

## Blood-Gas Analysis from Free-Ranging Eurasian Black Vultures (*Aegypius monachus*) in Mongolia and the Republic of Korea

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#### ABSTRACT

From 2011 thru 2014 the Denver Zoological Foundation, in collaboration with colleagues from Mongolia and the Republic of Korea (R. of Korea), collected venous blood samples for blood-gas analyses, from free-ranging and rehabilitation Eurasian Black Vultures (EbV, *Aegypius monachus*). The birds were hand-captured, hand-netted, or canon-netted. The sample analyses were determined using the i-STAT® portable chemistry analyzer. We performed venous blood-gas analyses in Mongolia and hand-netted rehabilitation birds in the R. of Korea, within minutes of venipuncture. There was a 2-3 hr. delay prior to analysis for canon-netted birds in the R. of Korea. We cooled these samples prior to analysis. A variety of venous blood-gas analyses were performed including; acid-base analytes (pH, PCO<sub>2</sub>, and HCO<sub>3</sub>), in addition to TCO<sub>2</sub>, Base Excess, Anion Gap, and lactate levels, venous oxygenation status (PO<sub>2</sub> and sO<sub>2</sub>), and temperature, pulse and respiration rates (TPR). For pH, PCO<sub>2</sub>, and sO<sub>2</sub> samples we report temperature corrected and non-temperature corrected results. From the data we constructed a blood-gas analyte and TPR reference range with a 95% predictive index. Results from these analyses were useful in assessing the health status for two vultures adversely affected by capture.

#### INTRODUCTION

The availability of point-of-care chemistry analyzers to document blood-gas parameters in free-ranging animal species, in the field is now both affordable and realistic. Blood-gas analyses for free-ranging avian species have been recently described<sup>[1-7]</sup>. Developing species specific blood-gas reference ranges can assist field veterinarians in implementing safer captures and help in providing more directed treatment efforts for compromised captured individuals. The Denver Zoological Foundation in collaboration with colleagues from Mongolia and the Republic (R.) of Korea conducted a patagial tagging<sup>[8,9]</sup> and radio telemetry project (unpublished data) for Eurasian Black Vultures (EbV, *Aegypius monachus*) to study movement patterns. During captures we collected venous blood samples and performed blood-gas analyses. In this paper we present reference ranges with a 95% predictive index for venous blood-gas analytes and cloacal temperature and heart and respiratory rates for EbVs from Mongolia and the R. of Korea.

# MATERIALS AND METHODS

## Capture techniques

Free-ranging nestling (90-120-day-old) EbVs were hand-captured for a radio telemetry and a patagial tagging study, in the Ikh Nart Chuluu Nature Reserve, Dornogovi Province in southeastern Mongolia from 2011 to 2014. During a radio-telemetry study in the R. of Korea (2012) we hand-netted juvenile (1-5-year-old) vultures, recuperating at a rehabilitation center in Paju, Gyeonggi Province. Also, in the R. of Korea (2013 to 2014) we used bait and a locally manufactured gun powder-based canon net system (koEco, Korea Institute of Environmental Ecology, Daejeon City, Republic of Korea) to capture free-ranging juvenile EbVs habituated to a vulture restaurant in Goseong County in Gyeongsang Province.

## Animal capture details

All vultures (n=122) used for the determination of the reference ranges appeared to be healthy prior to capture. We hooded all birds during processing to minimize stress. In Mongolia, we hand-captured nestlings (n=85) in August, but worked in the early morning thru early afternoon to avoid the hottest part of the day. We moved captured birds to a shaded area for processing. In the R. of Korea (n=37) we captured in January. In a rehabilitation center in Paju (n=9), we evaluated several birds available for release and some with permanent injuries precluding release. For rehabilitation birds we chased and hand-netted the birds in aviaries prior to sampling. We sampled (n=28) free-ranging birds, after capture by canon-netting in Goseong. Once removed from the canon net, the birds were placed in sacks to minimize struggling and stress.

