Alb. (g/dl)	Sick	11	2.09±0.39	1.9	1.5–2.4	NS
	Recovered	11	1.88±0.17	1.9	1.6–2.2	
Glo. (g/dl)	Sick	11	1.15±0.49	0.8	0.5–1.2	NS
	Recovered	11	0.98±0.41	0.9	0.4–1.8	
ALP (U/l)	Sick	11	152.09±104.35	188	40–315	<0.025
	Recovered	11	71.33±34.04	79	15–139	
AST (U/l)	Sick	11	456.36±329.22	610	70–1,010	<0.025
	Recovered	11	371±107.53	410	175–507	
ALT (U/l)	Sick	11	144.36±67.01	139	28–268	<0.025
	Recovered	9	71.13±42.18	67	39–147	
CK (U/l)	Sick	11	1,309.46±942.47	984	449–3,200	NS
	Recovered	11	747.6±403.94	590	331–1,434	
Chol. (mg/dl)	Sick	11	195.73±49.46	207	151–300	NS
	Recovered	11	224.4±25.37	220	182–287	
Trig. (mg/dl)	Sick	11	136.64±101.08	95	42–297	<0.01
	Recovered	11	48.53±7.12	47	38–66	
CHE (U/ml)	Sick	10	3.54±1.03	3.96	2.00–5.47	NS
	Recovered	11	3.08±0.68	2.86	2.05–4.27	
Na^+ (mmol/dl)	Sick	10	129.78±5.54	126.5	123–141	NS
	Recovered	11	122.47±7.69	123	106–131	
K^+ (mmol/dl)	Sick	10	3.61±1.52	3.55	2.6–7.4	NS
	Recovered	11	4.0±0.65	3.9	2.8–5.2	
Cl^- (mmol/dl)	Sick	10	126.78±4.94	127	106–136	NS
	Recovered	11	118.47±14.65	121	79–130	
Ca^{2+} (mg/dl)	Sick	10	9.04±0.67	8.95	8.4–9.5	NS
	Recovered	11	9.07±0.64	9.1	8–10.2	
P (mg/dl)	Sick	10	6.22±3.4	4.55	0.9–12.2	NS
	Recovered	11	2.72±1.12	3.5	1.7–4.7	

[a] BUN = blood urea nitrogen; UA = uric acid; TP = total protein; ALP = alkaline phosphatase; AST = aspartate aminotransferase; ALT = alanine aminotransferase; CK = creatine kinase; Chol. = cholesterol; Trig. = triglycerides; CHE = cholinesterase.

[b] Differences between sick and recovered birds as determined by Student's paired t-test; NS = no significant differences detected; NT = not tested.

MCH, or MCHC ($P>0.05$). In addition, the body weights of 11 sick (1.52±0.13 kg) and recovered (1.57±0.18 kg) birds were not different ($0.25>P>0.1$; T=1.081, df=10).

Values for creatinine, UA, ALP, ALT, AST, and triglycerides all decreased ($P<0.025$) in recovered birds (Table 2). Median BUN, UA, ALP, ALT, and triglycerides where more than double the level observed in recovered birds; mean creatinine was eightfold higher before recovery. Birds with botulism had acute paralytic signs, short-term anorexia, and acute dehydration. In dead birds, focal vacuolar degeneration in the liver, Zenker's degeneration in the skeletal muscles, and cloudy swelling in the epithelium of

the renal distal tubules were observed (Chuang et al., 2005). Elevated creatinine, UA, ALP, ALT, AST, and triglycerides in affected birds is consistent with anorexia, dehydration, and liver, kidney, and muscle damage. Although not statistically significant ($P=0.1$), CK (a muscle-specific enzyme) and phosphorus means also were elevated by more than twofold in birds with botulism.

Higher levels of UA and BUN in birds with botulism may have resulted from anorexia, dehydration, and kidney damage, and all three of these factors may have contributed to these elevated levels. Both uric acid and urea were elevated in buzzards (*Buteo buteo*) after 13-day starvation, suggesting increased body protein catabolism fasting birds (García-Rodríguez et al., 1987a). Elevated BUN may indicate prerenal azotemia in some bird species, because it is removed by glomerular filtration, which depends on the bird's hydration status (Campbell, 2004). Finally, kidney damage was observed in affected spoonbills during these botulism outbreaks (Chuang et al., 2005).

Elevated triglyceride values in affected Black-faced Spoonbills may have resulted from the combined effects of anorexia and liver damage. Fasting may enhance the lipolytic pathway; most triglycerides are synthesized in the avian liver before being transported to various peripheral deposit sites (Hazelwood et al., 2000).

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