Running Head: Letters

Title: Use of portable i-STAT for venous blood gas and biochemistry analysis in free-ranging Indian flying foxes (*Pteropus giganteus*) in Myanmar

Authors: Jennifer C. Kishbaugh[1], Marc T. Valitutto[1], Ohnmar Aung[1], Kyaw Yan Naing Tun[2], Lee-Ann C. Hayek[3], Jennifer H. Yu[1*], Suzan Murray[1]

Affiliations and Addresses:
[1] Global Health Program, Smithsonian's National Zoological Park and Conservation Biology Institute, 3001 Connecticut Ave NW, Washington, DC 20008
[2] Livestock Breeding and Veterinary Department, Ministry of Agriculture, Livestock, and Irrigation, Yangon, Myanmar
[3] National Museum of Natural History, Mathematics and Statistics, Smithsonian Institution, MRC-121, Washington, DC 20560

[*] Corresponding Author: Jennifer Yu
Phone #: 408-410-5401
Email: YuJ@si.edu

Word Count: 1000

Abstract:
Venous blood gas, acid-base, and biochemical parameters were determined for thirteen free-ranging *Pteropus giganteus* in Myanmar, using a handheld i-STAT analyzer with CG8+ and CHEM8 cartridges. For field-based projects, portable blood analyzers enable identification and management of electrolyte and acid-base imbalances and collection of physiologic data, but present logistical challenges.

Obtaining reliable hematologic and biochemical parameters for wildlife can be challenging in the field, but portable blood analyzers can facilitate monitoring of health and physiology of free-ranging species. In the present study, an i-STAT 200 blood gas analyzer with CG8+ and CHEM8 cartridges[1] was used to measure acid-base status, blood gas, and biochemistry values in free-ranging Indian flying foxes (*Pteropus giganteus*).

Study activities occurred in Okkan, Myanmar, selected for its close wildlife-domestic animal-human interface. Our study population was sourced from a colony of approximately 250 *P. giganteus* known to roost above human residences. Colony size fluctuates seasonally, with a peak population of about 2000 individuals during the wet season. Ambient temperatures during the study period ranged from 27.8–35.0 °C, and humidity averaged 66%.

As part of a larger viral surveillance and movement tracking project, thirteen (13) apparently healthy males, three juveniles and ten adults, were captured over three nights in April 2018 using a size 11 nylon mist-net suspended between bamboo masts[2]. Each night, the nets were placed in a different location within 100 m of the same roost. Bats were captured in the evenings at the time of roost departure, then disentangled from the nets and placed within pillowcases within five minutes of capture. They remained in the pillowcases for up to 40 mins prior to handling. Animals were examined, sexed, and aged based on the presence of penile barbs. Body condition was scored based on pectoral muscle mass and sternum prominence (Hossain et al. 2013a,b; McLaughlin et al. 2007); hydration was assessed through skin turgor, salivation, and position of the eye in the socket. All animals were deemed in "good" body condition with appropriate hydration status. Since bats were also fitted with GPS collars for a separate study, recaptures were avoided. Species was later confirmed by DNA barcoding (Townzen, Brower, and Judd 2008).

Physical examination and sample acquisition occurred within a five-minute timespan. For each bat, 2 mL of blood was drawn from the brachial vein at the distal humerus and transferred to a lithium heparin vacutainer[3] . Blood gas measurements were obtained immediately from CG8+ cartridges from whole blood directly from the syringe. Within 20 minutes of sample acquisition, biochemistry values were measured using CHEM8 cartridges after storage in lithium heparin. The i-STAT unit and cartridges were kept inside a cooler with ice packs; cartridges were removed immediately prior to blood collection, and the i-STAT was brought out for cartridge placement but returned to the cooler during sample analysis. All animals received 10–20 mL of fruit juice for hydration and were released near the trap site, with flight monitored for normality.

Table 1 displays descriptive statistics for body weight, blood gas analysis, and biochemical analysis. While hematologic and biochemical values have previously been reported, to the authors' knowledge, this is the first report of blood gas and select electrolyte values in free-ranging *Pteropus giganteus* (Heard and Whittier 1997; Hossain et al. 2013a,b; McMichael et al. 2015; McLaughlin et al. 2007). Hematocrit, urea, bicarbonate, and select electrolytes (Na, K, Cl) in this study appeared similar to other pteropids; as expected for frugivorous bats with low-protein diets, BUN was relatively low (Heard and Whittier 1997; Hossain et al. 2013a,b; McMichael et al. 2015; McLaughlin et al. 2007). However, our small sample size warrants further evaluation; a minimum of 40 individuals is recommended for reference range determination and comparison between species, sexes, and seasons (McMichael et al. 2015; Solberg 1987).

Compared to reports from other pteropids, a broad range in blood glucose was observed in the present study (Table 1) from both the CG8+ (mean 224.50 ± SD 69.720, range 87–337 mg/dL) and CHEM8 (mean 238.10 ± SD 111.07, range 69–396 mg/dL) panels (Hossain et al.

