Endocrine responses to snouted cobra (*Naja annulifera)* and African puffadder *(Bitis arietans)* envenomation in dogs

By

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Submitted in partial fulfilment of the requirements for the degree of

MSc (Companion Animal Clinical Sciences)

In the Faculty of Veterinary Science

at the University of Pretoria

Supervisor: Prof. Johan Schoeman

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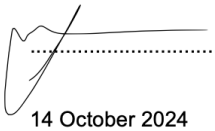
Declaration

I, Noeline Fourie-Viljoen, declare that the dissertation which I hereby submit for the degree MSc (Companion Animal Clinical Sciences), in the Faculty of Veterinary Science at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.



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LETTER OF APPROVAL

Ethics Reference No	**REC089-23**
Protocol Title	**Endocrine Allostasis in Snake Envenomation: changes in the Cortisol, Thyroxine and Thyroid Stimulating Hormone levels of dogs envenomed by the snouted cobra and African puffadder**
Principal Investigator	**Dr N Viljoen**
Supervisors	**Prof JP Schoeman**

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Yours sincerely

PROF M. OOSTHUIZEN
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Summary

The Hypothalamic-Pituitary-Adrenal (HPA) and Hypothalamic-Pituitary-Thyroidal (HPT) axes are pivotal in the pursuit of homeostasis in critical illness. The goal of this study was to investigate the host endocrine response in the snake-envenomed canine patient.

This prospective study included 17 client-owned dogs naturally envenomed by either a snouted cobra (*Naja annulifera*) (n=9) or a puffadder (*Bitis arietans*) (n=8), that presented within 6 hours of envenomation. The dogs were further subdivided clinically into a neurological (n=5) and non-neurological group (n=4). Serum samples were collected at admission, and thereafter at 12-, 24-, and 36-hours post envenomation. At each time point, the serum total thyroxine (TT4), thyrotropin (TSH), C-reactive Protein (CRP) and cortisol concentrations were measured.

Compared to control dogs, the median serum TT4 concentrations of all the snake-envenomed dogs were significantly lower at all time points ($P<0.05$). The non-neurological cobra subgroup recovered to serum TT4 concentrations comparable to that of the controls within 24 hours of envenomation, while the puffadder and neurological cobra subgroup serum TT4 concentration remained significantly suppressed until 36 hours post envenomation. Serum TT4 concentration was negatively correlated with serum CRP concentration ($P<0.05$, $\rho=-0.326$)). The differences in TSH between groups failed to reach significance. The total serum cortisol concentrations of all envenomed dogs were highest at admission, but only the neurological cobra subgroup had a significantly higher concentration at admission compared to the controls. The neurological cobra subgroup had the highest peak in serum CRP concentration, but the correlation between total serum cortisol and CRP concentrations failed to reach significance.

Puffadder and snouted cobra envenomation is associated with significant suppression of serum TT4 concentrations that is correlated with the severity of the host inflammatory response. The only significant increase in total serum cortisol concentration was observed in the neurological snouted cobra envenomed subgroup at admission. This study provides novel insights into the temporal endocrine perturbations in Puffadder and snouted cobra envenomation, and the relation thereof to the degree of the host inflammatory response.

"... a typical syndrome appears, the symptoms of which are independent of the nature of the damaging agent, or the pharmacological type of the drug employed, and represent rather a response to damage as such …"

Hans Selye, *Nature.* July 4, 1936.

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Acknowledgements

It is with gratitude that I am able to submit this dissertation, and by no means would I have been able to do so without the guidance and support of others.

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A special thanks is also owed to all members of the PVR veterinary clinic team, who have without exception supported and enabled the completion of this dissertation.

I would penultimately like to thank my parents for shaping me with their dedication and deep-seated curiosity in the world around them. This dissertation is lastly and most importantly a reflection of the persistent patience, proof-reading, companionship and support of my husband, to whom I owe all my thanks.

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ACTH	Adrenocorticotrophic Hormone
AKI	Acute kidney injury
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUROC	Area under the receiver operating curve
CBC	Complete blood count
CIRCI	Critical Illness Related Corticosteroid Insufficiency
CNS	Central nervous system
CRP	C-Reactive Protein
cTn-1	Cardiac Troponin 1
DAMPS	Damage-Associated Molecular Patterns
EDTA	Ethylenediaminetetraacetic acid
GH	Growth Hormone
Hct	Haematocrit
HPA	Hypothalamic-Pituitary-Adrenal
HPT	Hypothalamic-Pituitary-Thyroidal
IL-6	Interleukin 6

MCP	Macrophage Chemoattractant Protein
NTIS	Non-Thyroidal Illness Syndrome
OVAH	Onderstepoort Veterinary Academic Hospital
PAMPS	Pathogen-Associated Molecular Patterns
Plt	Platelet Count
PRR	Pattern Recognition Receptors
RAAS	Renin-Angiotensin Aldosterone System
SIRS	Systemic Inflammatory Response Syndrome
T4	Total Serum Thyroxine
TNF	Tumour Necrosis Factor
TSH	Thyroid Stimulating Hormone
TSP	Total serum protein
WBC	White Blood Cell Count
WHO	World Health Organisation

Chapter 1. Literature review

1.1 Literature review

1.1.1 Canine snake envenomations in South Africa: clinical and laboratory changes

Snake envenomation is one of the most characteristic South African veterinary emergencies. The snakebite has been classified as a neglected tropical disease(1), and is overrepresented in rural areas and agrarian societies (2) where access to polyvalent antivenom is severely limited. In sub-Saharan Africa an average of 250 000 human- and animal snake envenomations take place annually (3). It is of growing international concern, and the developing world is in the centre of this socio-ecologically complex disease (4).

Despite the growing political momentum and the high incidence of snake bites in South Africa, there is a sparsity of knowledge on the subject in our veterinary scientific community. This silence by the scientific community on the topic is concerning in the face of a disease with consistent annual peaks in mortality and year-round prevalence (5). A global scoping review (6) found that only three articles on puff adder envenomation of domestic animals, and two on the snouted cobra have been published, in comparison to 19 on the European viper, which is counter-intuitive given the aforementioned focus areas of clinical disease. There are also significant global knowledge gaps on the incidence, socio-economic impact, epidemiology, pathophysiology, and complications of snake envenomations (6). It is the endocrine pathophysiology and complications that will form the focus of this study.

The pathophysiology of snake envenomation, specifically Puffadder and snouted cobra envenomation, is not only poorly understood and frequently oversimplified, but also venom type-, and degree of envenomation dependent. The venom type can be grouped on different levels, the most basic thereof being cytotoxic, neurotoxic and coagulopathic. The trichotomy is not absolute, though, with significant overlap in certain species (7). This literature review will discuss each species' associated clinicopathological and endocrine changes should be interpreted accordingly.

There are close to 200 medically relevant snake species, of which the majority are part of the Viperidae and Elapidae families (8). The two species included in this study, *Bitis arietans* and *Naja Annulifera* represent each of these families and is thus the best reference point from which to further investigate the endocrine patterns in snake envenomation. Usually, elapid envenomation

has mostly neurotoxic effects, while viperids are associated with local cytotoxic effects and systemic vascular toxicity (9), including coagulopathic effects, have also been described (10). Vasculotoxicity presents systemically in contrast to local cytotoxic effects, and impact the systemic ature leading to bleeding and bruising through proteolytic effects on clotting proteins (11). Signs reported for each of these species, similar to those in humans, tend to converge on a consistent clinical picture for each species (6).

Puffadder envenomation:

African Puffadder envenomations outnumber those of all other African snakes combined (12). Local signs associated with *B. arietans* envenomation include cytolysin-induced macroscopical swelling from bleeding into the subcutis as well as local tissue necrosis at the site of envenomation (13). Progressive swelling can cause significant local and ultimate systemic signs by causing interference with respiratory and cardiovascular functions(13).

Systemic signs can include coagulopathy, thrombocytopenia and vasculotoxicity (10). Thrombocytopaenia is a common finding in Puffadder envenomation (5). In earlier experimental research, the haemorrhage has been ascribed to an irreversible platelet aggregating factor and consumptive coagulopathy (14). It is now known that the venom contains both platelet aggregins and platelet inhibitors(15). Puffadder-envenomed dogs tend to show progressive hypercoagulability after 24 hours post-envenomation(16). The causal role of the acute inflammatory response in this coagulopathic pattern is becoming more apparent (16). This study will also investigate the role of systemic inflammation, as measured by serum C-Reactive protein (CRP) concentration, in the endocrine response to Puffadder and snouted cobra envenomation.

The inflammatory activation following Puffadder envenomation is well described, yet the underlying mechanism of toxicity is poorly understood (13). Pertinently, the presence of systemic inflammatory response syndrome (SIRS) in Puffadder envenomation remains largely unknown (17). In haematology, neutrophilia with a left shift is common (5) and leucocytosis was found to be predictive of mortality in *V. palaestinae* envenomations (18), hinting that inflammatory activation is a central part of the pathophysiology of viperid envenomation. A dog study performed on this dataset specifically evaluating the systemic inflammatory response found significant serum CRP concentration elevations but inconclusive associations with mortality or severity (19); further

begging the question of whether inflammation could be central in the systemic vasculotoxic and coagulopathic effects seen in certain viperid species. In an in vivo study in mice, a severe inflammatory response was demonstrated in a polymorphonuclear cell accumulation, and the systemic production of eicosanoids, Interleukin-6 (IL-6), and Monocyte Chemoattractant Protein-1 (MCP) (17). Their degree of response to gene blocking and pre-treatment with anti-inflammatories further support the inflammatory theory, which is gaining more and more traction as evidence accumulates. The involvement of stress as a confounding factor complicates this debate, though, which is to be supplemented by the endocrine insights obtained in this study.

Another complicating factor is the pain associated with puffadder envenomation. Chronic pain is known to cause elevations in total serum cortisol concentrations in humans(20). In dogs, there is evidence of acute surgical pain causing transient elevations in serum cortisol concentrations(21), that is independent of surgeon experience(22). It is thus possible that the endocrine changes associated with viperid snake envenomations are affected not only by the venom or inflammatory activation, but by pain as well. The nature of a clinical study limits the number of variables that can be fixed or controlled, which must be interpreted as possible confounders.

