Assessment of Corticosterone Levels in American Alligators (*Alligator mississippiensis*) with Dermatitis

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Abstract: Bacterial and fungal dermatitis is a common problem in captive reared alligators in commercial operations, and stress has been suggested as a predisposing factor. We compared corticosterone levels between alligators with dermatitis (Tx) and alligators without dermatitis (Cx). There was no statistically significant difference between the baseline corticosterone concentrations (time 0) of the Tx and Cx groups ($P = 0.272$). At 15 min postcapture, there was a statistically significant difference between treatment and control animals ($P = 0.006$), with the Tx alligators having higher corticosterone concentrations compared with the Cx alligators. At 3 h (after dexamethasone administration) values for both groups decreased from the 15 min levels, showing a functional negative feedback loop of the hypothalamic–pituitary axis; there was no statistically significant difference between groups ($P = 0.90$) at that time. The results do not show an association between stress and dermatitis, but suggest that animals may be more prone to increases in corticosterone release once dermatitis is present.

Key Words: *Alligator mississippiensis*, American alligator, corticosterone, dermatitis, dexamethasone, reptile.

Introduction

The authors have observed mixed bacterial and fungal dermatitis as a common health problem of captive reared American alligators, *Alligator mississippiensis,* in Louisiana since 2007 (Nevarez, 2007). Prior to 2007, there were sporadic cases of dermatitis but it now seems to be more commonly reported. It is unclear whether these observations reflect a true increase in prevalence or simply increased reporting.

In our experience, the disease is mostly observed in hatchling alligators, but also occurs in alligators over 1 yr old (Nevarez, 2007). This presentation of dermatitis is characterized by a perceived loss or change of skin pigment, which is due to bacterial and/or fungal colonization and epithelial destruction in the acute phase, and later results in obliteration of the epidermis and dermis with exposure of underlying bone (Fig. 1) (Nevarez, 2007). In some instances, the destruction of melanocytes by the infection is responsible for the white color of the skin. There is typically a mixed bacterial and fungal flora associated with the dermatitis (Nevarez, 2007). Secondary septicemia and death may result in advanced cases (Nevarez, 2007). The authors have used systemic antibiotics and water treatments (salt, soap, potassium permanganate, bleach) in alligators with dermatitis; however, this treatment is largely unsuccessful (Nevarez, 2007).

With regard to pathogenesis, it is hypothesized that a stressor event or combination of variables leads to immunosuppression and predisposes alligators to opportunistic bacterial and/or fungal infections (Nevarez, 2007). A number of stressors have been identified clinically as potentially associated with the occurrence or severity of the dermatitis, including strong water influx into a pen, fires, cold exposure, and other events that may cause perceived stress (Nevarez, 2007). These stressors have been observed in clinical cases, but have not been studied in detail.

Stress response in crocodilians has been examined in relation to restraint, long-term corticosterone implants, cold shock, and stocking densities (Elsey *et al.*, 1990; Morici *et al.*, 1997; Lance and Elsey, 1999a, 1999b; Rooney and Guillette, 2001). Lance *et al.* (2001) provide an overview of the physiology and endocrinology of stress in crocodilians, which appears to be similar to that of mammals. Catecholamine release, glucocorticoid secretion, elevation in blood glucose, and elevation in plasma lactate have been identified during the stress response of crocodilians. Lance *et al.* (2001) also emphasize the importance of temperature in the stress response. Although the optimal temperature range for crocodilians is 25–35°C (77–95°F), a range of 30–32°C (86–90°F)