## Sample collection

Free-flowing venous blood was collected from the ulnar vein (basilic vein) using a 3 ml syringe and 25 G needle. Prior to lactate analysis, it is important to collect free flowing blood to avoid vascular stasis, which may introduce a non-basal artificial increase in lactate levels or other artifacts<sup>[10]</sup>. Collected blood samples were immediately transferred to a lithium heparin green-top tube (Capiject, Terumo Medical Corp., Elkton, Maryland 21921, USA).

Avian blood cells continue to consume oxygen necessitating prompt analysis<sup>[11,12]</sup>. In Mongolia and at the R. of Korea's rehabilitation center we analyzed samples within minutes of venipuncture. For canon-netted birds in the R. of Korea there was a 2-3 hr. delay, between venipuncture and analysis. Animal welfare was our highest priority so we extracted birds from the net and processed all birds prior to blood sample analysis. We kept samples in a shaded and cool area, prior to analysis. Immediately following capture, we also recorded cloacal temperature, heart and respiratory rate (TPR). We determined that two birds were adversely impacted by the capture and therefore were excluded from the reference ranges.

## Equipment, sample processing, and testing details

We used the i-STAT® portable chemistry analyzer (Abaxis North America, Union City, California 94587, USA) for blood-gas analyses. The cartridges were stored at 2 to 8°C prior to use and the analyzer was maintained between 16 to 30°C during analysis (i-STAT® 1 User Manual, 16 Oct 2012, Abbott Point of Care Inc., Abbott Park Illinois 60064, USA). We used instant hot or cold packs, within an insulated bag and a portable 12 volt cooler (RoadPro®, Palmyra, Pennsylvania 17078, USA) in the field to keep the analyzer and cartridges within the required temperature ranges. Blood-gas analyzers, including the i-STAT® (VetScan® i-STAT®1 handheld analyzer, Operator's Manual, December 2009), are calibrated to operate at the normal human body temperature of 37°C<sup>[13-15]</sup>. We used the i-STAT® CG4+ (pH, PCO<sub>2</sub>, HCO<sub>3</sub>, Base Excess [BE], sO<sub>2</sub>, and lactate) and CHEM8+ (Hct, Hgb, BUN, Crea, iCa, Glu, Cl, Na, K, TCO<sub>2</sub>, and Anion Gap [AG]) cartridges for analysis. The pH, PCO<sub>2</sub>, PO<sub>2</sub>, and lactate are measured analytes while HCO<sub>3</sub>, TCO<sub>2</sub>, BE, and AG are calculated values ((i-STAT® 1 User Manual). For two EbVs, one obese and the second hyperthermic, we present the analyte results as case reports later in the Discussion.

## Statistical analyses

Descriptive statistics (mean, standard error [SE], and n) were determined for vultures from Mongolia and the R. of Korea. Due to a small sample size (n=<120) some statistical bias<sup>[16]</sup> may be present. We examined all variables for normality using the Shapiro and Anderson tests. We computed reference ranges for the two populations with a 95% prediction interval (PI). We eliminated outliers and used square root and natural logs to transform nonparametric data prior to determining the Predictive Interval. For samples (n=<20) we report a complete numeric range (maximum and minimum) but did not calculate a reference range<sup>[17]</sup>. We used simple t-tests, with pooled variance to compare temperature corrected and temperature non-corrected data. We accounted for the effects of location, year, age, class, and the impact of temperature adjustments on blood-gas values using General Linear Models. We set significance at P <.05.

# RESULTS

The blood-gas values for 122 EbVs are reported in **Table 1** (Mongolia; n=85 free-ranging nestlings, R. of Korea; n=37 [n=9 captive rehabilitation plus n=28 free-ranging EbVs]). Blood-gas analyzers, including the i-STAT® (VetScan® i-STAT®1 handheld analyzer, Operator's Manual, December 2009), are calibrated for a patient temperature of 37 °C<sup>[13-15]</sup>. If the patient's temperature

is not 37 °C it changes the results for pH, pCO<sub>2</sub>, and PO<sub>2</sub> [13,15,18]. Therefore, we report the results for pH, pCO<sub>2</sub>, and PO<sub>2</sub> as temperature corrected (TC) and non-temperature corrected (nTC).