Snouted Cobra envenomation:

Naja annulifera envenomations are common and dangerous, yet largely unexplored. In South Africa, snouted cobras are responsible for approximately 60% of canine snake envenomations (23), but studies on the mechanism of toxicity are scarce. On a family level, Elapid envenomation is characterised by flaccid paralysis and ascending weakness (13). Their toxins include neurotoxins, cardiotoxins, as well as haemorrhagic toxins (24). The most important toxins, α-neurotoxins, like α-bungarotoxin, act by blocking the neuromuscular junction at the pre- and postsynaptic nicotinic acetylcholine receptor (25). The neuromuscular blockade is usually progressive, and manifests as gradual loss of function starting with local nerve paralysis and progressing centrally until mechanical ventilation is necessary due to respiratory arrest (26). Although these neurotoxins were long thought to be the main or only manifestation of Elapid envenomations, additional toxins with pro-inflammatory, anti-coagulant, cytotoxic, cardiotoxic and haemorrhagic effects are becoming increasingly apparent (27). Haematological changes

observed in puffadder-envenomed dogs in South Africa include thrombocytopenia and leukocytosis (5). Through elevations found in serum CRP and Cardiac Troponin-1 (CTn-1) concentrations (19), it is clear that the classification as purely neurological is an oversimplification. Coagulation disturbances (16), and local necrosis (13) has also been observed. Evidence of systemic inflammatory effects have been demonstrated in elevations of serum IL-6 and MCP-6 (27), which was associated with a leucocytosis in viperid envenomations. The elimination of inflammatory components in an experimental model of snouted cobra envenomation yielded significant reductions in clinical pain and oedema (23). In the aforementioned study, histological changes such as vascular congestion and haemorrhagic foci were also observed in post-mortem lung parenchyma (23). This brought the sole causal relationship between neurotoxicity and respiratory arrest into question. There is thus a significant need for a more in-depth understanding of the internal perturbations brought about by *Naja annulifera* envenomation.

If the pathophysiology of snake envenomation, specifically Puffadder and snouted cobra envenomation, is to be better understood, the pursuit of patterns in patient response to envenomation is imperative. A few common denominators are present in the literature that are independent of snake species.

The serum biochemical changes associated with snouted cobra and Puffadder envenomation is largely unexplored, but other Viperidae species are known to cause hypoalbuminemia, hyperglobulinaemia, mild hyperglycaemia, hypocholesterolaemia, and increases in markers of hepatocellular damage (24). This is evidence of not only a significant acute phase response, but concurrent stress presence, illustrating the complexity of snake envenomation pathophysiology and the need for further investigation. Haemoconcentration is another haematological parameter in both *B. arietans* and *N. annulifera* envenomations (5). The different causal contributors include catecholamine-related splenic contraction and capillary leak from hemorrhagin-induced vasculotoxicity, among others (18). Hyperglycaemia is also observed in envenomations by other African snakes (28). Acute kidney injury, one of the major sequelae of snake bites, is thought to be secondary to both microthrombosis in coagulopathies, as well as vasoconstriction(29). The gravity of these changes and sequelae provides an impetus for the further exploration of markers and measures of the pathophysiological changes secondary to snake envenomation.

The investigation of biomarkers have been increasing in the field of veterinary science, in an attempt to find measurable defining characteristics that represent either physiological or pathophysiological processes, and provide prognostic or diagnostic information(30). An important principle regarding biomarkers, is that they should have dual correlatory and explanatory functions (31). Biochemical variables can correlate accurately with specific changes to the internal environment, but they cannot paint an integrated picture of allostasis. Endocrine variables seem promising in their ability to prognosticate as biomarkers in canine critical illness(32).

The intricacies of neuroendocrinology has long been one of the prime pursuits of medicine, as all the alterations made to physiology are intricately bound to the endocrine system (32). Endocrine parameters could thus provide a mechanism of signposting progression and severity in patients, as biomarkers. It could also, in producing reliable patterns and associations with inflammatory processes and pathophysiological mechanisms. This improved understanding could guide clinicians in informed treatment decisions. The investigation of cortisol, TT4 and TSH serum concentration changes and patterns in Puffadder and snouted cobra envenomation is undertaken to highlight lacunae for future research. There is a need for dependable proxies of severity of envenomation, and if endocrine parameters perform as well as they do in other acute inflammatory models of critical illness, clinical decision making could be substantively improved.

1.1.2 Endocrine changes in snake envenomation: veterinary literature

The endocrine changes induced in canine snake envenomation are virtually unknown. From the murine studies and experimental rabbit models, patterns of endocrine changes distinct from those observed in humans, which will be discussed below. The little endocrinological data available focuses on different snake species to those investigated in the current study. The general patterns of snake envenomation are thus discussed and compared to the species-specific data that was obtained in this study.

The endocrine components of the acute phase response, including elevations in adrenocorticotropic hormone and thus serum cortisol concentrations, are to be expected (33), since significant activation of the acute phase has been proven, especially in African snake envenomation (34). The little endocrine data available is mainly centred around adrenal activity,

because the adrenal gland was thought to be most prominently affected by bee (35), and scorpion(36) envenomation.

Interestingly, a decrease in circulating mineralocorticoids has been found. This suppression of the Renin-Angiotensin Aldosterone system (RAAS) is thought to be mediated by phospholipase-A(37). No histological evidence of decreased mineralocorticoid activity was found in the zona glomerulosa of the adrenal gland (38), but the effects were reflected in electrolyte changes.

Glucocorticoid perturbations exhibit the opposite. One endocrine study investigated the effect of *Naja haje* (Snouted Cobra) venom on the adrenal activity of rabbits (39) and found a significant increase in total serum cortisol concentrations. A few studies focusing on the histological changes of the adrenal gland have also found significant depletions of cortical lipids and ascorbic acid (38), also indicative of increased adrenocortical activity.

The exact mechanism of hypothalamic-pituitary-adrenal axis stimulation is, however, unknown. There is evidence that neurotoxins such as alpha-bungarotoxin bind with great affinity to the cells of the CNS and the adrenal gland (40), but the exact pattern of secretion that follows the binding of these common elements of snake venom has not been determined. The possibility of toxin-induced increases in ACTH (39) by different mediators has also been postulated. Epinephrine from the adrenal medulla causing adenohypophyseal ACTH release has been implicated (41), as well as the direct action of histamine on the zona fasciculata (42). The unfortunate confounding factor complicating the consolidation of these different theories, is the fact that these mediators are present in stress, acute phase, and pain responses. The serial evaluation of the hypothalamic-pituitary-adrenal and -thyroidal axes will provide valuable insight into the temporal relationship between these observed variables.

There is also no true consensus on the cortisol patterns obtained in the above studies. In canine envenomation by *Waltersinia aegyptia*, or Desert cobra, sublethal doses cause increased urine ketosteroids, but decreased urine ketosteroids at lethal doses (43). These studies all provide a foundational body of knowledge upon which further studies can improve, but little has been added in recent years to the understanding of these patterns.

Previous studies performed on the same study population, of which some findings have been discussed above, have investigated other aspects of envenomation (16, 19, 44, 45). The first study initially found significant leukocytosis, thrombocytopaenia and clotting abnormalities in puffadder-envenomed dogs, while snouted cobra envenomation was characterized by normocoagulability to hypercoagulability(16, 44). After 24 hours post-envenomation, both groups became hypercoagulable(16, 44). Another focused on myocardial injury in snake-envenomed patients, and found significant cardiac troponin I (cTnI) elevations in all species(19). In Puffader and snouted cobra envenomation, no correlation of cTnI with CRP concentrations could be demonstrated(19). Snake bites were also used as comparative model of inflammatory disease in a study on serum amyloid-A and its utility in the detection of systemic inflammation.

1.1.3 Envenomation-induced endocrine dysfunction in humans

Endocrine disorders observed in snake-envenomed humans include adrenal, anterior- and posterior pituitary dysfunction and dysglycemia (9). Although in humans these are lesser-known manifestations, they contribute significantly to morbidity and mortality(46).

Pituitary dysfunction following snake envenomation, initially likened to Sheehan's syndrome, was first described in 1958 (47, 48). Sheehan's syndrome is a delayed postpartum pituitary apoplexy after hypoxia in an enlarged and vulnerable gland state (49). The most characteristic species causing pituitary dysfunction is the Russell's viper (*Vipera ruselli*), and viper bites in general (50). The clinical manifestation of acute pituitary insufficiency following snake envenomation includes hypotension responsive to glucocorticoid therapy, and hypoglycaemia (51). These patients are often asymptomatic, and the hypopituitarism is only detected on dynamic HPA or HPT function testing (52). Acute pituitary insufficiency may be transient or persistent, while chronic pituitary dysfunction is thought to be a delayed clinical manifestation of occult acute pituitary damage (9). Diagnosis is often approached by evidence of cortisol deficiencies, and then the investigation of pituitary-level ACTH and TSH insufficiencies, followed by pituitary imaging to detect the presence of empty sellae(9). The first priority in these cases is the detection of hypocortisolaemia, given the clinical implication of glucocorticoid supplementation(9). This diagnostic process and the adaptation thereof for veterinary use could prove valuable, not only in the debate surrounding

corticosteroid use in snake envenomation, but also in the broader controversy of corticosteroid insufficiency in critically ill animals.

The pathophysiology of hypopituitarism following snake envenomation remains largely uncharted. It is further complicated by enormous inter- and intraspecies venomic variation (53), with most of the reports on hypopituitarism following snake envenomation originating from the Indian subcontinent (9). In true Sheehan's syndrome, the vulnerability stems from a period of hyperstimulation and -secretion (54). Following snake envenomation, a few mechanisms leading to pituitary vulnerability to hypoxia are suggested. Firstly, the capillary leak or vasculotoxicity seen in many Viperidae bites are implicated (55), that leads to congestion and oedema of the gland. Another proposed mechanism that is perhaps more congruent with current veterinary knowledge, is that envenomation by these species first causes a transient increase in the secretion of growth hormone (GH), ACTH, and TSH (56). This is then postulated to cause the same vulnerable gland state, that in pregnancy is caused by lactotroph hyperplasia (57), is induced in these hypersecretory pituitary states (9). The cause of the apoplexy, made possible by this stimulated and engorged state, is also controversial. The vascular insult could firstly be due to systemic coagulopathies, whether consumptive or otherwise, independently present in the snake envenomed patient (58). These coagulopathies have also been illustrated in animals (59). Other possibilities include the intravascular pressure oscillations brought about by capillary leak (60), hypotension from circulatory shock(55), as well as increases in intracranial pressure, and continued auto-antibody mediated destruction which has not been investigated in snakebite(61). Veterinary insight into this phenomenon, given that the presence of coagulopathies and the presence of acute pituitary hypersecretion has been demonstrated, could add value to this pathophysiological debate.