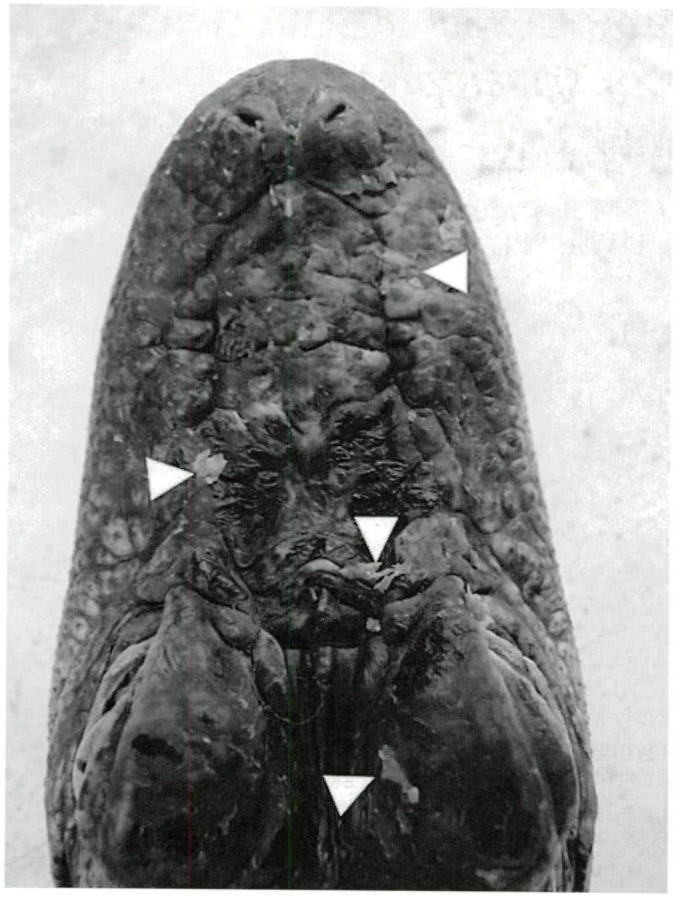

Figure 1. Dermatitis in a captive reared alligator. Areas of epidermal flaking can be observed over the eyes and snout region (white arrowheads).

provides a better secretion of corticosterone from the adrenal cells and lymphocyte activity *in vitro* (Lance *et al.*, 2001). Some studies have also reported changes in the white blood cells (WBC) associated with stress response (Morici *et al.*, 1997; Lance and Elsey, 1999a, 1999b; Lance *et al.*, 2001). A study on exposure to cold shock in juvenile alligators revealed a significant increase in total WBC with an increase in lymphocytes and heterophils and a decrease in azurophils and basophils 48 h after 20 min in an ice bath (Lance and Elsey, 1999a). A study on the effects of restraint in juvenile alligators reported an increase in heterophils and a decrease in all other cell types without an increase in total WBC through 48 h of the study (Lance and Elsey, 1999b).

Despite substantial evidence that stress plays an important role in the physiology of crocodilians, a role between stress and disease, specifically infections perceived to be opportunistic, has yet to be clearly demonstrated in captive reared alligators in Louisiana. The objective of this study was to study the association between stress and dermatitis by comparing the corticosterone levels between alligators with bacterial dermatitis and alligators without dermatitis. Our hypothesis was that alligators with dermatitis would have higher plasma concentrations of corticosterone and a disrupted negative feedback, 2 indications of chronic stress (Romero, 2004).

Materials and Methods

This study was approved by the Louisiana State University Institutional Animal Care and Use Committee. Alligators used in this study were selected from a single Louisiana alligator ranch where cases of bacterial/fungal dermatitis had recently been encountered. All sampling was conducted in May of 2009. Alligators were from mixed clutches and housed indoors with a water temperature range between 29.4 and 31.6°C (85 and 89°F). A total of 50, 7-month-old alligators were included in this study. Twenty-five alligators were from a single pen in which dermatitis was present (Tx) as confirmed by gross examination by one of the authors (JGN). The additional 25 alligators were from a single pen in which dermatitis was not observed (Cx). The 2 groups also happened to be from different buildings at the facility. Husbandry parameters were equal for both groups. The Tx group was sampled within 7 days of the onset of dermatitis, and the Cx group was sampled 3 wk after sampling of the Tx group.