2013a,b; McLaughlin et al. 2007). High glucose values likely reflect acute or prolonged physiological stress in response to capture and handing, mediated by a glucocorticoid surge. As reported in *Pteropus* bats, capture, restraint, and handling-induced stress can induce elevations in plasma cortisol even at 90 mins post-capture (McMichael, Edson, and Field 2014; Reeder, Kunz, and Widmaier 2004; Smythe, Pascoe, and Storlien 1989; Widmaier and Kunz 1993). Another possible explanation is fruit juice administration before sampling of some individuals, in response to ambient heat and observed panting. Conversely, lower values could be attributed to artifact from delayed sample analysis (manufacturer instructions recommend within 10 mins for CHEM8), or a fasting period (Day, Heard, and LaBlanc 2001; Heard, Ruiz, and Harr 2006; Widmaier and Kunz 1993).

It is noteworthy that not all sample analyses yielded viable results. Manufacturer guidelines recommend cartridge storage at 2–8°C and analyzer operation at 16–30°C and below 90% humidity; however, ambient field temperatures reached 37.8°C. Unfortunately, temperatures within the cooler were not recorded, and it is uncertain how heat may have affected the results.

Inhalant anesthesia and short-term physical restraint have been associated with measurable hematologic and biochemical changes in Old World fruit bats (Heard and Huft 1998). In the present study, it was decided that the benefits of quick restraint and handling times outweighed anesthetic risks. Potential consequences include hyperthermia and stress, which surely impacted these results and impede interpretation. Additionally, some bats were observed panting, which can mask acid-base disturbances. Interpretation is also complicated by venous sampling, which precludes interpretation of oxygenation status; however, it is a more feasible option for wildlife due to potential hazards of arterial sampling in field conditions.

Despite logistic challenges, field blood gas and biochemical analysis may still have value; physiologic data collection can contribute to projects linking physiologic states with disease status. When considered with physical exam and hematocrit, inferences may be made regarding renal health and function and hydration status from BUN, creatinine, and metabolic acid-base disturbances. Additionally, electrolyte derangements can be corrected for in the field with selection of appropriate fluids.

This project was supported by Smithsonian Women's Committee (SWC grant 40); the Morris Animal Foundation (MAF) and Dennis and Connie Keller through a training partnership; and the Judy and John W. McCarter Global Health Internship.

References

1. Day RL, Heard DJ, LaBlanc D. 2001. The effect of time at which plasma separation occurs on biochemical values in small island flying foxes (*Pteropus hypomelanus*). *J Zoo Wildl Med* 32:206–208. DOI:10.1638/1042-7260(2001)032[0206:TEOTAW]2.0.CO;2
2. Heard DJ, Huft VJ. 1998. The effects of short-term physical restraint and isoflurane anesthesia on hematology and plasma biochemistry in the island flying fox (*Pteropus hypomelanus*). *J Zoo Wildl Med* 29:14–17.
3. Heard DJ, Ruiz MM, Harr KE. 2006. Comparison of serum and plasma for determination of blood biochemical values in Malaysian flying foxes (*Pteropus vampyrus*). *J Zoo Wildl Med* 37:245–248. DOI:10.1638/02-064.1
4. Heard DJ, Whittier DA. 1997. Hematologic and plasma biochemical reference values for three flying fox species (*Pteropus sp.*). *J Zoo Wildl Med* 28:464–470.
5. Hossain MB, Islam MN, Shaikat AH, Yasin MG, Hassan MM, Islam SKMA, Rahman A, Mamun MA, Khan SA. 2013a. Biochemical profile of wild-captured Indian flying fox (*Pteropus giganteus*) in Bangladesh. *Bangladesh Journal of Veterinary Medicine* 11:75–79.
6. Hossain MB, Islam MN, Yasin MG, Hassan MM, Islam SKMA, Khan SA. 2013b. Hematological profile of wild-captured Indian flying fox (*Pteropus giganteus*) in Bangladesh. *Int J Nat Sci* 3:12–14.
7. McLaughlin AB, Epstein JH, Prakash V, Smith CS, Daszak P, Field HE, Cunningham AA. 2007. Plasma biochemistry and hematologic values for wild-caught flying foxes (*Pteropus giganteus*) in India. *J Zoo Wildl Med* 38:446–452.
8. McMichael LA, Edson D, Field H. 2014. Measuring physiological stress in Australian flying-fox populations. *EcoHealth* 11:400–408. DOI:10.1007/s10393-014-0954-7
9. McMichael L, Edson D, Mayer D, McLaughlin A, Goldspink L, Vidgen ME, Kopp S, Meers J, Field H. 2016. Temporal variation in physiological biomarkers in black flying-foxes (*Pteropus alecto*), Australia. *EcoHealth* 13:49–59. DOI:10.1007/s10393-016-1113-0
10. McMichael L, Edson D, McLaughlin A, Mayer D, Kopp S, Meers J, Field H. 2015. Haematology and plasma biochemistry of wild black flying-foxes, (*Pteropus alecto*) in Queensland, Australia. *PLOS ONE* 10:e0125741. DOI:10.1371/journal.pone.0125741
11. Reeder DM, Kunz TH, Widmaier EP. 2004. Baseline and stress-induced glucocorticoids during reproduction in the variable flying fox, *Pteropus hypomelanus* (Chiroptera: Pteropodidae). *J Exp Zool A Comp Exp Biol* 301:682–690. DOI:10.1002/jez.a.58
12. Smythe GA, Pascoe WS, Storlien LH. 1989. Hypothalamic noradrenergic and sympathoadrenal control of glycemia after stress. *Am J Physiol* 256:E231–E235. DOI:10.1152/ajpendo.1989.256.2.E231
13. Solberg H. E. 1987. Approved recommendation (1987) on the theory of reference values. Part 5. Statistical treatment of collected reference values. Determination of reference limits. *Clin Chim* 170:S13–S32. DOI:10.1016/0009-8981(87)90151-3
14. Townzen JS, Brower AVZ, Judd DD. 2008. Identification of mosquito bloodmeals using mitochondrial cytochrome oxidase subunit I and cytochrome b gene sequences. *Med Vet Entomol* 22:286–292. DOI:10.1111/j.1365-2915.2008.00760.x
15. Widmaier EP, Kunz TH. 1993. Basal, diurnal, and stress-induced levels of glucose and glucocorticoids in captive bats. *J Exp Zool* 265:533–540. DOI:10.1002/jez.1402650509