Acute kidney injury in humans caused by vasculotoxic snake envenomation is also associated with endocrine manifestations (59). Acute hypopituitarism occurred in 26% of patients with snake envenomation induced AKI (59). AKI was thus found to be a significant predictor of secondary hypopituitarism, although the relationship is not necessarily causal. Another case series(52) found the occurrence of acute hypopituitarism to be independent of coagulopathy, renal failure, and clinical severity, demonstrating the degree to which the scientific community has been unable to reach consensus on the matter.

Adrenal disorders also occur, albeit less frequently, as a direct sequela of snake envenomation. One study on humans in Myanmar showed that adrenal haemorrhage on autopsy was present in 36% of snake envenomed patients mostly envenomed by Russel's vipers (62). Similar to the hypophysis, controversies surrounding pathophysiology persists around adrenal haemorrhage (63). Snake venom again causes increased secretion in these glands during the acute phase, and that in combination with the simultaneous pro-coagulant and coagulopathic elements of snake venom is theorised to predispose to haemorrhage and necrosis(64). The appearance is similar to that of Waterhouse–Friedrichsen syndrome, an uncommon human syndrome involving adrenal haemorrhage, usually secondary to meningococcaemia, that can have multiple aetiologies(65). Other causes, such as capillary leak discussed above, have not definitively been ruled out nor in.

Case reports of electrolyte and other metabolic derangements are present in human literature, but they fall outside the scope of this dissertation. The goal of the study was to gain insight into the changes in cortisol, TT4 and TSH associated with Puffadder and snouted cobra envenomation in dogs, and whether there were patterns emerging that were similar to those found in human literature. The reasoning behind this focus is the clinical and therapeutic implication of hypopituitarism and adrenal insufficiency, that might potentially change the way envenomation cases are managed. Another goal was to contribute to the broader understanding of endocrine allostasis in critical illness, so that critical care in companion animals can improve in the long term.

1.1.4 Cortisol and Thyroxine allostasis in canine critical illness

The Hypothalamic-Pituitary-Adrenal (HPA) and Hypothalamic-Pituitary-Thyroidal (HPT) axes are central in the pursuit of homeostasis in health, although the role of these axes critical illness is still surrounded by controversy. A comparative discussion of perturbations brought about by other models of acute critical illness serves to raise questions regarding the possible patterns that might emerge should snake envenomation endocrinology be more thoroughly investigated. It also serves to illustrate the value of investigating endocrine changes as integrative biomarkers with significant prognostic value.

A decreased serum TT4 concentration is characteristic in critical illness as part of the adaptive attenuation of metabolic activity in tissues, explained by a defined state of euthyroid sick syndrome (66, 67), known more recently as non-thyroidal illness syndrome (NTIS)(68).

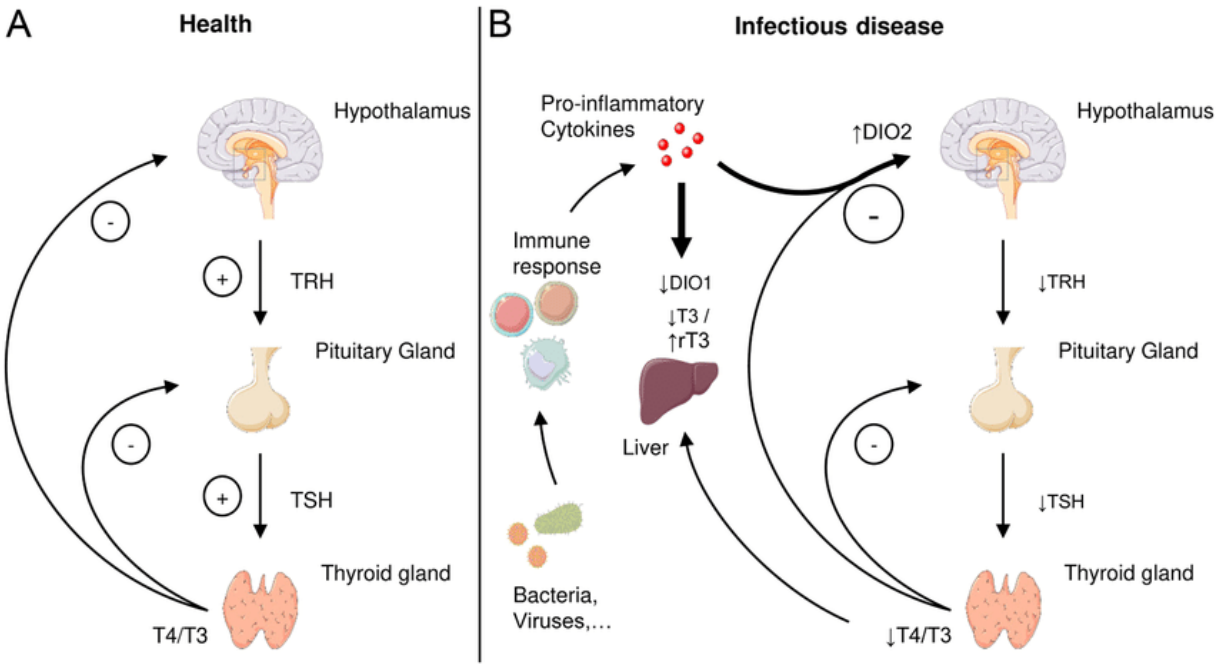

Figure 1: Diagrammatic illustration of the hypothalamic-pituitary-thyroidal axis in health and disease. Image A represents the HPA in health. Image B represents NTIS (69).

Most acute inflammatory models of critical illness in humans are associated with a decrease in thyroxine that is proportional to severity, and predictive of mortality (70-82). In dogs, he suppression of serum thyroxine concentrations take place in a plethora of disease states, although the adaptive-maladaptive debate is still ongoing (81, 83-95). The magnitude of this decrease in thyroxine has been associated with different measures of severity (81, 83, 85, 86, 89, 95-98). Additionally, the magnitude or persistence of this decrease in serum thyroxine has been associated with mortality (84, 86, 91, 96, 99). The recovery of thyroxine serum concentrations to normal ranges take up to 4 weeks post nonthyroidal illness, but patterns vary and longitudinal research on this aspect is sparse (87, 88, 92, 95, 100) (91).

The HPA contributes arguably more to the systems-integration that is acute critical illness, especially where inflammatory processes are involved (101). The classical endocrine response to critical illness is characterised by elevated serum cortisol, proportional to severity of illness(102-107). In humans, the acute HPA activation results in a temporary increase in cortisol production, followed by a sustained hypercortisolaemia mediated by several peripheral adaptations (104). Peripheral adaptations include significant decreases in carrier proteins such as cortisol binding globulins (CBG) and albumin that result in increased free, unbound cortisol(108). This can enable

increased systemic cortisol availability and activity without affecting total serum cortisol concentrations(104). Since total serum cortisol concentrations are measured in this study, the risk of type II error caused by the insensitivity of total serum cortisol in critical illness must be kept in mind. Even if total serum cortisol might not show significant elevations or alterations, metabolically significant changes in cortisol activity and availability might go undetected.

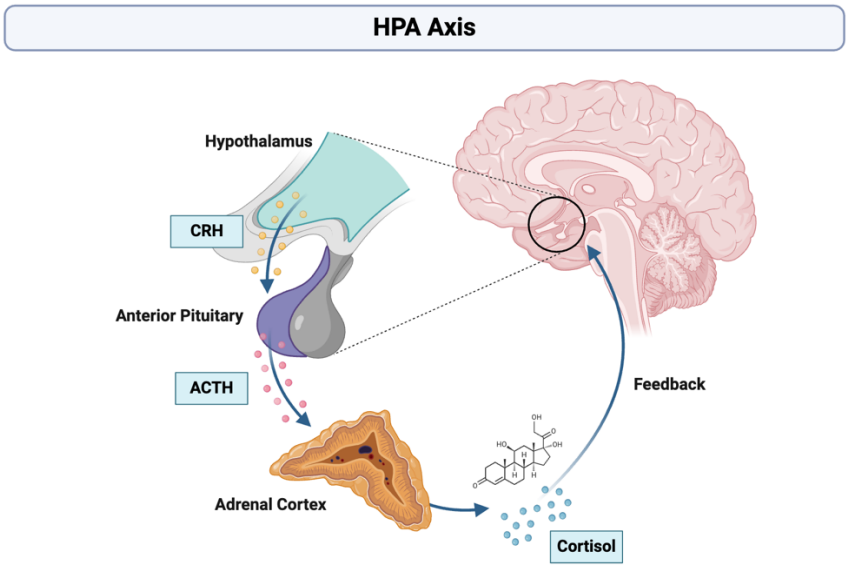

Figure 2: Diagrammatic illustration of the hypothalamic-pituitary-adrenal axis (109).

Hypercortisolaemia in critically ill dogs is associated with severity or outcome in a variety of critical illness states (82, 95, 96, 99, 110-115). Total serum cortisol concentration has been shown to be a valuable prognostic biomarker, predictive of mortality in a heterogenous population of critically ill dogs with an area under the receiver operating curve (AUROC) of 0.72(111). In parvoviral enteritis, serial serum cortisol concentrations was shown to be highly predictive of mortality, with none of the survivor group concentrations exceeding 224 nmol/L at 48 hours (99). In staphylococcal pneumonia, significant HPA activation after the onset of sepsis, but not at admission, proved indicative a poor prognosis (110). Babesiosis, as another acute model of critical illness, also demonstrates the association of increased total serum cortisol concentration with mortality (96, 113). In experimental canine babesiosis, higher cortisol serum concentrations with a more rapid incline were associated with both severity and parasite dose inoculated (95). Another

study investigating various inflammatory and neoplastic conditions also showed that increased basal or stimulated total serum cortisol concentration was predictive of mortality(115).