Individual animals were selected at random from within the pen. Sampling was performed in the same manner for all animals in both groups. One milliliter of blood was collected from the lateral occipital sinus using a 25-gauge needle and a 3-ml syringe; the sample was divided into 2 lithium heparin microtainer tubes (Becton Dickinson, Franklin Lakes, NJ). One alligator was selected at a time and blood was collected immediately upon capture (Time 0). The animals were then housed individually in a clear plastic container. A second restraint and venipuncture was performed 15 min after the first sample was collected (time 15). Immediately after the 15 min sample was obtained, alligators were injected with 1 mg/kg dexamethasone sodium phosphate (Dexamethasone Sodium Phosphate Injection USP, 4 mg/ml, APP Pharmaceuticals LLC, Schaumburg, IL) intramuscularly in the proximal forelimb with a 22-gauge needle. After injection, the animals were placed back in the container until a third blood sample was obtained at 3 h (time 180). The blood tubes were placed on ice immediately after collection. Within 1 h of collection, the blood tubes were centrifuged at 1,900 *g*/4,000 rpm for 10 min to obtain plasma for corticosterone analysis. The plasma was kept on ice for 4 h and then frozen at −20°C (−4°F) until being shipped on dry ice to Tufts University (Medford, MA) for analysis of corticosterone concentrations. Samples were assayed for corticosterone with the use of a previously described radioimmunoassay (Wingfield *et al.*, 1992; Romero and Wikelski, 2001). Briefly, plasma was equilibrated with a small amount of titrated corticosterone to measure subsequent recovery, and then steroids were extracted with redistilled dichloromethane. Each sample was then assayed in duplicate with the final concentration adjusted by the percent recovery. Intra-assay variation was 9% and samples were assayed in a single assay.

After the last blood sample was collected, 3 full-thickness skin biopsies were obtained from the skin over the dorsal aspect of the snout with the use of a 4-mm punch biopsy. The skin samples were placed in individual cassettes and preserved in 10% buffered formalin. All specimens were decalcified for approximately 4 h, bisected, processed into paraffin for sectioning, and stained with hematoxylin and eosin (H&E) using routine methods. Sections of select cases were also stained with the use of Brown & Brenn and Gomori methenamine silver methods (GMS).

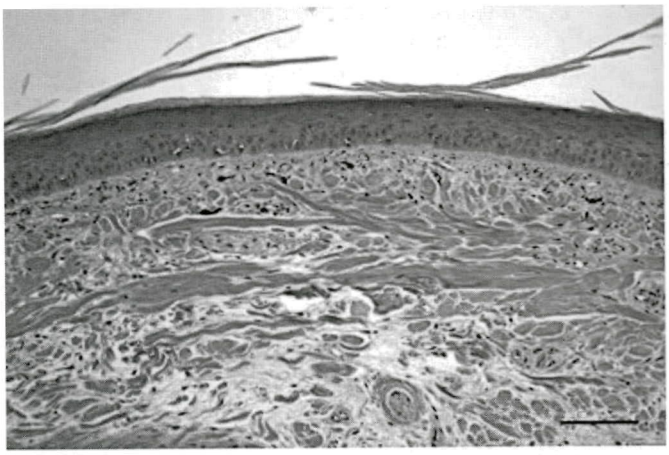

Figure 2. Alligator skin (Case no. 28): No significant findings. H&E. Scale bar = 100 μm.

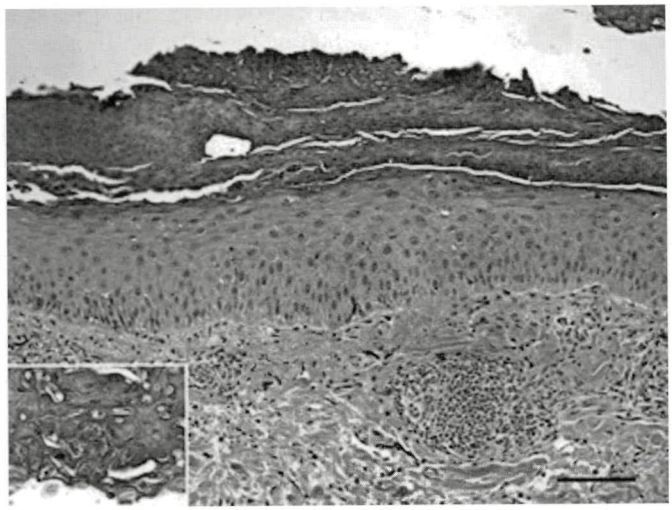

Figure 3. Alligator skin: Exudative fungal and bacterial dermatitis. A thick crust of heterophils admixed with fungal hyphae and bacteria (inset) covers the hyperplastic epidermis. A perivascular infiltrate comprised of lymphocytes, histiocytes, and heterophils surrounds dermal vessels. H&E. Scale bar = 100 μm.

