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Electrolyte Depletion in White-nose Syndrome Bats

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ABSTRACT: The emerging wildlife disease white-nose syndrome is causing widespread mortality in hibernating North American bats. White-nose syndrome occurs when the fungus *Geomyces destructans* infects the living skin of bats during hibernation, but links between infection and mortality are underexplored. We analyzed blood from hibernating bats and compared blood electrolyte levels to wing damage caused by the fungus. Sodium and chloride tended to decrease as wing damage increased in severity. Depletion of these electrolytes suggests that infected bats may become hypotonically dehydrated during winter. Although bats regularly arouse from hibernation to drink during winter, water available in hibernacula may not contain sufficient electrolytes to offset winter losses caused by disease. Damage to bat wings from *G. destructans* may cause life-threatening electrolyte imbalances.

Key words: Bats, dehydration, electrolytes, *Geomyces destructans*, white-nose syndrome.

White-nose syndrome (WNS) is a disease of hibernating North American bats that is causing unprecedented population declines (Blehert et al., 2009; Frick et al., 2010; Langwig et al., 2012). The disease is caused by a cold-growing fungus, *Geomyces destructans*, which infects living skin tissues of bats during hibernation (Meteyer et al., 2009). Although *G. destructans* causes WNS (Lorch et al., 2011; Warnecke et al., 2012), the exact processes by which fungal skin infection lead to death are not known. Bat wings are thin membranes composed of two layers of skin covering a delicate inner layer of connective tissue, blood vessels, and nerves. Because of their disproportionally large surface area, bat wings play important roles in maintaining physiologic homeostasis, and may be particularly important during hibernation (Cryan et al., 2010). Wings are the primary site of infection by *G. destructans*, and complications associated with dehydration and electrolyte imbalance caused by fungal damage to wing skin may contribute to morbidity and mortality from infection (Meteyer et al., 2009; Cryan et al., 2010; Willis et al., 2011). In the course of examining blood and urine from hibernating bats in the context of WNS, we observed evidence for possible life-threatening electrolyte depletion. Electrolytes are positively (e.g., Na^+, K^+) or negatively (e.g., Cl^-, HCO_3^-) charged solutes found within cells and in the fluid compartment of circulating blood. Proper concentrations of electrolytes are critical for maintaining physiologic homeostasis.

We analyzed whole blood from captive ($n=18$) and wild hibernating ($n=35$) little brown bats (*Myotis lucifugus*). Captive bats originally came from a wild population hibernating in a mine in northwestern Wisconsin during autumn of 2009 and were sampled on 9 May 2010 after having been maintained in hibernation for 187 days in dark environmental chambers at approximately 6.3 C and 84% relative humidity (control group: 6.4 C [SD 0.3 C], 82% [SD 7%]; infected group: 6.2 C [SD 0.6 C], 85% [SD 3%]), similar to the experiment reported by Lorch et al. (2011). Wild bats were sampled from a natural cave and an abandoned mine, both with *G. destructans*,

in Centre and Fayette counties, Pennsylvania, USA, respectively. Bats were sampled from the cave on 23 March 2010, and from the mine on 24 March 2010 and then again on 10 March 2011, each year about 130–160 days into hibernation. Cave temperature was approximately 5 C at time of collection. Mine temperature was approximately 2 C during collection in 2010 and 2011. Captive bats were moved to room temperature for up to 2 hr prior to euthanasia and administered isoflurane anesthetic prior to decapitation and blood collection. Wild bats were minimally handled, were quickly removed from their hibernation sites, and remained in deep torpor prior to decapitation without anesthetic.

Whole blood was collected into plastic centrifuge tubes using 200-μL lithium-heparinized capillary tubes (StatSpin StatSampler, model SS2H, IRIS International, Inc., Chatsworth, California, USA) within 30 sec of decapitation. Approximately 95 μL of whole blood was pipetted from the centrifuge tube to a diagnostic cartridge (model i-STAT EC8+, Abaxis, Union City, California, USA) and analyzed with a portable clinical analyzer (i-STAT VetScan, Abaxis) within 8 min of collection ($\bar{x}$ [95% confidence interval (CI)]=2.0 min [1.3–3.0 min]). The 200-μL capillary tubes contained 6 USP units of lithium heparin, which when filled with at least 125 μL of whole blood resulted in a calculated lithium concentration of <50 kIU/L, well below the concentration known to bias sodium results with this type of analyzer (Vuillaume et al., 1999). When possible (n=25), urine specific gravity was measured with a handheld refractometer. Urine was collected from wild and captive bats by cystocentesis 12–24 h and 0.5–1.0 h, respectively, after they were euthanized.