Inflammation is central in the clinicolaboratory manifestation of snake envenomation, which implies direct activation of the HPA axis (116). Inflammation is a coordinated response to potential threats from the environment, characterized by the release of chemokines and cytokines from macrophages and neutrophils, which leads to a cascade of leukocytosis and acute phase protein release (117). This is triggered by patterns known as pathogen-associated molecular patterns (PAMPS), or damage-associated molecular patterns (DAMPS)(118). These patterns are recognized and the PAMPS bound by pattern recognition receptors (PRRs) in the innate immune system(117). It has been suggested that snake venoms contribute directly and indirectly to the initiation of systemic inflammation, via secretory phospholipases A2(119) and DAMPS released by damaged skin or skeletal muscle(120). This activation of the DAMPS-PRR complex catalyses the acute phase response – a rapid elevation of positive acute phase proteins(34). Among these acute phase proteins like fibrinogen, serum amyloid-A and alpha-globulins is CRP, rapidly released in quantities proportional to the severity of the insult(121). CRP will thus be used in this study as indicator of these complex pathophysiological processes involved in the acute inflammatory response to snake envenomation.

HPA activation is to be expected in these cases as part of the acute phase response and are repeatedly demonstrated in murine snake envenomation (34). The degree to which these changes are adaptive is debatable. Corticosteroid therapy for critical illness states have been oscillating in and out of vogue for time immemorial, whether it be large suppressive dosing regimens or the more modern replacement theories(122). The concept of critical illness-related corticosteroid insufficiency (CIRCI) is based on the presupposition that HPA axis activations and serum cortisol concentration elevations are adaptive(123), and thus that the level of adrenal activity should be commensurate with the degree of inflammatory activation (124). This theory is let down by the repeatable association of hypercortisolaemia with mortality, even in CIRCI-aimed studies (114). In one study, high serum cortisol concentrations were strongly correlated with both severity and mortality, a finding that speaks to the inherent nature of the critical illness endocrine response as a stress response(122).

The change in serum cortisol concentrations over time paints a representative and informative picture of homeostasis in critical illness. The degree to which these patterns are repeated in snake envenomed patients would provide insight not only into the inflammatory pathophysiology of snake envenomation, but also whether the hypopituitarism seen in human patients can be congruent with inflammatory HPA activation. The adrenal and pituitary dysfunction seen secondary to snake envenomation as an established clinical and histological occurrence in humans, could provide valuable context for the interpretation of serum cortisol concentration perturbations in general critical illness.

Chapter 2: Study Objectives

2.1 Research questions

Are changes in serum cortisol, TT4 and TSH concentrations in canine Puffadder and snouted cobra envenomation related to venom type?

Are changes in serum cortisol, TT4 and TSH concentrations in canine Puffadder and snouted cobra envenomation related to the degree of systemic inflammation, as measured by serum CRP concentration?

2.2 Hypotheses

Primary hypotheses:

H_0: Canine Puffadder and snouted cobra envenomation does not cause a rise in serum total cortisol concentration.

H_1: Canine Puffadder and snouted cobra envenomation causes a rise in serum total cortisol concentration.

H_0: Canine Puffadder and snouted cobra envenomation in dogs does not cause a decrease in serum TT4 concentration.

H_1: Canine Puffadder and snouted cobra envenomation in dogs causes a decrease in serum TT4 concentration.

Secondary hypotheses:

H_0: Changes in serum cortisol, TT4 and TSH concentrations in canine Puffadder and snouted cobra envenomation are not related to venom type.

H_1: Changes in serum cortisol, TT4 and TSH concentrations in canine Puffadder and snouted cobra envenomation are related to venom type.

H_0: Changes in serum cortisol, TT4 and TSH concentrations in canine Puffadder and snouted cobra envenomation are not related to the degree of systemic inflammation as measured by serum CRP concentration.

H_1: Changes in serum cortisol, TT4 and TSH concentrations in canine Puffadder and snouted cobra envenomation are related to the degree of systemic inflammation as measured by serum CRP concentration.

2.3 Objectives

The goal of this study is to characterize the internal endocrine environment with regards to changes in serum cortisol, TT4 and TSH concentrations in the snake envenomed patient, both as critically ill patients and as distinct clinical grouping. The aim is to crystallize either a confirmatory or a comparative endocrine model of a critical illness. More research is needed on the pathophysiology of snake envenomation, specifically Puffadder and snouted cobra envenomations, in domestic animals. There is a dearth of knowledge on the endocrine elements, with most veterinary research done on laboratory animals(39). In humans, very specific endocrine patterns have emerged in snake envenomation cases, and more research is imperative in understanding the inflammatory nature of canine Puffadder and snouted cobra envenomation as well as the therapeutic implications thereof.

Specific objectives include:

To investigate the endocrine perturbations in dogs envenomed by *B. arietans* and *N. annulifera.*

To determine whether a characteristic pattern of changes in serum cortisol, TT4 and TSH concentrations arise from Puffadder and snouted cobra envenomation in dogs.

To determine whether changes in serum cortisol, TT4 and TSH concentrations in Puffadder and snouted cobra envenomation are related to the degree of systemic inflammation, as measured by serum CRP concentration.

2.4 Benefits arising from the study

This study aims to contribute to the veterinary body of knowledge in two parallel planes.

In part, this study forms part of the pursuit of an understanding of endocrine allostasis in canine acute critical illness, which has been the subject of some controversy in veterinary research. There is ongoing debate surrounding the role and function of cortisol and thyroxine in canine critical illness. There has been significant insight gained from studies on the endocrine perturbations in acute inflammatory disease processes, leading to advances in both prognostication and treatment. The investigation of endocrine patterns in an acute, toxic insult could lend valuable perspective so that conclusions on more diverse critical disease states can be drawn.

The second important factor lending gravity to the objectives of this study is the globally growing relevance of snake envenomation as a neglected tropical disease. The socio-ecological complexity of snakebites and its patterns of occurrence has historically led to the neglect thereof as a global disease burden, in political and scientific communities. There has been a recent global realisation of the gravity of the situation, leading to the publication of the 2019 global “Strategy for the Prevention and Control of Snakebite Envenoming” by the World Health Organisation (125). A clinical inquiry into the pathophysiology and the therapeutic implications thereof would thus improve the way we understand snake envenomations in general. A better understanding of the characteristic changes in serum cortisol, TT4 and TSH concentration patterns in veterinary patients might inform the interpretation of endocrine findings observed as sequelae of human snake envenomation. In doing so it will contribute to the global scientific momentum in this multidisciplinary field.

Chapter 3. Materials and Methods

3.1 Model system

The study was a prospective, observational study, specifically case-controlled. Data that has been collected between November 2010 and April 2011 from 23 naturally envenomed, client-owned dogs presented to the Onderstepoort Veterinary Animal Hospital (OVAH).

3.2 Study design

A prospective study evaluated the data of dogs naturally envenomed by snouted cobra (*Naja annulifera*) and African puffadder (*Bitis arietans*) and presented to an academic hospital between November 2010 and April 2011. Previous publications on the same study population focused on the inflammatory and haemostasis variables (16, 19, 44, 45). The endocrine assays were performed in February 2012 and is the first to utilise the endocrine data collected as part of this dataset.

Two control groups were used. Healthy dogs were selected for control groups so that statistic comparisons with the patient population could be performed. The CRP control group comprised of 10, and the endocrine control group of 13 client-owned dogs in good health, that presented for routine procedures such as blood donation or neutering, from the same background population at the same hospital. Both healthy control groups were initially included as controls in other studies, and the data was made available for use in this project, and thus the CRP and endocrine control groups consist of different individuals (44, 126). Health status was determined by owner history, physical exam, complete blood count and serum biochemistry. If no abnormalities could be found in any of the above screening tests, the dog was eligible for inclusion in the control group, and owner consent was obtained.

Inclusion criteria:

Animals of all breeds and genders were included. Cases were only included if owners were able to obtain a positive identification of snouted cobra (*Naja annulifera*), or puffadder (*Bitis arietans*). A positive identification of the snake could be obtained via photo or dead specimen, given the owner had witnessed the envenomation directly. Additional criteria included a minimum age of 6 months and a minimum weight of 5 kg. Further, the dog had to be presented to the OVAH within 6 hours post envenomation as the chronobiology of parameters measured could lead to significant systematic errors when interpreting results.

Exclusion criteria:

Exclusion criteria precluded any dogs not clinically healthy, according to the owner, prior to envenomation. Any concurrent disease states or inflammatory conditions as could be established in the history or with a full physical examination, serum biochemistry, complete blood count, and blood smear resulted in exclusion. Any treatment with steroidal- or non-steroidal anti-inflammatories also served as grounds for exclusion due to interference with endocrine parameters and correlatory blood panels. Another exclusion criterium was any history of prior veterinary treatment for Puffadder and snouted cobra envenomation, or any other disease within the preceding week.

Informed owner consent was obtained for participation in the study. Ethical approval was obtained from the Research Ethics Committee, Faculty of Veterinary Science, University of Pretoria (REC089-23). The original study published on this data also obtained Animal ethics approval from the animal ethics committee, Faculty of Veterinary Science, University of Pretoria (V058–10).

3.3 Experimental procedure

The cases were identified on presentation, and a thorough history was obtained, including questioning for the presence of any exclusion criteria, and details regarding the envenomation. These details include the time from envenomation, the positive identification of snake species, and method of identification. A physical examination was performed immediately thereafter, and physical data recorded, including site of envenomation, degree of swelling. The full examination recording sheet is available in annexure C.

Treatment Protocol:

Treatment was standardised specifically to different venom types relevant to the snake species, as far as owner finances and consent allowed. The spectrum of treatment included polyvalent antivenom, intravenous fluids (including crystalloids and colloids), blood products, and other supportive treatments as necessitated by the clinical state of the dog. Antibiotics were reserved for dogs that were critical, and corticosteroids were excluded from the protocol altogether.

Polyvalent Antivenom used consists of refined equine serum immunoglobulins. The treatment given to each patient was recorded to allow for interpretation within each dog's specific clinical context. Clinical monitoring was performed and recorded until discharge or death.