Tables

Table 1: Comparative weight, hematologic, biochemistry, and venous blood gas values reported for *Pteropus giganteus*. Values have been converted where necessary to enable comparison. Additional values not analyzed in the current study were excluded from the table. SD = standard deviation; SEM = standard error of the mean.

* Includes only males to facilitate comparison with data from this study.

† Values are depicted as reported, but were not converted due to concerns with validity.

Parameter	This study, CG8+ cartridges			This study, CHEM8 cartridges			McLaughlin et al. 2007			Hossain et al. 2013a,b*	
	N	Mean ± SD	Range	N	Mean ± SD	Range	N	Mean ± SD	Range	N	Mean ± SEM
Weight (g)	13	651.54 ± 93.53	500–770				39	789 ± 119	511–1023	52	505.5
Na (mmol/L)	11	149.73 ± 5.46	139–159	10	145.8 ± 3.7	142–155					
K (mmol/L)	11	4.67 ± 0.41	4.0–5.2	10	4.89 ± 0.82	3.8–6.5					
Cl (mEq/L)				10	121 ± 4.45	115–130					
iCa (mmol/L)	11	1.2 ± 0.08	1.05–1.31	9	1.05 ± 0.12	0.87–1.36					
Glu (mg/dL)	10	224.5 ± 69.72	87–337	10	238.1 ± 111.07	69–396				43	129.6 ± 17.2
BUN (mg/dL)				9	7.78 ± 2.39	4–13	30	7.0 ± 2.52	3.4–12.3		
Urea (mmol/L)†										19	65.2 ± 9.8
Crea (mg/dL)				10	0.95 ± 0.31	0.5–1.4					
HCT (%)	10	47.6 ± 3.06	41–51	10	48.3 ± 2.95	44–52				51	52.2 ± 1.5
HGB (g/dL)	10	16.19 ± 1.04	14–17	10	16.43 ± 0.99	15–17.7				51	14.9 ± 0.3
pH	11	7.36 ± 0.05	7.274–7.484								
pCO_2 (mmHg)	11	32.63 ± 7.76	21.5–48								
TCO_2 (mmol/L)	10	19.4 ± 3.86	14–26	10	13.9 ± 2.85	7–16					
HCO_3 (mmol/L)	9	17.97 ± 3.8	13.3–24.3								
BE (mmol/L)	11	-7.45 ± 3.33	-13–-2								
AG (mEq/L)				9	16.67 ± 3.04	12–21					
sO_2 (%)	10	85.5 ± 10.11	63–95								

Footnotes:

1. Abbott Point of Care Inc., Princeton, NJ
2. All fieldwork was conducted in accordance with the Institutional Animal Care and Use Committees of the University of California at Davis (Protocol 19300) and Smithsonian's National Zoological Park (Protocol 16–05), and with approvals of Myanmar's Ministry of Agriculture, Livestock and Irrigation (MOALI) and Ministry of Natural Resources and Environmental Conservation (MONREC).
3. BD Vacutainer™, Becton, Dickinson and Company, Franklin Lakes, NJ

Abbreviations:

AG – Anion gap

BE – Base excess

BUN – Blood urea nitrogen

Cl – Chloride

Crea – Creatinine

Glu – Glucose

HCT – Hematocrit

HGB – Hemoglobin

iCa – Ionized calcium

K – Potassium

Na – Sodium

pCO_2 – Partial pressure of carbon dioxide

TCO_2 – Total carbon dioxide

HCO_3 – Bicarbonate

sO_2 – Oxygen saturation