The results obtained were organized into four categories; acid-base, additional supportive acid-base analytes, oxygenation, and TPR. The reference ranges we determined are wide but the sample sizes for each analyte are small. The analytes were significantly different, when comparing nestlings hand-caught in Mongolia to juveniles chased and hand-netted or canon-netted in the R. of Korea.

We report the venous acid-base (pH, PCO<sub>2</sub>, and HCO<sub>3</sub>) results and additional analytes in **Table 1**. They are similar to results published for several other published avian blood-gas studies [1-4,7]. Venous blood-gas samples are typically not clinically helpful in determining oxygen status, but in small animals a venous PO<sub>2</sub> value <30 mmol/L is a cause for concern [15,19]. In this study EbVs had a mean PvO<sub>2</sub> (Mongolia; 72 and 77 mmol/L, R. of Korea; 77 and 82 mol/L, nTC and TC respectively) and mean svO<sub>2</sub> (Mongolia; 93%, R. of Korea; 95%) that appeared high for venous samples perhaps even approximating arterial levels (**Table 1**). In small animals 90-95% arterial oxygen saturation (sO<sub>2</sub>) is approximately equivalent to a PaO<sub>2</sub> of 60-80 mmol/L [20]. All vultures in this study fall within that range.

**Table 1.** Mean, standard error [SE], n, and reference range (95% prediction interval, PI) for venous blood-gas analytes and cloacal temperature and heart and respiratory rates (TPR) for Eurasian Black Vultures (EbV, *Aegypius monachus*) in Mongolia and the Republic (R.) of Korea. The analytes are categorized as venous acid–base (pH, PCO<sub>2</sub>, and HCO<sub>3</sub>), additional analytes; (TCO<sub>2</sub>, base excess [BE], Anion Gap [AG], and lactate), oxygenation (PO<sub>2</sub>, sO<sub>2</sub>), and TPR. We present pH, PCO<sub>2</sub>, and sO<sub>2</sub> results as non-temperature corrected<sup>1</sup> and temperature corrected<sup>2</sup>. We report minimum and maximum values and not reference interval for analytes with less than 20 samples designated by superscript<sup>3</sup>.

Analyte	Mongolia EbV Mean, ±SE, n	Reference range 95% (PI)	R. Korea EbV Mean, ±SE, n	Reference range 95% (PI)
Acid-base				
pH (Log <sub>10</sub> )	7.427, 0.01, 85 <sup>1</sup> 7.394, 0.01, 85 <sup>2</sup>	7.306–7.546 <sup>1</sup> 7.274–7.535 <sup>2</sup>	7.484, 0.01, 37 <sup>1</sup> 7.444, 0.01, 37 <sup>2</sup>	7.374–7.599 <sup>1</sup> 7.341–7.543 <sup>2</sup>
PCO <sub>2</sub> (mmol/L)	29.0, 0.43, 85 <sup>1</sup> 32.2, 0.48, 85 <sup>2</sup>	21.1–36.8 <sup>1</sup> 20.8–41.5 <sup>2</sup>	25.4, 0.58, 37 <sup>1</sup> 29.0, 0.58, 37 <sup>2</sup>	18.1–32.7 <sup>1</sup> 21.7–36.3 <sup>2</sup>
HCO <sub>3</sub> (mmol/L)	19.1, 0.23, 85	14.9–23.2	19.1, 0.33, 37	15.0–23.1
Additional analytes				
TCO <sub>2</sub> (mmol/L)	20.0, 0.24, 85	15.8–24.9	19.9, 0.33, 37	16.1–24.3
BE (mmol/L)	-5.2, 0.29, 85	-10.5–0.0	-4.4, 0.40, 37	-9.4–0.6
AG (mmol/L)	12.9, 0.39, 56	8.7–18.8	14.9, 0.47, 35	9.9–21.5
Lactate (mmol/L)	3.46, 0.11, 85	1.84–5.99	2.64, 0.19, 37	1.06–5.57
Oxygenation				
PO <sub>2</sub> (mmol/L)	72, 2.62, 85 <sup>1</sup> 77, 2.81, 85 <sup>2</sup>	43–105 <sup>1</sup> 48–107 <sup>2</sup>	77, 3.40, 37 <sup>1</sup> 82, 3.65, 37 <sup>2</sup>	47–121 <sup>1</sup> 50–130 <sup>2</sup>
sO <sub>2</sub> (%)	93, 0.47, 85	84–100	95, 0.43, 37	90–100
TPR				
Cloacal Temp (°C)	39.3, 0.12, 85	38.1–40.6	39.8, 0.35, 37	37.4–42.3
Heart Rate (b/min)	168, 3.04, 84	116–227	172, 5.10, 12	144–204 <sup>3</sup>
Resp. Rate (b/min)	28, 0.59, 84	17–37	47, 5.87, 12	24–88 <sup>3</sup>

