

Original Study

## Postcapture Point-of-Care Blood Lactate Measurement in Zoo-Housed Cattle Egrets (*Bubulcus ibis*)

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**Abstract:** Blood lactate concentration is a fast and reliable point-of-care metric used in both human and veterinary medicine to indirectly assess tissue perfusion. Blood lactate concentration is also frequently used as a prognostic indicator for multiple disease processes in veterinary medicine. The goals of this study were to evaluate point-of-care venous lactate concentrations in a group of cattle egrets (*Bubulcus ibis*) maintained in a zoo to determine if order of net capture had a significant effect on blood lactate concentrations and evaluate the association between lactate concentration and routine blood diagnostic test results. Blood samples were obtained from 10 cattle egrets housed together in a holding area as part of routine quarantine procedures. Animals were captured via net in pairs, quarantine examinations and sample collection were completed, and birds were placed in a crate for holding prior to net capture of the next pair. All birds had blood samples collected for a complete blood cell count, avian biochemistry panel, and lactate concentration. Blood lactate concentrations were obtained with the Nova Biomedical Lactate Plus point-of-care lactate meter. The mean blood lactate concentration was 12.6 mmol/L (range, 9.3–17.4 mmol/L). Capture order was not significantly associated with blood lactate concentration ( $P = 0.60$ ). There was a significant positive correlation between lactate concentration and sodium ( $P = 0.04$ ). There were no significant relationships between blood lactate concentration and standard complete blood cell count or biochemical values. This study found post-capture blood lactate concentrations in the cattle egrets that were unexpectedly high when compared with published avian values and found that the order in which the animal was processed in the capture event was not associated with blood lactate concentration. Further research is warranted to determine the value of blood lactate concentration trends in this species.

**Key words:** lactate, postcapture, avian, cattle egret, *Bubulcus ibis*

### INTRODUCTION

The cattle egret (*Bubulcus ibis*) is a species of heron (family Ardeidae) found in the tropics, subtropics, and warm temperate zone. Their bright white color, flocking nature, and propensity to perch high in trees makes them appealing exhibit birds within zoos. These birds are also commonly presented to wildlife rehabilitation clinics. Due to the excitable nature of this species, capture stress is commonly encountered in both zoological institutions and rehabilitation settings. Acquiring patient-side data that have the potential to aid in determining a disease prognosis (eg, point-of-care lactate concentration) can help guide patient care and resource allocation.

Lactate is the byproduct of anaerobic metabolism of pyruvate during glycolysis.<sup>1</sup> Elevations in lactate concentrations are characterized as hypoxic (type A) or nonhypoxic (type B) elevations. Hypoxic (type A) elevations occur secondary to obstructive shock, cardiogenic shock, distributive shock, and hypovolemic shock.<sup>2</sup> Nonhypoxic (type B) elevations occur with disease (eg, liver disease, neoplasia), toxin exposure, and mitochondrial dysfunction.<sup>2</sup> In most mammals, normal concentrations of blood lactate are between 1 and 2 mmol/L but can quickly rise to over 20 mmol/L during intense exertion.<sup>3–5</sup> Lactate concentrations have also been shown to positively correlate with corticosterone concentrations.<sup>6,7</sup> Corticosterone is a steroid hormone produced by the adrenal glands during times of stress and is the major stress hormone in birds.<sup>6</sup> With this association, lactate concentration has been used as an indirect measure of stress during handling and transport of multiple avian species,

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including chickens (*Gallus gallus domesticus*), American flamingos (*Phoenicopterus ruber*), and Dalmatian pelicans (*Pelecanus crispus*).<sup>7–10</sup>

Point-of-care analyzers allow for targeted information and rapid results in a patient-side manner with a small sample volume. Measurement of blood lactate concentration is a frequently performed patient-side clinical test that can help identify tissue hypoxia, capture stress, and aid clinicians in determining prognosis.<sup>3,7,11,12</sup> Previous studies investigating lactate as an indirect measure of stress or as a prognostic indicator in avian species include studies on whooping cranes (*Grus americana*), double-crested cormorants (*Nannopterum auritum*, previously known as *Phalacrocorax auritus*), American flamingos, mourning doves (*Zenaidura macroura*), boat-tailed grackles (*Quiscalus major*), and house sparrows (*Passer domesticus*).<sup>4,8,13,14</sup>

The purpose of this study was to document post-capture lactate concentrations and hematologic data in clinically healthy cattle egrets and to determine the effect of order of capture on venous blood lactate concentrations in these birds. We hypothesized that the birds captured later would experience prolonged stress and would have higher lactate concentrations than birds captured earlier in the event.

## MATERIALS AND METHODS

This retrospective study was performed from reviewed values obtained during routine quarantine examinations. Use of data was approved by the Research Review Committee at the Saint Louis Zoo. Because this study was retrospective in nature, an Institutional Animal Care and Use Committee proposal was not required.