We used a histology scoring system published elsewhere (Reeder et al., 2012) to classify WNS-associated wing damage. Briefly, this scoring system is based on the histopathology determination of severity of fungal erosion and ulceration combined with the extent to which lesions were distributed over the surface area of wing skin on a scale of 0 (no lesions associated with WNS) to 4 (severe damage to wing membrane).

Bat capture and sampling procedures were approved by the Institutional Animal Care and Use Committees of the US Geological Survey (protocol EP081118-A1) and Bucknell University (to D.M.R.). Wild bats were captured under authority of a scientific collecting license issued to D.M.R. by the Pennsylvania Game Commission, and laboratory bats were collected under the authority of the Wisconsin Department of Natural Resources.

We used generalized linear modeling to model sodium (Na^+) and chloride (Cl^-) levels in bats as a function of site (lab, cave, mine), type (wild vs. captive), and wing damage score ("glm" function; R Development Core Team, 2012). We then used Akaike information criteria to select among models to determine the combination of variables that best explained variance in Na^+ and Cl^-.

Best models of Na^+ and Cl^- included the effects of site and wing damage. Parameter estimates of the effect of wing damage on Na^+ (estimate [95% CI]=−3.3 [−5.06–−1.48]) and Cl^- (−1.08 [−4.7–2.6]) indicated a negative relationship between wing erosion and Na^+ in blood (Fig. 1). Among the bats with moderate to severe WNS infection (erosion scores 2–4; n=22), average concentrations of Na^+ ($\bar{x}$ [95% CI]=132 mmol/L [128–135 mmol/L]) and Cl^- (103 mmol/L [99–106 mmol/L]) were lower than the concentrations observed in uninfected bats (n=28; Na^+, 146 mmol/L [143–149 mmol/L]; Cl^-, 109 mmol/L [107–112 mmol/L]) (Table 1). We are not aware of any reliable baseline information on normal Na^+ and Cl^- concentrations in blood of bats during hibernation (Riedesel, 1977), and presume that values we measured from uninfected bats represent normal concentrations for this species during late hibernation among the populations sampled. Urine specific gravity values of the bats tested (1.020 [1.018–1.022]) indicated they were

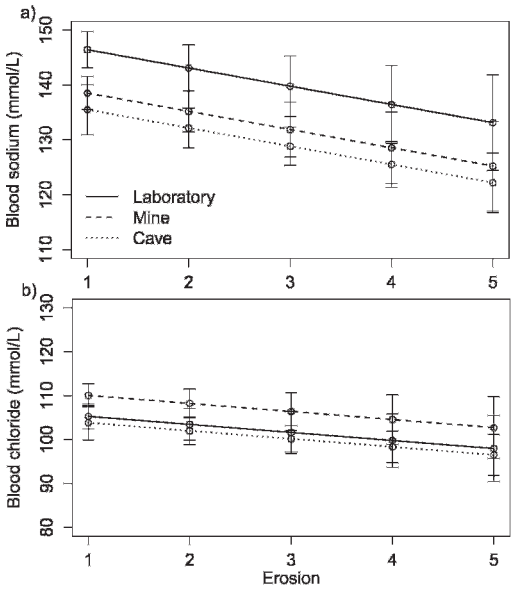

FIGURE 1. Concentration of sodium [Na^+] and chloride [Cl^-] ions (mmol/L) in whole blood of hibernating little brown bats (*Myotis lucifugus*), in relation to severity of wing infection by the fungus *Geomyces destructans*. Solid lines represent values from bats that hibernated under laboratory conditions and dashed and dotted lines represent values from bats sampled from a mine and cave, respectively, where they hibernated in the wild. Severity of fungal erosion of wing skin in each individual was determined by histopathology and scored on a scale of 0 (no infection) to 4 (severe skin erosion).

able to concentrate urine, and kidneys of captive bats examined histologically ($n=15$) were unremarkable and without lesions.