Sample Collection:

A physical examination was performed on presentation and blood samples were collected by jugular venepuncture into 3 mL ethylenediaminetetraacetic acid (EDTA) and serum vacutainer tubes using vacuum assistance. Serum samples were allowed to clot and then centrifuged within 45 minutes of collection at 2100g for 8 minutes. The serum samples were stored at -80° C.

The serum blood samples were collected at admission, and then at 12-, 24-, and 36-hours post-envenomation to investigate longitudinal changes. The 12-, 24- and 36-hour time points were calculated from envenomation using the time of envenomation provided by the owner during history taking.

3.4 Observations:

The following endocrine variables were measured using a previously validated (127) solid-phase competitive chemiluminescent immunoassay: TT4 (Canine Total T4, Siemens Medical solutions diagnostics, Los Angeles, USA), TSH (Immulite® Canine TSH, Diagnostic Products Corporation, Los Angeles, USA), and Total cortisol (Immulite® 1000 Cortisol, Siemens Medical solutions diagnostics, Los Angeles, USA) were measured according to the manufacturer's instructions. The above assays have minimum limits of detection specific to each assay: the total cortisol assay has a calibration range of 28 – 1380 nmol/L, and concentrations below the minimum limit of detection were reported as 28 nmol/L; the TT4 assay has a calibration range of 6.4 – 193 nmol/L, and concentrations below the minimum limit of detection were reported as 6.4 nmol/L; TSH assay has a calibration of up to 12 ng/dl, and has an analytical sensitivity of 0.01 ng/dL. Concentrations below 0.01 ng/dL were taken as 0.01 ng/dl for statistical purposes. Complete cell counts were performed within 30 minutes of admission on the EDTA samples on the ADVIA 2120 (Siemens), and serum biochemistry was also performed on serum samples to rule out any comorbidities that could affect the results of this study. Serum CRP concentration was also determined using a previously validated (128) automated turbidimetric immunoassay (Cobas Integra 400 Plus

analyser, Roche, Basel, Switzerland) that had been calibrated to ensure species-specific measurement with the heterologous assay. The minimum detection limit of C-Reactive Protein in this assay was 5.1mg/L, and all readings below the minimum detection limit was homogenised to 5.1mg/L.

Some components of this dataset, including the coagulation and inflammatory parameters, have been published before (16, 19, 45). The focus of this study is thus the endocrine variables in this dataset, specifically the longitudinal changes in serum TT4, TSH, and cortisol concentrations.

3.5 Data analysis

Data was captured in an excel spreadsheet format (Microsoft Excel 2021. Microsoft Corporation). Endocrine concentrations below the limit of detection for their respective assays were taken as 28 nmol/l for cortisol, 5.1 mg/L for CRP, and 6.4 nmol/l for thyroxine.

Statistical analysis was performed using two different commercial software packages, SPSS (SPSS 28.0, 2023, SPSS Inc.), and Stata (StataCorp. 2023. Stata Statistical Software: Release 18. College Station, TX: StataCorp LLC.). The assumption of normality on the endocrine data was tested using the Shapiro-Wilke test.

Patients were grouped on two levels after being compared to controls as a combined case population. Firstly, the snake species served as grounds for comparison between puffadder-envenomed and cobra-envenomed dogs, defined as the cobra (n=9) and puffadder subgroups (n=8). The cobra group was sub-divided for further comparison into a group that presented with neurological signs (n=5) and a group without neurological signs (n=4). Longitudinal comparisons between dependent samples from the same patient group at different time points were compared using linear mixed modelling (129). Once an acceptable model with the lowest Akaike information criterion was found, assumptions were checked using standard residual testing, and pairwise comparisons within this model were performed. Each time point was then compared to admission, and to every time point thereafter. Thereafter, pairwise comparisons between groups (i.e. puffadder, cobra, neurological and non-neurological groups, as well as cases and controls) within each time point were performed to evaluate time-group interactions. For all comparisons, $P < 0.05$ were considered significant.

The correlations between different variables were evaluated by calculating the Spearman rank correlation coefficient, because of the non-parametric nature of the data. A Spearman's rho value of (+/-)0.1 – (+/-)0.3 was regarded a weak correlation, (+/-)0.4 - (+/-)0.6 was regarded a moderate correlation, and (+/-)0.7 - (+/-)0.9 was regarded a strong correlation. A P-value of <0.05 was regarded as significant throughout.

Control dogs were established to be of similar age sex and breed of the study population using ANOVA, and the control samples were all taken at a single time point.

Data is presented as median concentrations in the tables below, with the interquartile range as indicator of data dispersion.

Chapter 4: Results

4.1 Signalment

The records of 23 naturally envenomed dogs were evaluated and five dogs were excluded based on the criteria above, i.e. presentation over 6 hours post-envenomation, treatment with corticosteroids, or incomplete data. The sex distribution of the final 17 dogs consisted of 9 females and eight males, three females in the puffadder group and six females in the cobra group. The neurological cobra group consisted of three females and two males, while the non-neurological cobra group consisted of three females and one male. The endocrine control group consisted of 9 females and 4 males, and the CRP control group of 4 males and 6 females. The weight and age of the study population are described in table 1. No significant differences in age, weight, or gender were found between case, control, or different envenomation groups. A variety of breeds were represented in the study population. The full dataset is included in Annexure E below. The data was originally recorded as part of a study investigating the effect of Puffadder and snouted cobra envenomation on coagulation (44).

Table 1: Descriptive statistics of the signalment of the case and control populations. Q1 denotes the lower quartile (25th percentile), Q3 the upper quartile (75th percentile), and IQR the interquartile range.

	Puffadder Group (n=8)	**Snouted Cobra Group (n=9)**	**Combined Study Population (n=17)**
Variable	Median (Q1-Q3)	Median (Q1-Q3)	Median (Q1-Q3)
Age (months)	45 (35.5 – 60)	42 (24 – 48)	43.5 (24 – 54.75)
Weight (Kg)	10.2 (7.55 – 30)	17.8 (6.6 – 30.2)	11.9 (7.25 – 30)
	Neurological Snouted Cobra Group (n=5)	**Non-neurological Snouted Cobra Group (n=4)**	
Variable	Median (Q1-Q3)	Median (Q1-Q3)	
Age (months)	48 (48 – 58.8)	24 (22.8 – 28.8)	
Weight (Kg)	11.2 (6 – 17.8)	31.6 (30.9 – 39.9)	
	Endocrine control Group (n=13)	**CRP control Group (n=10)**	
Variable	Median (Q1-Q3)	Median (Q1-Q3)	
Age (months)	37.50 (22.5 – 79.5)	51.5 (38.5 – 58.5)	
Weight (Kg)	27 (19 – 30.5)	17.6 (14.5 – 19.75)	

Basic clinicopathologic changes:

Table 2: Descriptive statistics of the signalment of the study population. Q1 denotes the lower quartile (25th percentile), Q3 the upper quartile (75th percentile), and IQR the interquartile range. "WBC" denotes White Blood Cell count, and "Hct" denotes Haematocrit.

	Puffadder Group	Snouted Cobra Group	Overall Study Population
Variable	Median (Q1-Q3)	Median (Q1 - Q3)	Median (Q1 - Q3)
Hct (L/L)	0.53(0.40 - 0.54)	0.53(0.48 - 0.56)	0.53 (0.45 - 0.55)
WBC ($\times 10^9$/L)	15.82(14.11 - 24.9)	8.54 (7.33 - 12.5)	13 (8 - 15.47)

4.2 Clinical Outcome findings

Three of the 18 dogs died (17% mortality rate). Of the 3 mortalities, one was a neurological cobra-envenomed dog that was euthanized at 72 hours after admission, due to a poor prognosis. The two mortalities from the puffadder groups were spontaneous deaths, of which one died just before 36 hours post envenomation, and the other before 24 hours post envenomation.

The clinical treatment took place in accordance with a protocol as suggested in a review article by Leisewitz et. al. (13). The protocol is attached in annexure D. In summary, this included mainly supportive therapy for the entire population, with the addition of specific therapy for the puffadder group - including fresh frozen plasma or whole blood transfusions if indicated. Only 2 dogs were treated with polyvalent antivenom, and only one each received fresh frozen plasma and whole blood transfusions. These small numbers tie in with some of the significant limitations of this study, as will be discussed below.

4.3 Endocrine results

4.3.1 Total thyroxine

Serum TT4 concentrations were lower than that of control dogs in all snake-envenomed cases in this study population, at all time points. The descriptive statistics of thyroxine results are presented in Table 3 below. The interpretive statistics are presented on the following page.

Table 3: Descriptive statistics of serum TT4 concentrations over time. Median, lower quartile(Q1), and upper quartile(Q3) values are given in nmol/L. One asterisk (*) indicates a significant difference in comparison to control dogs at a significance level of P<0.05. Two asterisks (**) indicate a significant difference in comparison to control dogs at a significance level of P<0.01. "Cases" represent the combined puffadder and snouted cobra envenomation groups.

		Time since envenomation			
		Admission	12h post-envenomation	24h post-envenomation	36h post-envenomation
		TT4 (nmol/L)	TT4 (nmol/L)	TT4 (nmol/L)	TT4 (nmol/L)
		Median (Q1-Q3)	Median (Q1-Q3)	Median (Q1-Q3)	Median (Q1-Q3)
Group	Cases (n=17)	20.80* (15.2 – 25)	7.71** (6.4 – 19.7)	11.50** (6.4 – 18.5)	12.30** (6.4 – 16.6)
	Controls n=12)	24.85 (19.55 – 32.5)	-	-	-
	Puffadder (n=8)	20.30* (11.55 – 24.4)	10.90** (6.4 – 22.1)	9.15** (6.4 – 14.65)	10.30** (6.4 – 12.8)
	Snouted Cobra (n=9)	20.80 (15.2 – 25.5)	7.71** (6.4 – 17.8)	12.80** (6.4 – 20.2)	15.25** (6.4 – 22.7)
	Neurological Snouted Cobra (n=5)	20.8 (16.1 – 25.2)	6.4** (6.1 – 7.4)	6.40** (6.4 – 14.5)	6.40** (6.4 – 15.6)
	Non-Neurological Snouted Cobra (n=4)	21.85 (13.8 – 25.45)	14.6* (9.4 – 21.45)	21.55 (14.8 – 19.35)	21.65 (12.45 – 30.6)

At admission, the serum TT4 concentration of the combined case population was found to be significantly lower than control dogs (P<0.05). The puffadder subgroup serum TT4 concentration at admission was also shown to be significantly (P<0.05) lower compared to controls, where the cobra subgroups failed to reach significance at the first time point. In the neurological cobra subgroup, admission serum TT4 concentrations were found to be lower than every time point thereafter (P<0.05). In the puffadder group, admission serum TT4 concentrations were found to be significantly higher than at 24 (P<0.01), and 36 (P<0.05) hours post-envenomation.