Ten male cattle egrets ranging in age from 6 to 8 years were housed together in a 3-m-long by 2.8-m-wide by 3.2-m-tall outdoor stall. The stall was partially flooded to mimic normal habitat and contained ample perching, both partially submerged and affixed in the air. The diet was fed in multiple pans near ground level twice per day and consisted of 0.32 kg feline diet (Milliken Meat Products, Markham, Ontario, Canada), 0.16 kg Mazuri flamingo breeder pellets (Mazuri Exotic Animal Nutrition, St Louis, MO, USA), 0.32 kg frozen thawed capelin, and 0.55 kg frozen thawed smelt. All animals were evaluated as part of routine quarantine procedures 12 days into their quarantine period after arrival from another institution. Birds ranged in weight from 345 to 460 g, with an average weight of 406 g. No animal had any significant prior medical history or clinical concerns during their quarantine period. All animals had been housed in a free-flight aviary at the previous

institution and were not accustomed to handling or netting. The flock was not fasted prior to the physical examinations.

Animals were netted in pairs out of the group of 10 birds by a team of 2 trained animal care staff, and order of handling was recorded. After netting, each bird was manually restrained, and blood was immediately collected from the right jugular vein followed by a thorough physical examination. Once examinations were completed, the birds were released into crates, and the team restarted the capture process for the next pair. The amount of time required to net each bird was approximately 2 minutes per bird, and the physical examination lasted approximately 3 minutes per bird. The amount of time between the netting of the first pair of birds and the netting of the final pair was approximately 45 minutes. There was no difference appreciated by animal care staff in difficulty of netting any individual bird over another.

Once obtained, blood was immediately placed into lithium heparin microtubes (BD Microtainer, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) for a complete blood cell count (CBC), plasma biochemical analysis, and lactate. Blood lactate concentrations were measured for all samples immediately following the examinations via the Nova Biomedical Lactate Plus point-of-care analyzer (Nova Biomedical, Waltham, MA, USA) with lactate test strips (Nova Biomedical). One drop of heparinized whole blood was placed on the test strips. All hematologic and plasma biochemical analyses were performed at the Midwest Veterinary Laboratory (St Louis, MO, USA) within 6 hours of the examinations. Hematologic analyses were performed via the Leukopet system (Jorgensen Laboratories LLC, Loveland, CO, USA).

Mean, median, and standard deviations were calculated for all CBC and plasma biochemical values in this study population. Lactate concentrations were normally distributed. Accordingly, a simple linear regression was used to determine if capture order affected lactate concentrations. Additionally, simple linear regressions were used to assess the relationships between lactate concentration and the other CBC and biochemical parameters. Significance was set at  $P \leq 0.05$ . Linear models were analyzed by the lme4 package<sup>15</sup> in R.

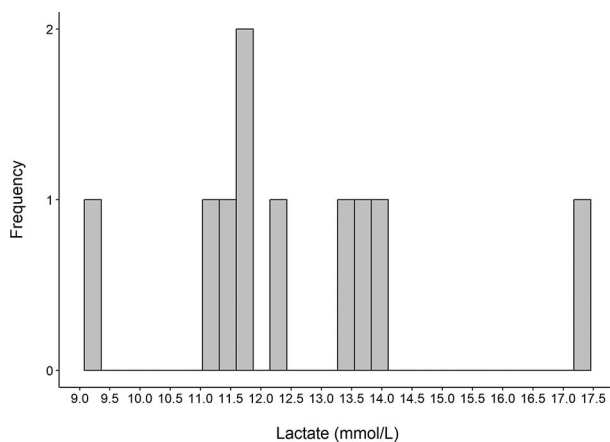
## RESULTS

Cattle egret mean  $\pm$  standard deviation postcapture blood lactate concentration was  $12.6 \pm 2.05$  mmol/L, and the median was 12.05 mmol/L (Table 1; Fig 1). All

**Table 1.** Linear model results for correlations between blood lactate concentrations and other complete blood cell count and biochemical parameter values in clinically healthy cattle egrets (*Bubulcus ibis*). Significance was set at  $P \leq 0.05$ , and only sodium (bold) was significant.