Finally we report TPR results in **Table 1**. The most hyperthermic birds (n=9) in this study corresponded to chased and hand-netted specimens, at the aviary in the rehabilitation center (R. of Korea). The mean cloacal temperature was 41.3°C SE ± 0.15. The lowest cloacal temperatures corresponded to nestlings (n=85) hand-caught in Mongolia, with a mean cloacal temperature of 39.3 SE ± 0.07. We believe nestlings in Mongolia represent the normal cloacal temperature for undisturbed vultures.

## DISCUSSION

In another study the i-STAT® chemistry analyzer was found to be accurate for blood samples from avian patients [21]. This point-of-care technology allows veterinarians the ability to perform in the field chemistry analyses, while the patient is restrained. The major impediment for the i-STAT® as field tool is to keep the cartridges and analyzer within their required storage and operating temperature ranges. We detailed our strategy for temperature control for the cartridges and analyzer in the Materials and Methods section.

Due to the difficulties in obtaining an arterial blood sample from a large unanesthetized bird, in the field, we opted to use the more easily obtainable venous sample. A high degree of agreement for pH, PCO<sub>2</sub>, HCO<sub>3</sub>, base excess, and lactate is reported when comparing arterial and central venous values, while there is poor correlation comparing arterial PO<sub>2</sub> with venous PO<sub>2</sub> [19,22-26]. We believe a venous PO<sub>2</sub> reference range may still be useful information for patients with very low venous PO<sub>2</sub> values.

Blood-gas analyzers calculate pH, PCO<sub>2</sub>, and PO<sub>2</sub> assuming a patient temperature of 37 °C [13-15]. Patient temperatures outside of 37 °C will change those results [13-15]. However, the clinical relevance for correcting pH and PCO<sub>2</sub> for body temperatures

outside of 37 °C is debatable [13-15]. The validity of temperature correction algorithms in non-human patients is unknown. Some clinicians consider it unnecessary to correct pH and pCO<sub>2</sub> in cases of hyperthermia [13-15]. In this study all birds had cloacal temperatures exceeding 37 °C (mean=39.5 SE ± 0.08 °C, n=122). The recommendation for PO<sub>2</sub> is to temperature correct the results if the body temperature difference is more than 1 degree variance from 37 °C [13,27]. Therefore we present venous pH, PCO<sub>2</sub>, and PO<sub>2</sub> results as TC and nTC in **Table 1**.