The admission serum TT4 concentrations in the combined study population was significantly higher than every time point thereafter($P<0.05$).

All group serum TT4 concentrations were significantly lower than controls at 12 hours post-envenomation, including puffadder- ($P<0.01$), combined cobra- ($P<0.001$), neurological cobra- ($P<0.001$) and non-neurological cobra- ($P<0.05$) -envenomed group TT4 concentrations. At the 12-hour time point the serum TT4 concentrations of both the combined population and the non-neurological cobra subgroup reached its nadir. The non-neurological cobra subgroup median TT4 concentration at this point was significantly lower in comparison to 24- and 36-hours post-envenomation ($P<0.05$).

At 24 hours post-envenomation, the overall median TT4 concentration of the study population had started to increase but was still significantly lower than admission ($P<0.05$), and controls ($P<0.001$). The non-neurological cobra subgroup's median TT4 serum concentration had started to increase in comparison to the 12-hour time point ($P<0.05$), but no longer significantly lower than controls ($P<0.01$). The puffadder subgroup reached its nadir at this point and was significantly lower than both admission ($P<0.05$), 12 hours post-envenomation($P<0.05$), and controls ($P<0.001$). At this point, the non-neurological cobra subgroup median serum TT4 concentration was significantly higher than that of both puffadder ($P<0.05$), and neurological cobra ($P<0.05$) groups.

At 36 hours post-envenomation, the combined case population, neurological cobra and puffadder subgroup serum TT4 concentrations were still significantly lower than both admission ($P<0.05$) and controls ($P<0.001$). In contrast, the non-neurological cobra subgroup serum TT4 concentration had increased significantly compared to the 12-hour time point ($P<0.05$) and was still significantly higher than that of the puffadder and neurological cobra subgroups ($P<0.05$). No difference between non-neurological cobras and controls could be detected at this time point. A boxplot is presented below to illustrate the overlap in range of these groups.

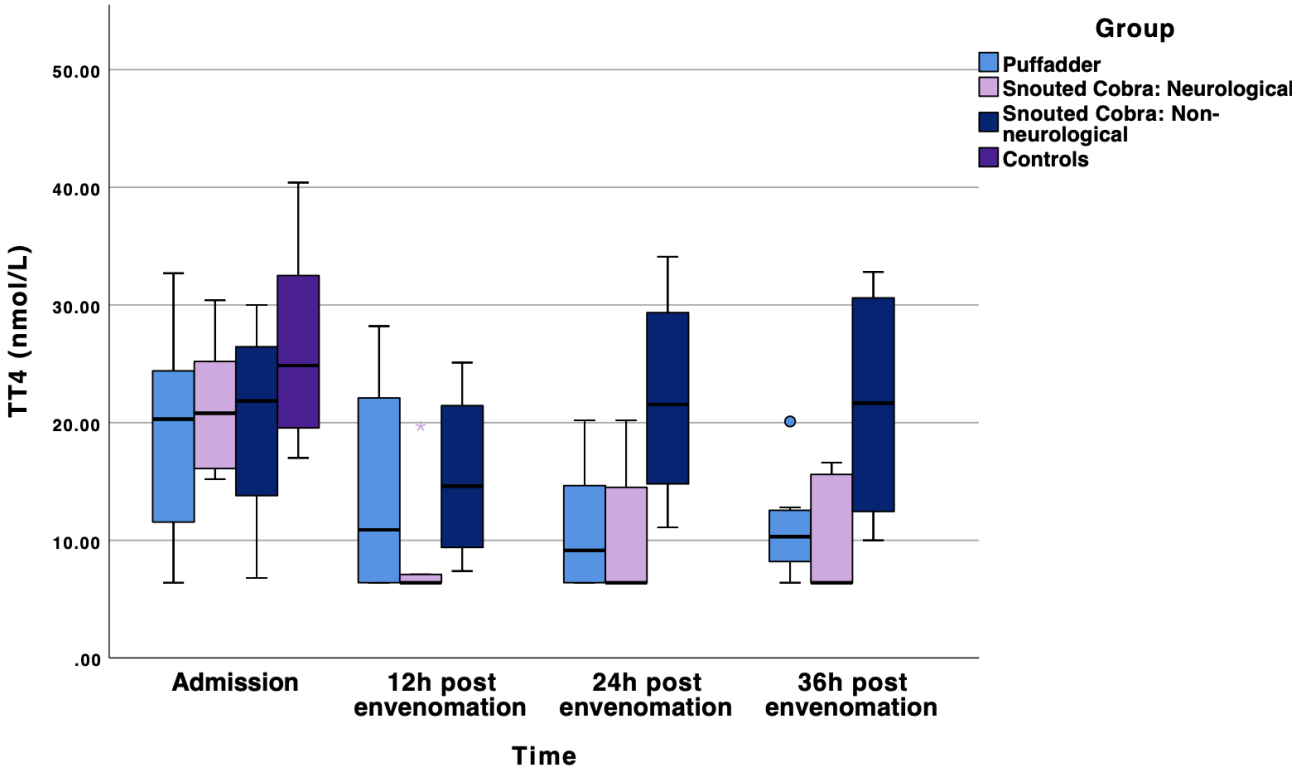

Figure 3: A clustered boxplot of serum TT4 concentration in nmol/L at admission, and at 12, 24 and 36 hours post envenomation of dogs naturally envenomed by the African puffadder (Bitis arietans) or snouted cobra (Naja annulifera), and of control dogs. The line in the middle of the box represents the median, with the box itself representing the interquartile range(IQR). The whiskers extend to the upper and lower fence values.The dots represent outliers that fall outside 1.5 times the IQR.

As anecdotal observation, the case with the highest serum cortisol concentration (579 nmol/l), as well as the highest serum CRP concentration (197.3 mg/l), also had the lowest sustained serum TT4 concentration (below the minimum limit of detection) at the 36-hour time point.

4.3.2 Thyroid stimulating hormone

The descriptive statistics of serum TSH concentrations are presented in table 5 below. The findings of interpreted statistics will be presented thereafter.

Table 4: Descriptive statistics of serum TSH concentrations over time. Median, lower quartile(Q1), and upper quartile(Q3) values are given in ng/mL. One asterisk (*) indicates a significant difference in comparison to control dogs at a significance level of P<0.05. Two asterisks (**) indicate a significant difference in comparison to control dogs at a significance level of P<0.01. "Cases" represent the combined puffadder and snouted cobra envenomation groups.

		Time since envenomation			
		Admission	12h post envenomation	24h post envenomation	36h post envenomation
		TSH (ng/mL)	TSH (ng/mL)	TSH (ng/mL)	TSH (ng/mL)
		Median (Q1 - Q3)	Median (Q1 - Q3)	Median (Q1 - Q3)	Median (Q1 - Q3)
Group	Cases (n=17)	.12 (.07 -.23)	.08 (.05 - .17)	.10 (.05 - .21)	.11 (.06 - .21)
	Controls (n=13)	.16(.11 - .28)	-	-	-
	Puffadder (n=8)	.124 (.085 - .222)	.078 (.048 - .161)	.103 (.057 - .148)	.126 (.059 - .211)
	Snouted Cobra (n=9)	.123 (.062 - .332)	.079(.043 - .194)	.137 (.051 - .245)	.108 (.055 - .208)
	Neurological Snouted Cobra (n=5)	.092 (.062 - .332)	.069 (.043 - .088)	.212 (.122 - .273)	.103 (.055 - .293)
	Non-Neurological Snouted Cobra (n=4)	.155 (.109 - .248)	.171 (.099 - .207)	.114 (.064 - .198)	.118 (.088 - .165)

Although an apparent rise was seen in neurological cobra group between 12- and 24 hours post envenomation, and in the non-neurological group between 0- and 12 hours post-envenomation (24 hours before the increase in serum TT4 concentration in both groups), these increases failed to reach significance. When comparing serum TSH concentrations of cases to that of control dogs, the comparisons failed to reach significance. The difference in TSH concentrations between time points, within each group, was insignificant. There was also no difference observed between the two envenomation groups at any given time point.

When comparing serum TSH concentrations of cases to that of control dogs, the comparisons failed to reach significance. The subgroups were also compared to controls, and no difference was found. There was also no difference observed between the two groups at any given time point.

A boxplot is presented below, to allow more detailed visualisation of data.