Statistical analysis was performed with PASW Statistics 18.0 program (SPSS Inc., Chicago, IL). Data were evaluated for normality with the use of the Shapiro-Wilk test. Comparisons between groups were performed with the Mann-Whitney U-test and within groups with the Wilcoxon signed-rank test. Statistical significance was established at 0.05.

Results

Histopathologic findings: There were notable differences between Tx and Cx groups. Findings in the Cx were limited to minor histopathologic findings in 18 of the 25 cases, and no histological lesions in 7 cases (Fig. 2). Minor findings interpreted as background lesions included minimal or mild, perivascular pleocellular dermatitis ($n = 8$), dermal fibroplasia ($n = 5$), microgranuloma formation associated with embedded keratin ($n = 4$), and minimal or mild, superficial heterophilic dermatitis ($n = 4$) (no organisms observed). Many of these findings were focal lesions. In contrast, all Tx cases had notable exudative dermatitis. Most had both fungi and bacteria within lesions ($n = 22$); however, bacteria alone were observed in 2 cases, and no organisms were observed in one case. The surface exudate had largely been lost in those cases in which no fungi were observed, so there is a possibility that these may have been infected by fungi as well.

Histopathologic features included infiltration of the superficial epidermis by large numbers of intact and degenerate heterophils, which formed a densely cellular crust (Fig. 3). Numerous fungal hyphae and bacteria, predominantly bacilli, were distributed throughout the exudate. Fungal hyphae were septate and characterized by parallel walls and dichotomous branching (ascomycete type). The underlying epidermis was diffusely hyperplastic, and dermal vessels often were surrounded by a perivascular infiltrate comprised of histiocytes, lymphocytes and heterophils. Bacteria from 3 representative cases were characterized as Gram negative. In 3 cases from the Tx group, small numbers of small (2–3 μm), round unidentified organisms with an eccentric internal basophilic body were present within the superficial exudate. These organisms did not stain with GMS (possible protozoa).

Corticosterone analysis: The data were not normally distributed. Descriptive data are presented in Table 1. There was no statistically significant difference between the baseline corticosterone levels (time 0) of the Tx and Cx groups ($P = 0.272$). At 15 min (time 15), there was a statistically significant difference between treatment and control animals ($P = 0.006$), with the Tx alligators having higher corticosterone levels when compared with the Cx alligators. At 3 h (time 180) (after dexamethasone administration) values for both groups decreased from the 15 min levels, showing a functional negative feedback of the hypothalamic pituitary axis; there was no statistically significant difference between the groups ($P = 0.90$) at that time. In the Cx group there was a statistically significant difference in corticosterone levels between time 0 and time 15 ($P = 0.0001$) and between time 0 and time 180 ($P = 0.008$). There was no difference between time 15 and time 180 ($P = 0.389$). In the Tx group, there was

Table 1. Corticosterone (ng/ml) results from alligators with (Tx) and without (Cx) dermatitis.

Group	Time (min)	Mean	95% confidence index	Median	Minimum	Maximum
$Cx^{a,b}$	0	1.18	0.56–1.79	0.6	0	5.4
Tx^{d}	0	1.19	0.84–1.54	1.3	0	2.9
$Cx^{a,c}$	15	3.6	2.57–4.63	3.3	0.3	11.6
$Tx^{c,d}$	15	5.65	4.52–6.78	5.6	1.3	11.1
Cx^{b}	180	3.2	1.70–4.70	2.2	0	15.7
Tx^{d}	180	2.7	1.67–3.72	1.9	0	10.4

Superscript a,b,c,d denote statistically significant difference between groups.

a significant difference between values at all time periods: time 0 and time 15 ($P = 0.0001$), time 0 and time 180 ($P = 0.009$), time 15 and time 180 ($P = 0.0001$).