The initial step for blood-gas analysis is to evaluate the pH to determine if there is evidence for an acid-base disorder [3,19,28]. For small animals, life-threatening pH is reported to range from <7.1 to >7.7 [19]. If there is pH evidence, for an acid-base disturbance, PCO<sub>2</sub> and HCO<sub>3</sub> are determined. This will indicate if the cause is respiratory or metabolic in nature [3,22,23,28,29]. PCO<sub>2</sub> levels determine if the disturbance is respiratory, while HCO<sub>3</sub> TC0<sub>2</sub>, BE, and AG aid in determining if the disturbance is metabolic in origin [3,22,23,28,29]. However, a normal pH does not completely rule out an acid-base disorder [19,28]. Compensatory responses can return the pH to the normal range. For humans full compensation for a respiratory disorder requires 6 to 12 hr, while a metabolic disturbance requires 3 to 5 days [30]. We hypothesized that the stress of capture could result in a mixed acid-base disorder. We expected a metabolic acidosis due to increased exercise during capture and a respiratory alkalosis from hyperventilation. This might result in partial or full compensation for an acid-base disorder resulting in a normal pH.

The pH, PCO<sub>2</sub>, and HCO<sub>3</sub> results in this study were similar to the nTC i-STAT® results for 40 rehabilitated red-tailed hawks (*Buteo jamaicensis*) bled from the jugular vein and 35 captive quaker parrots (*Myiopsitta monachus*) captured by mist net and also bled from the jugular vein (**Table 2**) [4,7]. Avian venous PCO<sub>2</sub> levels are typically lower than mammals [31]. In a study with five unanesthetized domestic dogs venous PCO<sub>2</sub> values from three different venous sites determined a mean of 43.1 mmol/L (mixed venous), a mean of 42.1 mmol/L (jugular vein), and a mean of 43.0 mmol/L (cephalic vein) [32]. The lower venous PCO<sub>2</sub> levels (mean=29.0 TC mmol/L, mean=32.2 nTC mmol/L) determined in this study (**Table 1**) may be a normal for avian species. Possible explanations are hyperventilation following capture and/or more complete ventilation for avian species compared to mammals [31].

**Table 2.** Mean pH, PCO<sub>2</sub>, HCO<sub>3</sub>, lactate, PO<sub>2</sub>, sO<sub>2</sub> venous blood comparisons for Eurasian Black Vultures (EbV, *Aegypius monachus*), red-tailed hawks (*Buteo jamaicensis*), quaker parrots (*Myiopsitta monachus*), Passeriformes, Australasian goshawk (*Accipter fasciatus*), domestic goose, broiler breeders. All samples, except domestic geese (radiometry), were analyzed with i-STAT® chemistry analyzer at 37 °C except lactate (not affected by patient temperature).

Species	pH (Log <sub>10</sub> )	PCO <sub>2</sub> (mmol/L)	HCO <sub>3</sub> (mmol/L)	Lactate (mmol/L)	PO <sub>2</sub> (mmol/L)	sO <sub>2</sub> (%)
EbV (Mongolia) n=85	7.427	29.0	19.1	3.46	72	92.9
EbV (R. of Korea) n=37	7.484	25.4	19.1	2.64	77	95.4
Red-tailed hawk <sup>7</sup> n=40	7.43	26.8	18.1	ND	ND	ND
Quaker parrot <sup>1</sup> n=35	7.43	28.6	19.2	ND	41.0	77.9
Passeriformes <sup>2</sup> n=91	7.583	26.1	24.5	4.55	38.4	81.1
Australasian goshawk <sup>46</sup> n=9	ND	ND	ND	3.53	ND	ND
Domestic goose <sup>45</sup> n=3	ND	ND	ND	0.94	ND	ND
Broiler breeders <sup>5</sup> n=165	ND	ND	ND	ND	46.2	82.0

<sup>7</sup>Heatley et al. 2005, <sup>1</sup>Rettenmund et al. 2014, <sup>2</sup>Heatley et al. 2013, <sup>46</sup>Maho et al. 1992, <sup>45</sup>Holz and Naisbitt 2000, <sup>5</sup>Martin et al. 2010. ND=not done.

The venous acid-base analytes we determined in Mongolia had lower pH, higher PCO<sub>2</sub>, and similar HCO<sub>3</sub> when compared to the R. of Korea. In a study that examined the effect of age on venous blood-gas results in Passeriformes found that juvenile birds had higher lactate and lower BE, HCO<sub>3</sub>, and TC0<sub>2</sub> compared to adults [2]. Perhaps the difference in pH and PCO<sub>2</sub> we determined reflect age (nestlings in Mongolia vs. juveniles in the R. of Korea) and/or capture technique effect (hand-caught in Mongolia vs. ground-chased and hand or canon-net captured in the R. of Korea).