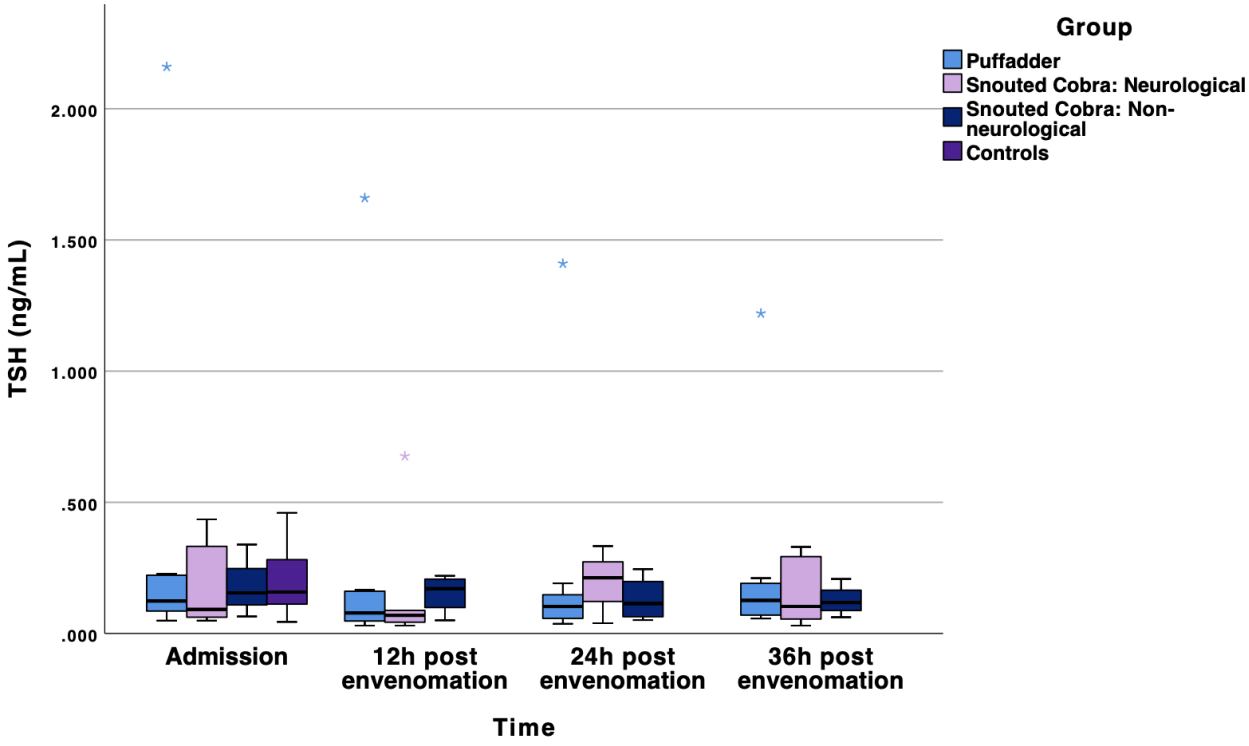

Figure 4: A clustered boxplot of serum TSH concentrations (in ng/mL) at admission, and at 12, 24 and 36 hours post envenomation. The line in the middle of the box represents the median, with the box itself representing the interquartile range(IQR). The whiskers extend to the upper and lower fence values.The dots represent outliers that fall outside 1.5 times the IQR.

4.3.3 Cortisol

The serum cortisol concentrations of this study population were at their highest at admission but were not significantly higher than control dogs. From that point, a consistent decrease was observed. The descriptive statistics for cortisol are presented in table 2, and a description of interpretive statistics follows below.

Table 5: Descriptive statistics of serum cortisol concentrations over time. Median, lower quartile(Q1), and upper quartile(Q3) values are given in nmol/L. One asterisk (*) indicates a significant difference in comparison to control dogs at a significance level of P<0.05. Two asterisks (**) indicate a significant difference in comparison to control dogs at a significance level of P<0.01. "Cases" represent the combined puffadder and snouted cobra envenomation groups.

		Time since envenomation			
		Admission	12h post-envenomation	24h post-envenomation	36h post-envenomation
		Cortisol (nmol/L)	Cortisol (nmol/L)	Cortisol (nmol/L)	Cortisol (nmol/L)
		Median (Q1-Q3)	Median (Q1-Q3)	Median (Q1-Q3)	Median (Q1-Q3)
Group	Cases (n=17)	227.50 (153 – 361)	108.00 (49.1 – 191)	111.00 (54.4 – 180)	85.80 (58.2 – 153)
	Controls (n=13)	135.00 (53.7 – 183)	-	-	-
	Puffadder (n=8)	264.5 (173 – 338)	114.5 (68 – 184)	128.5 (88.3 – 188.5)	80.3 (63.7 – 134)
	Snouted Cobra (n=9)	163.0 (136 – 483)	87.6 (30.9 – 191)	69.8 (46.9 – 117)	133.0 (38.4 – 372)
	Neurological Snouted Cobra (n=5)	483.0* (153 – 549)	191 (30.9 – 206)	117.0 (66.8 – 469)	58.2 (38.4 – 406)
	Non-Neurological Snouted Cobra (n=4)	163 (146.5 – 248.5)	87.6 (47.7 – 115.5)	63.6 (50.7 – 88.9)	148.5 (133 – 267)

At admission, the combined case population median serum cortisol concentration (Table 1) concentration was at its highest. Only the neurological subgroup of the Snouted Cobra envenomed dogs at admission had significantly higher serum cortisol concentrations in comparison to controls (P<0.05). Admission concentrations in the case population were significantly higher than at 12- (P<0.05), 24- (P<0.05), and 36- hours post-envenomation (P<0.01). In the puffadder subgroup, only the comparison of serum cortisol concentrations at admission versus 36 hours post-envenomation proved significant (P<0.01), with the admission values being higher. The neurological cobra subgroup's serum cortisol concentrations were significantly higher at admission than at the 12- (P<0.01), 24- (P<0.05) and 36 (P<0.01) hour time points. None of the

fluctuations observed in the non-neurological subgroup reached significance. No differences between groups were found within any of the time point comparisons.

At 12 hours post envenomation, the median serum cortisol concentration of the cases approached that of control dogs, and no significant differences between case and control populations were observed(fig.4). The combined case population median serum cortisol concentration was significantly lower at 12 hours post envenomation in comparison to admission($P<0.05$).

At 24 hours post envenomation, the serum cortisol concentrations of the combined case population as well as the cobra subgroup were still significantly lower than their admission concentrations ($P<0.05$). No differences between cases and controls were observed at this point.

At 36 hours post envenomation, the combined study population serum cortisol concentration was still lower than admission($P<0.05$). The puffadder subgroup serum cortisol concentrations also decreased significantly below admission concentrations($P<0.05$) for the first time.

As anecdotal observation, the case with the highest serum cortisol concentration at 36 hours post-envenomation (579 nmol/l), as well as the highest serum CRP concentration (197.3 mg/l), also had the lowest sustained serum TT4 concentration (below the minimum limit of detection) at the 36 hours after envenomation.
Another important anecdotal observation is the presence of two hypocortisolaemic patients within the broader dataset. Although one was excluded from the study based on admission after more than 6 hours since envenomation, it presents an interesting pattern for discussion. Both case 7 and case 19 were envenomed by a Snouted Cobra and had sub-normal cortisol serum concentrations at every time point, that were below the minimum limit of detection at every time point except case 7 at admission, which had a concentration of 30.6 nmol/L. Case 7 was a 5-year-old male Staffordshire bull terrier that was presented in respiratory paralysis at 4.5 hours after envenomation. Case 19 was a 9-year-old French poodle that had been excluded from the study population, presented at 18 hours post envenomation in respiratory paralysis and was referred for

ventilation. Both recovered to discharge despite consistent hypocortisolaemia for the duration of the study period.

A boxplot is presented below to demonstrate the higher admission serum concentrations with a wide range, in comparison to the 12-, 24-, and 36-hour time points.

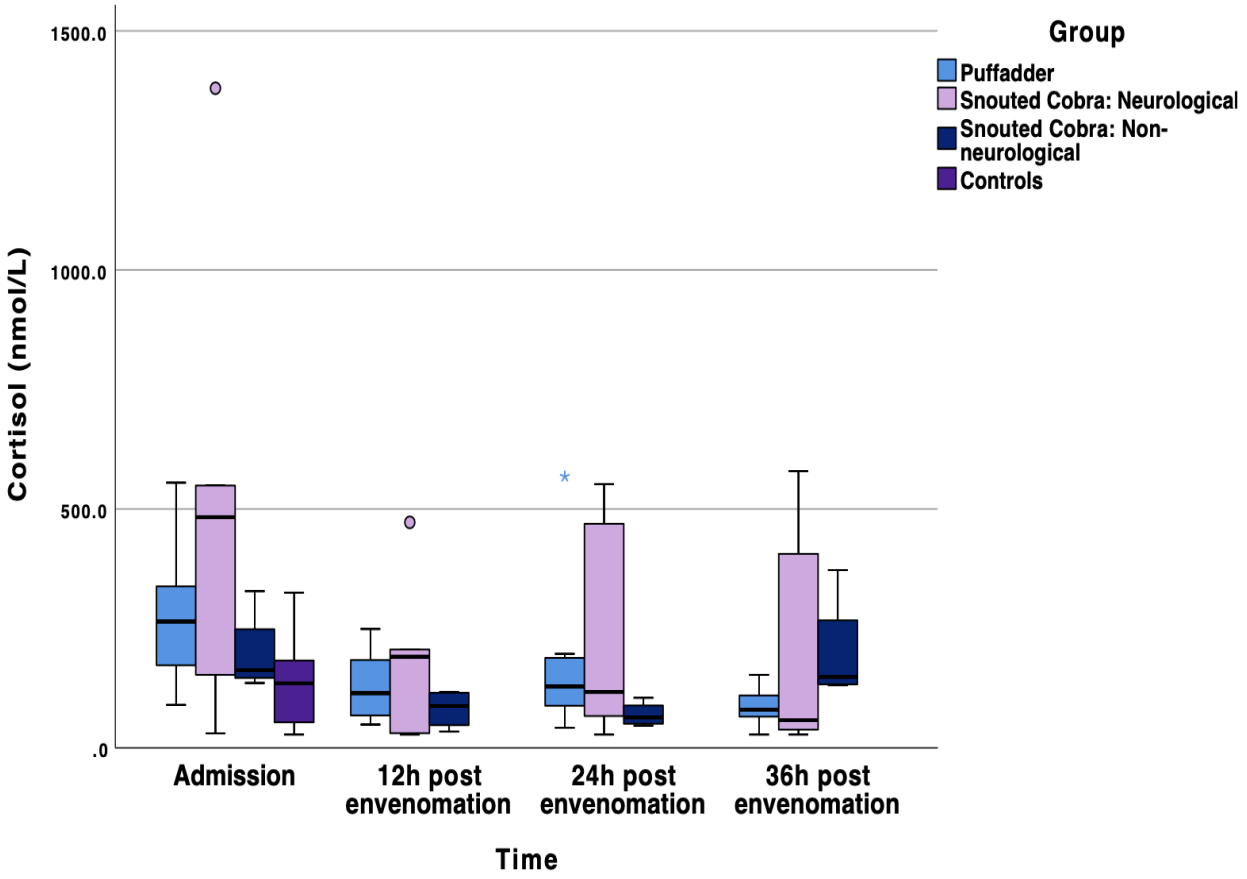

Figure 5: A clustered boxplot of serum cortisol concentration in nmol/L at admission, and at 12, 24 and 36 hours post envenomation. The line in the middle of the box represents the median, with the box itself representing the interquartile range(IQR). The whiskers extend to the upper and lower fence values.The dots represent outliers that fall outside 1.5 times the IQR.