Discussion

The similar baseline corticosterone concentrations in animals with and without dermatitis did not support our hypothesis that stress predisposes alligators to a presentation of mixed bacterial and fungal dermatitis. However, the higher corticosterone concentration at all other bleeding times suggests that alligators with dermatitis have a hypersensitive response and may become more stressed than animals without skin disease. Thus, alligators may be more prone to stress once dermatitis is present, which could lead to immunosuppression and compound an infection.

Two aspects of this study must be considered. First, corticosterone levels could not be measured until 7 days after the development of dermatitis. It is possible that corticosterone concentration was elevated immediately before or after the development of the dermatitis. At this time, we are not able to predict the occurrence of dermatitis in a manner that would support prospective study or practically sample animals in commercial operations until a disease occurs. For this reason we must either make retrospective analysis after the occurrence of disease, as was done in this study, or develop consistent methodologies for experimental induction of disease in captive alligators. Second, the control group was not sampled until 3 wk after the treatment group, which may have introduced unintended variables. We do not believe, however, that this difference in timing was significant because the alligators were maintained at the same temperature and the study was conducted on the same age/size cohort within the same season.

Clinically, the authors' have seen alligators develop dermatitis after stressful events (e.g., a fire in the building where they were housed) and changes in husbandry practices (e.g., the addition of strong water streams into pens). Also, it is common practice by alligator farmers and ranchers to minimize any event that they perceive as potentially stressful to the alligators due to concerns that such disturbances negatively affect growth and the general health of the animals. Although this study did not demonstrate that stress predisposes alligators to the clinical presentation of dermatitis, higher elevations of corticosterone following a stressful event in alligators with dermatitis suggests that stress response is altered and may influence the course of disease. Based on our current knowledge about stress in alligators from both the peer-reviewed literature and clinical observations, it is still important to consider the potential effects of stressors as predisposing factors for disease and it is worthy of further study.

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Literature Cited

Elsey RM, Joanen T, McNease L, Lance V. 1990. Growth rate and plasma corticosterone levels in juvenile alligators maintained at different stocking densities. J Exp Zool 255:30–36.

Lance VA, Elsey RM. 1999a. Hormonal and metabolic response of juvenile alligators to cold shock. J Exp Zool 283(6):566–572.

Lance VA, Elsey RM. 1999b. Plasma catecholamines and plasma corticosterone following restraint stress in juvenile alligators. J Exp Zool 283(6):559–565.

Lance VA, Morici LA, Elsey RM. 2001. Physiology and endocrinology of stress in crocodilians. *In* Grigg GC, Seebacher F, Franklin CE (eds): Crocodilian Biology and Evolution. Surrey Beatty and Sons, Chipping Norton, Australia:327–340.

Morici LA, Elsey RM, Lance VA. 1997. Effects of long-term corticosterone implants on growth and immune function in juvenile alligators (*Alligator mississippiensis*). J Exp Zool 279(2):156–162.

Nevarez JG. Unpublished data from clinical cases dealing with captive reared alligators in Louisiana, 2007–2011.

Romero LM. 2004. Physiological stress in ecology: Lessons from biomedical research. Trends Ecol Evol 19:249–255.

Romero LM, Wikelski M. 2001. Corticosterone levels predict survival probabilities of Galápagos marine iguanas during El Niño events. Proc Natl Acad Sci 98:7366–7370.

Rooney AA, Guillette LG. 2001. Biotic and abiotic factors in crocodilian stress: The challenge of a modern environment. *In* Grigg GC, Seebacher F, Franklin CE (eds): Crocodilian Biology and Evolution. Surrey Beatty and Sons, Chipping Norton, Australia:214–228.

Wingfield JC, Vleck CM, Moore MC.1992. Seasonal changes of the adrenocortical response to stress in birds of the Sonoran Desert. J Exp Zool 264:419–428.