TCO<sub>2</sub>, BE, and AG are additional analytes used to evaluate the metabolic component when there is an acid-base disturbance [28,33-37]. Ninety five percent of TCO<sub>2</sub> is HCO<sub>3</sub> [28,36,37]. Therefore, clinicians occasionally use TCO<sub>2</sub> as a metabolic indicator for an acid-base disturbance [28,36,37]. The mean TCO<sub>2</sub> result in this study is at the low end of the range (20-30 mmol/L) for that recommended as normal for many species but consistent with i-STAT® studies involving quaker parrots and red-tailed hawks [1,3,7,35]. Low TCO<sub>2</sub> values support a diagnosis of metabolic acidosis or compensatory respiratory alkalosis while high values indicate metabolic alkalosis or compensatory respiratory acidosis [35,37].

BE is the amount of strong acid or base used to titrate 1 L of blood to a pH of 7.40 at 37°C with the PCO<sub>2</sub> held at 40mmol/L [28,38]. In humans, the reference range for BE is -2 to +2 mmol/L [34,39]. A decrease in TCO<sub>2</sub> and a negative BE are consistent with metabolic acidosis [28,34].

AG is the difference between commonly measured cations and anions ( $\text{Na}^+ + \text{K}^+ - (\text{Cl}^- + \text{HCO}_3^-)$ ) [19,28,40]. Increases in the production of organic acids cause an increase in the AG [38]. The AG reference range for humans is 8 to 16 mmol/L and dogs 12 to 24 mmol/L [19,28,34,41]. High AG values are associated with metabolic acidosis [28,42]. Clinicians have also used AG to assist in calculating sodium bicarbonate therapy for severe acidosis [39,43]. The mean AG reference ranges determined in this study (**Table 1**) fall within the normal mammalian range.

Severe physical activity can lead to lactic acidosis [28,19]. Capturing free-ranging species often involves increased physical activity. In humans, persistently elevated blood lactate concentrations ( $>5$  mmol/L) are consistent with lactic acidosis [10,28, 34]. Levels less than 2 mmol/L are normal for dogs [10,19]. In a study with two exercise conditioned red-tailed hawks lactate levels peaked at 2 min and returned to baseline in 10 min following exercise emphasizing the critical relationship between venipuncture timing and lactate analysis [44]. Venous lactate levels in this study were higher than arterial samples remotely retrieved from unanesthetized domestic geese but lower than Passeriformes captured by mist netted and exercised conditioned rehabilitated Australasian goshawks (*Accipiter fasciatus*) (**Table 2**) [2,45,46]. In this study lactate levels for canon-netted juvenile EbVs in R. of Korea were lower than the nestlings hand-caught in Mongolia (**Table 2**). We determined birds for lactate analysis in Mongolia within minutes of capture, while in the R. of Korea there was a 1 to 2 hr. delay following venipuncture while we extracted and processed all the birds captured in the canon net. Lactate samples should be analyzed promptly following collection but in this case the delay was necessary for animal welfare considerations. In an effort to minimize these effects we kept the samples cool. In the R. of Korea lactate levels may have been returning to baseline levels prior to venipuncture.

The avian lung is extremely efficient in regards to gas exchange due to several unique avian anatomic and physiologic features [47-52]. Compared to mammals avian species are more efficient at extracting oxygen from ambient air and are more tolerant of hypoxia [51,53]. Oxygen demands can increase by several orders of magnitude when transitioning from rest to flight [54,55]. The altitude record for an avian species was an aircraft collision with a Rüppell's Griffon Vulture (*Gyps rueppelli*) at 11,000 meters [56]. A study replicating oxygen conditions at 6100 m with species of similar mass demonstrated that a sparrow (*Passer domesticus*) was alert and active while a mouse (*Mus musculus*) appeared to be near death [57].