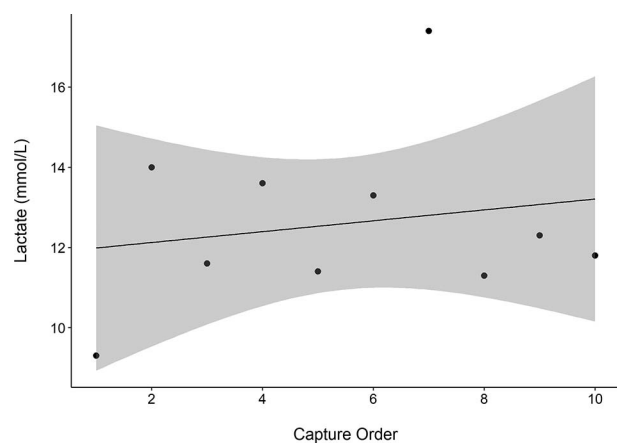
Biomarker/analyte	Model results		
	$\beta$	SE	$P$
White blood cell count, K/ $\mu$ L	-0.024	0.113	0.835
Hematocrit, %	0.344	0.363	0.371
Heterophils, %	0.063	0.042	0.171
Lymphocytes, %	-0.063	0.036	0.118
Monocytes, %	0.147	0.191	0.463
Eosinophils, %	0.152	0.355	0.681
Basophils, %	0.361	0.396	0.389
Heterophil : lymphocyte ratio	1.291	0.621	0.071
Sodium, mmol/L	<b>0.595</b>	<b>0.234</b>	<b>0.038</b>
Potassium, mmol/L	0.511	2.187	0.821
Chloride, mmol/L	-0.170	0.289	0.572
Glucose, mg/dL	0.015	0.022	0.529
Calcium, mg/dL	2.004	1.418	0.195
Phosphorus, mg/dL	0.447	0.478	0.377
Total protein, g/dL	-0.513	2.999	0.868
Albumin, g/dL	-6.862	5.381	0.238
Albumin : globulin ratio	-24.043	12.140	0.083
Globulin, g/dL	3.581	4.029	0.400
Aspartate aminotransferase, U/L	0.017	0.022	0.473
Total bilirubin, mg/dL	-2.620	18.340	0.890
Uric acid, mg/dL	0.022	0.039	0.597
Creatine kinase, U/L	-0.001	0.001	0.187
Bile acids, $\mu$ mol/L	0.001	0.039	0.981

CBCs and biochemical parameters were considered normal for available species reference intervals (ZIMS version 2.3).<sup>16</sup> Capture order did not predict lactate concentration ( $\beta = 0.14$ , SE = 0.25,  $P = 0.60$ ; Fig 2). There was a significant positive correlation ( $R^2 = 0.44$ ,  $P = 0.04$ ) between lactate and sodium concentrations (Table 1; Fig 3); no other correlations were found

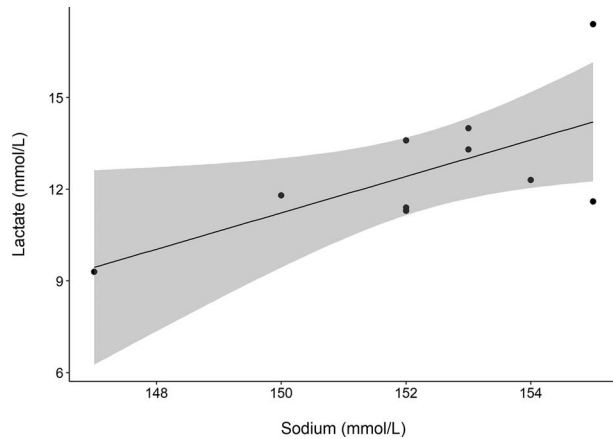


**Figure 1.** Histogram of lactate concentrations for 10 clinically healthy cattle egrets (*Bubulcus ibis*).

between blood lactate concentration and other CBC or biochemical values (Table 1). All birds had unremarkable physical examinations, although due to the retrospective nature of the study, full vital parameters were not available for review.



**Figure 2.** Relationship between lactate and capture order. There was no significant variation in blood lactate concentrations by capture order in clinically healthy cattle egrets (*Bubulcus ibis*) ( $\beta = 0.136$ , SE = 0.248,  $P = 0.600$ ).



**Figure 3.** Relationship between lactate and sodium. There was a significant positive correlation between blood lactate and sodium concentrations in clinically healthy cattle egrets (*Bubulcus ibis*) during capture ( $\beta = 0.595$ ,  $SE = 0.239$ ,  $P = 0.038$ ).

## DISCUSSION

This study documented single-time-point postcapture lactate concentrations in cattle egrets with the Nova Biomedical Lactate Plus analyzer. The Nova Biomedical Lactate Plus meter uses an electrochemical lactate oxidase biosensor for measurement of lactate in whole blood. This unit requires 0.07  $\mu\text{L}$  of whole blood and performs the analysis in approximately 13 seconds. The test strips do not require calibration codes and contain 2 levels of quality control solution to ensure proper analysis.<sup>17</sup> In our experience, this analyzer is a frequently used tool in nondomestic species' medical care.