Our results indicate that bats with increasingly severe erosion and ulceration of wing membrane caused by *G. destructans* (WNS severity scores 2–4) may become clinically hyponatremic (low Na^+) and hypochloremic (low Cl^-). Although uncomplicated clinical dehydration would result in increased concentrations of Na^+ and Cl^+, we hypothesize that our data indicate hypotonic dehydration (total body water is sufficient, but Na^+ and Cl^- are low). Hypotonic dehydration can result from loss of electrolytes without proportional water loss, or from replacement of body water by drinking electrolyte-deficient water without uptake of electrolytes from other sources (e.g., food). It remains to be determined how wing damage caused by *G. destructans* might result in loss of electrolytes. Fungal erosion and ulceration of wing membranes may cause Na^+ and Cl^- to leak from damaged tissues along with fluids—similar to the impacts of extensive skin burns when the epidermis is destroyed. Alternatively, *G. destructans* might sequester salts from bat wing fluids. Hyponatremia and hypochloremia can also be caused by failure of the kidneys to properly conserve electrolytes and water. However, kidney histology and urine osmolality of WNS bats we examined appeared normal.

Dehydration resulting from fungal damage to skin is hypothesized to cause WNS bats to drink more during winter (Cryan et al., 2010; Willis et al., 2011), and possibly cause the more frequent arousals from hibernation associated with WNS mortality (Reeder et al., 2012; Warnecke et al., 2012). Although bats regularly arouse from hibernation to drink, water available in

TABLE 1. Average (95% confidence interval) concentrations of sodium [Na^+] and chloride [Cl^-] ions in whole blood of hibernating little brown bats (*Myotis lucifugus*) in relation to severity of wing infection by the fungus *Geomyces destructans*. Units are mmol/L. Severity of fungal erosion of wing skin in each individual was determined by histopathology and scored on a scale of 0 (no infection) to 4 (severe skin erosion). Samples pooled among sites.

Severity of skin erosion	[Na^+]	[Cl^-]	Sample size
0	146 (143–149)	109 (107–112)	28
1	142 (133–151)	106 (99–114)	3
2	134 (129–138)	105 (101–110)	9
3	132 (126–137)	101 (96–106)	10
4	126 (97–155)	99 (74–124)	3

hibernacula may not contain sufficient electrolytes to offset winter losses. Hibernating bats are obligate insectivores and obtain important minerals, including electrolytes, from their insect prey during early spring through late autumn (Studier et al., 1994). If certain electrolytes are lost during hibernation due to WNS, bats may be unable to replace them when insects are inactive and unavailable as prey.

It is difficult to assess potentially life-threatening health effects of the lower electrolyte concentrations we observed because few baseline data are available for bats, especially during hibernation (Riedesel, 1977). However, imbalance of blood electrolyte concentrations is known to cause serious physiologic problems (e.g., impaired neural and heart function) in other mammals, as well as in amphibians infected with skin-damaging chytrid fungus (*Batrachochytrium dendrobatidis*; Voyles et al., 2009).

Blood of bats hibernating under natural conditions had lower levels of Na^+ and Cl^-, suggesting hibernacula conditions influenced electrolyte balance independently of fungal damage. More information is needed on normal electrolyte concentrations in the blood of hibernating bats, as well as how WNS or environmental conditions in hibernacula (e.g., humidity and availability of drinking water) influence hydration status and electrolyte balance.

White-nose syndrome is one of the most devastating wildlife diseases to emerge in recorded history, and there are currently no clear methods for increasing the survival of bats affected by this disease. Our preliminary findings suggest that further research is needed to determine whether electrolyte depletion is a consistent clinical sign of WNS and is directly caused by *G. destructans*; establish the role of electrolyte depletion in WNS mortality; and assess whether naturally occurring sources of electrolyte-replete water in hibernacula might contribute to survival of infected wild bats.

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