The gold standard for assessing oxygenation status is an arterial blood sample [24,28,34,37]. However, arterial samples are much more difficult to obtain than venous samples in the field and there are more potential negative health complications and side effects from arterial venipuncture [24]. Venous blood is usually not helpful in evaluating oxygen status because the range for venous oxygen levels is too wide and variable [20,41,58]. EbVs from Mongolia and the R. of Korea in this study had higher mean venous PO<sub>2</sub> and sO<sub>2</sub> levels when compared to several other i-STAT® venous oxygen studies (**Table 2**) [1,2,4,5]. Increased hemoglobin affinity for O<sub>2</sub> in some avian species when compared to mammalian species, may partly explain the high svO<sub>2</sub> levels we determined [48,50]. We obtained the samples from the ulnar vein in the wing, so perhaps the muscles of the vulture wing reflect high levels of oxygen for high altitude soaring. We believe a reference range for venous oxygen levels may still provide useful information when as previously described levels are very low.

A study with the great tit (*Parus major*) found that increases in temperature and heart and breathing rate were indicators of acute emotional stress during capture and handling [59,60]. Although in Mongolia we immediately obtained a TPR following capture we found that heart and breathing rates would increase just from handling. Since we measured the cloacal temperature within minutes of capture we believed a rise in cloacal temperature during processing would be a predictor for emotional stress from capture and restraint. We believe the mean temperature of 39.3°C we determined for hand-caught nestlings in Mongolia approximates the resting undisturbed EbV cloacal temperature (**Table 1**). The EbV mean cloacal temperature was also consistent with body temperature values determined for avian species in other studies [18,61]. The mean cloacal temperature for canon-net captured birds in the R. of Korea was only 0.5°C higher at 39.8°C than hand-caught nestlings. Perhaps the cloacal temperatures were initially elevated but returned close to baseline while restrained in sacks prior to processing.

Hyperthermia is often associated with exertional or capture myopathy in mammals and presumably in avian species as well [62]. At the conclusion of restraint in Mongolia, we obtained a second cloacal temperature. We used the difference between the initial cloacal temperature and the final cloacal temperature just prior to release as a measure for processing stress. For hand caught EbV nestlings (n=85), the mean increase in cloacal temperature from capture to release was only 0.4°C. Perhaps by hooding and working quickly in a shaded area we were able to minimize the emotional stress following capture and processing. We believe the reference range we determined from Mongolia will assist in identifying stressed and hyperthermic birds. Since in the R. of Korea we evaluated  $<20$  birds for heart and breath rates we did not construct a reference range but present the whole range (minimum to maximum for all samples)

Blood-gas and TPR reference ranges are useful in evaluating the effects of capture. They are also useful in directing treatment efforts for animals adversely affected by capture. We used the reference ranges to evaluate two outliers that experienced difficulty following capture. Results from these two cases were not included in constructing the reference ranges but are described in the following case reports.

## CASE REPORTS

**Case 1** was an obese bird housed in an aviary at the Paju rehabilitation center in the R. of Korea. Following a ground-chase

and hand-netting we positioned it on its back for TPR and venipuncture. The respirations were slow and shallow and it appeared cyanotic. We report the blood-gas results in **Table 3**. Comparison with the reference range determined for EbVs in the R. of Korea demonstrated ↓vpH (acidemia), ↑PvCO<sub>2</sub> (respiratory acidosis), and a metabolic component with ↑vlactate. Evaluation of additional analytes demonstrated a negative BE and elevated AG further supporting a diagnosis of lactic acidosis (**Table 3**). Although venous blood samples don't typically provide reliable information regarding oxygenation status in this case venous PO<sub>2</sub> and sO<sub>2</sub> levels were significantly less than the R. of Korea reference levels indicating possible oxygenation issues. Following repositioning to a standing position it quickly returned to normal.