This study evaluated the relationship between capture order and lactate concentrations in cattle egrets. It was hypothesized that birds captured later would experience greater stress due to repeated netting events as opposed to the birds processed earlier, which experienced fewer events. Our hypothesis was found to be incorrect, with capture order not having a significant effect on lactate concentration. It is important to note that this flock of egrets was not accustomed to routine handling or net capture and therefore stress was expected to occur with this capture event. Blood lactate concentrations might not have been associated with capture order if elevations were associated with restraint and handling for exams rather than with disruption of the flock with netting.

Although corticosterone was not measured in this study, blood lactate concentration is proven to positively correlate with corticosterone concentrations in other avian species and therefore is frequently used as an indirect measure of stress.<sup>6,7</sup> Stress causes a release of catecholamines, which, in turn, increases glycolytic rate and production of pyruvate beyond the mitochondria's

ability to utilize it. This results in increased lactate production and subsequent hyperlactatemia, which can lead to lactic acidosis if not addressed.<sup>7,18</sup> The higher blood lactate concentrations recorded in the cattle egrets compared with other birds following capture suggest this species might have a more robust stress response or different metabolic processes.

Past studies on various avian species, including hawks, falcons, pigeons, ratites, passerine birds, macaws, and chickens, have demonstrated positive correlations between heterophil : lymphocyte ratio and stress in avian species.<sup>19–26</sup> In this study, we observed a nonsignificant positive relationship between heterophil : lymphocyte ratio and blood lactate concentration ( $P < 0.10$ ). Given the known positive correlation between blood lactate and corticosterone concentrations, these values are likely both influenced by stress in cattle egrets. The small sample size likely affected this result, and a larger number of samples would be needed to reduce the risk of a type II error. Our present study also showed a significant positive correlation between plasma sodium and blood lactate concentrations; although at this time the relationship between these values is unknown, it is likely clinically insignificant, as all sodium concentrations were within species reference ranges (ZIMS version 2.3).<sup>16</sup>

This study also established that post-net capture blood lactate concentrations in cattle egrets measured  $12.6 \pm 2.05$  mmol/L with a median of 12.05 mmol/L and were not influenced by capture order. This measurement would be considered moderately to severely elevated compared with other avian species based on current available literature.<sup>4,8,10,13,14</sup>

Post-mist net capture studies in house sparrows, mourning doves, and boat-tailed grackles found mean blood lactate concentrations of 4.77 (2.66–12.03), 7.72 (3.94–14.14), and 5.74 (3.09–8.75) mmol/L, respectively. At these values, lactic acidosis was noted on blood gas analysis, particularly in the house sparrows.<sup>14</sup> Another study evaluating blood lactate concentrations in American flamingos found that concentrations positively correlated with length of time required for the initial capture but did not increase significantly as capture difficulty increased.<sup>8</sup> Burgdorf-Moisuk and colleagues<sup>8</sup> reported a mean blood lactate concentration of 8.61 mmol/L (3.10–19.00 mmol/L), a higher value than had been previously published in other bird taxa. In contrast to that work in American flamingos, we did not observe changes in blood lactate concentration with capture order in cattle egrets. However, mean blood lactate concentration in these cattle egrets was higher than in those other species.

Blood lactate concentration has also been used as a prognostic indicator in rehabilitation settings.<sup>13,21,26</sup>

One such study used blood lactate concentration to assess the ability of raptors to be returned to the wild postrehabilitation. Postflight blood lactate concentrations of raptors that were not conditioned to routine flight measured over double the concentrations of flight-conditioned raptors.<sup>26</sup> Clinicians who are presented with cattle egrets for rehabilitation should be aware of the higher lactate concentration in this species for clinically normal individuals following brief capture.

This study was intended to aid clinicians when evaluating single-time-point blood lactate concentration measurements postcapture in cattle egrets. Limitations of this study are the small sample size and that the point-of-care analyzer is not currently validated for use in this species. Further testing with a larger sample size and multiple time points postcapture could be used to evaluate blood lactate concentration as a prognostic indicator for diseases such as capture stress. Additionally, validation studies on the Nova Biomedical Lactate Plus analyzer for cattle egrets should be performed.

Per the American Society for Veterinary Clinical Pathologists, these hematologic data do not meet the guidelines for establishing species-specific reference intervals. However, in zoo-housed species this is often not feasible and therefore smaller sample sizes are used for clinical guidance, as is presented in this article.

Postcapture point-of-care blood lactate concentrations in the cattle egret were higher than values reported in other avian species, which should be considered when interpreting single lactate concentrations in this species. In contrast to expected results, lactate concentrations were not associated with capture order.<sup>2,5,7,8,10,13</sup> Clinicians who are presented with a cattle egret for rehabilitation should be aware of the higher blood lactate concentration in this species for clinically normal individuals following brief capture.

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