**Table 3.** Clinical case reports for an obese and hyperthermic Eurasian Black Vulture (EbV) in the Republic (R.) of Korea. The analytes are categorized as venous acid–base (pH, PCO<sub>2</sub>, and HCO<sub>3</sub>), additional analytes (TCO<sub>2</sub>, BE, Anion Gap, and lactate), oxygenation (PO<sub>2</sub>, sO<sub>2</sub>), and cloacal temperature and pulse and respiratory rates (TPR). We present pH, PCO<sub>2</sub>, and sO<sub>2</sub> results as non-temperature corrected<sup>1</sup> and temperature corrected<sup>2</sup>. We report the minimum and maximum values and not reference interval for analytes with less than 20 samples designated by superscript<sup>3</sup>.

Analyte	R. Korea EbV Reference range	R. Korea EbV Obese	R. Korea EbV Hyperthermic
Acid-base			
pH (Log <sub>10</sub> )	7.374–7.599 <sup>1</sup> 7.341–7.543 <sup>2</sup>	7.038 <sup>1</sup> 6.996 <sup>2</sup>	7.603 <sup>1</sup> 7.499 <sup>2</sup>
PCO <sub>2</sub> (mmol/L)	18.1–32.7 <sup>1</sup> 21.7–36.3 <sup>2</sup>	73.1 <sup>1</sup> 83.8 <sup>2</sup>	18.7 <sup>1</sup> 26.2 <sup>2</sup>
HCO <sub>3</sub> (mmol/L)	15.0–23.1	19.7	18.5
Additional analytes			
TCO <sub>2</sub> (mmol/L)	16.1–24.3	22	19
BE (mmol/L)	-9.4–0.6	-11	-3
AG (mmol/L)	9.9–21.5	20	17
Lactate (mmol/L)	1.06–5.57	16.22	2.55
Oxygenation			
PO <sub>2</sub> (mmol/L)	47–121 <sup>1</sup> 50–130 <sup>2</sup>	45 <sup>1</sup> 48 <sup>2</sup>	70 <sup>1</sup> 5 <sup>2</sup>
sO <sub>2</sub> (%)	90–100	59	97
TPR			
Cloacal Temp (°C)	37.4–42.3	39.8	43.9
Heart Rate (bmp)	144–204 <sup>3</sup>	122	ND
Resp. Rate (bmp)	24–88 <sup>3</sup>	28	ND

**Case 2** illustrates the issue comparing TC vs. nTC analytes in acid-base analysis. Lethal hyperthermia in avian species occurs at deep core temperatures (measured from the proventriculus or large intestine) ranging between 46 and 47°C [18]. Following canon net capture we noted one bird had a cloacal temperature of 43.9°C. This is 1.6°C higher than the upper end of the cloacal temperature reference range we determined in this study (**Table 3**). In a project involving domestic chickens increasing hyperthermia is associated with respiratory alkalosis and lactic acidosis [63]. In this bird we found an increase in the venous nTC pH and a decrease in PCO<sub>2</sub> indicating respiratory alkalosis. In this case lactate levels appeared to be normal. However, the TC results for this same bird were within the reference range we determined. But, this bird was not normal and was unable to fly off following processing. We easily approached and restrained it. Treatment consisted of removing the bird to a cooler environment. It required several days in a rehabilitation center before release. The nTC values supported clinical intervention while the TC values did not.

In conclusion, the availability of portable chemistry analyzers provide field veterinarians with the capability to monitor field captured free-ranging animals, while still restrained. Capture is a stressful event and predisposes captured animals to potentially lethal physiologic consequences. In regards to blood-gas analyses, we believe venous samples provide useful information under field conditions. Knowledge of normal blood-gas analyte values and reference ranges can assist field veterinarians in making assessments, in real time, prior to instituting therapeutics measures. In this study we presented venous blood-gas data for EbVs in Mongolia and the R. of Korea. These values were useful in establishing a reference range for blood-gas analytes, evaluating the effects of capture techniques, evaluating the effects on individual captured vultures, and assisted with critical care for two individuals negatively impacted by capture.

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