4.3.4 C-Reactive Protein

Although CRP formed part of the secondary hypotheses of this study, correlations with endocrine variables were performed to gain insight into the endocrine pathophysiology of Puffadder and snouted cobra envenomation. The reporting will be minimal in this dissertation, but previous publications on the dataset can be consulted for in-depth analysis(16, 19, 44, 45). The basic results are thus presented for contextualization of above endocrine findings. The descriptive statistics for serum CRP concentration are presented in table 6. The results of interpretive statistics will be presented thereafter.

Table 6: Descriptive statistics of serum CRP concentrations over time. Median, lower quartile(Q1), and upper quartile(Q3) values are given in mg/L. One asterisk (*) indicates a significant difference in comparison to control dogs at a significance level of P<0.05. Two asterisks (**) indicate a significant difference in comparison to control dogs at a significance level of P<0.01. "Cases" represent the combined puffadder and snouted cobra envenomation groups.

		Time since envenomation			
		Admission	12h post envenomation	24h post envenomation	36h post envenomation
		CRP (mg/L)	CRP (mg/L)	CRP (mg/L)	CRP (mg/L)
		Median (Q1 - Q3)	Median (Q1 - Q3)	Median (Q1 - Q3)	Median (Q1 - Q3)
Group	Cases (n=17)	5.10 (5.10 - 8.93)	64.68** (57.86 - 90.18)	61.73** (51.58 - 77.94)	56.67* (5.10 - 61.24)
	Controls (n=13)	5.10 (5.10 - 5.10)	-	-	-
	Puffadder (n=8)	264.5 (173.0 - 338.0)	114.5** (68.0 - 184.0)	128.5** (88.3 - 188.5)	80.3* (63.7 - 134.0)
	Snouted Cobra (n=9)	163.0 (136.0 - 483.0)	87.6** (30.9 - 191.0)	69.8** (46.9 - 117.0)	133.0* (38.4 - 372.0)
	Neurological Snouted Cobra (n=5)	5.10 (5.10 - 5.10)	62.98 (55.32 - 80.07)	93.27 (77.94 - 115.03)	5.10 (5.10 - 60.96)
	Non-Neurological Snouted Cobra (n=4)	-	62.68 (43.09 - 114.51)	60.99 (31.08 - 70.97)	35.09 (6.88 - 81.06)

The CRP of the combined case population was significantly higher than that of the control population at 12- (P<0.001), 24- (P<0.001), and 36 (P<0.01) hours post-envenomation. The neurological cobra subgroup showed the highest peak in CRP serum concentrations at 24 hours post-envenomation, which was significantly higher than the puffadder (P<0.001) and non-neurological cobra(P<0.01) groups at that time point. The puffadder and neurological cobra subgroup median serum CRP concentrations were also significantly higher than controls at 12- (P<0.01), 24- (P<0.001), and 36- (P<0.05) hours post-envenomation. The non-neurological cobra subgroup was only significantly higher than controls at 12- (P<0.05) and 24- (P<0.001) hours post envenomation. The puffadder and neurological cobra subgroups showed a significant increase between admission and 12- (P<0.001), as well as 24 (P<0.001) hours post-envenomation. A sharper decrease was seen between the 12- and 36-hour time points (P<0.05) in both cobra subgroups.

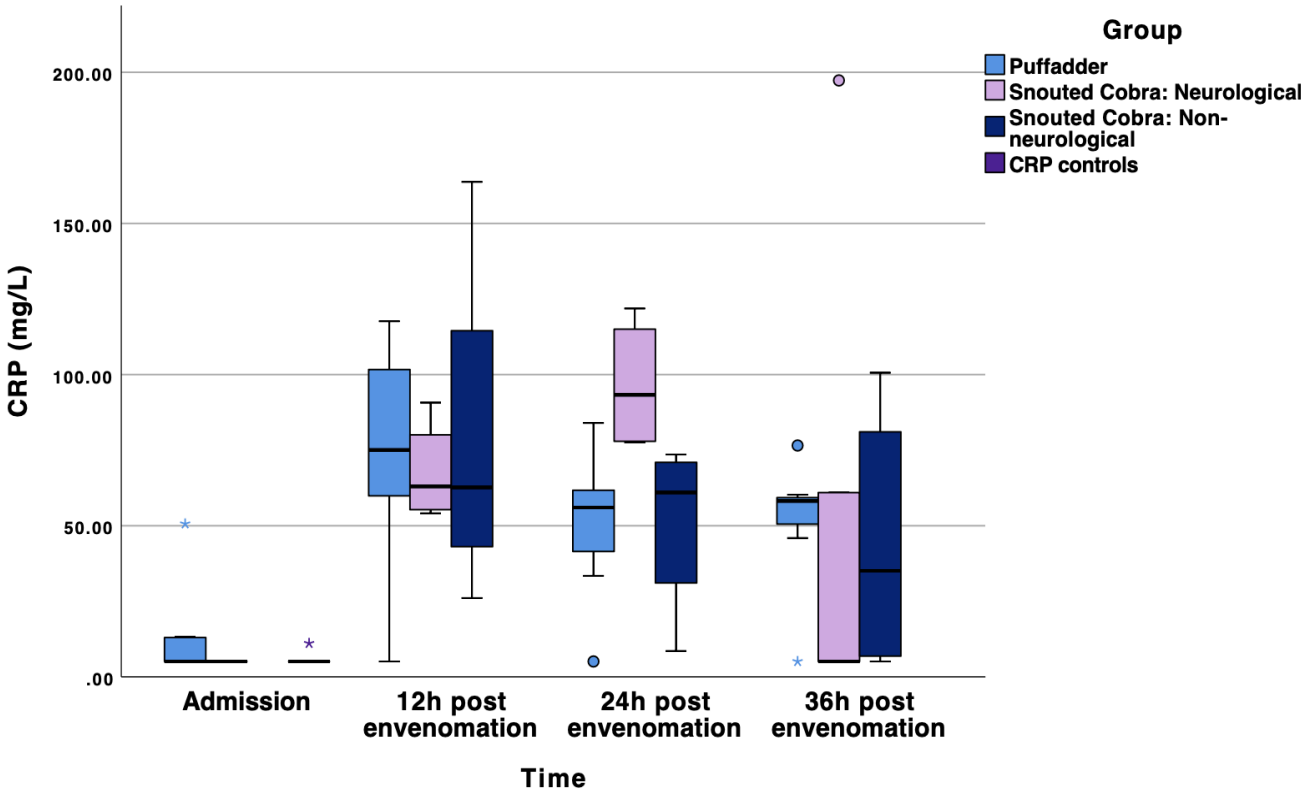

Figure 6: Clustered boxplot of serum CRP concentration (in mg/L) at admission, and at 12, 24 and 36 hours post envenomation. The line in the middle of the box represents the median, with the box itself representing the interquartile range(IQR). The whiskers extend to the upper and lower fence values.The dots represent outliers that fall outside 1.5 times the IQR.

4.3.5 Correlations

A weak significant negative correlation was found between serum TT4 and CRP concentrations ($P<0.05$). The Spearman's rho value was calculated as -0.326 for this correlation ($P<0.05$), classifying it as a weak correlation. The serum cortisol and CRP concentrations were also weakly correlated (Spearman's rho= -0.211), but this correlation failed to reach significance.

The correlations between other variables, TSH and CRP, cortisol and TT4 and even TT4 and TSH also failed to reach significance.

Chapter 5: Discussion

This study presents the first description of the longitudinal changes in serum TT4, TSH, and cortisol concentrations in response to canine Puffadder and snouted cobra envenomation. A significant suppression of serum TT4 concentrations were observed at all time points in the study population. Additionally, the association of TT4 suppression and degree of systemic inflammation as represented by serum CRP concentrations has not previously been demonstrated in dogs.

The longitudinal changes seen in Puffadder and snouted cobra envenomation are rapid, reflecting a more toxic nature (87) in contrast to most longitudinal endocrine studies that have been performed on infectious disease models (95, 99, 110, 130). This comparative model emphasises the scarcity of longitudinal data in critical illness endocrinology, which likely contributes to the incomplete and contrived understanding thereof in the literature.

5.1 TT4 and TSH

Serum TT4 concentration changes induced by Puffadder and snouted cobra were characterised by a significant suppression in comparison to control dogs at every time point, from admission to 36 hours post-envenomation. This demonstrates a longer lasting suppression than what has been reported for experimental endotoxin(87). The significant suppression seen in the combined case population was repeated in the puffadder subgroup at every time point, and in the neurological cobra subgroup from 12- to 36 hours post envenomation. The non-neurological cobra subgroup deviated from this pattern, with an early normalisation of serum TT4 concentrations between 12- and 24-hours post-envenomation. In this non-neurological group as a lower severity subgroup, the early recovery from NTIS might indicate that sustained serum TT4 suppression is associated with severity of the underlying condition.

In a dog bite study the onset of serum TT4 concentration suppression was similar to this study with a nadir at 16 hours, but the suppression was sustained - only reaching normal reference interval by 72 hours (92). Due to the limited duration of the current study, the timing of TT4 normalisation could not be evaluated. Another study on diverse mild nonthyroidal illness reported the lowest TT4 concentrations at admission, but in that study the time since onset of disease could not be standardised (88). In experimental babesiosis the first significant decrease compared to controls was only seen at 72 hours post infection, which lasted until 192 hours post infection (95).

Although this infectious disease model seems to have a later nadir in TT4 concentrations, the time taken for parasitaemia to develop after experimental inoculation must be factored in, which complicates comparison to Puffadder and snouted cobra envenomation. In our study, the serum TT4 concentration suppression preceded the first spike in serum CRP concentration, with the TT4 already demonstrating significant suppression in comparison to control dogs at admission, and the CRP only increasing significantly at 12 hours post-envenomation.

A weak significant negative correlation between serum TT4 concentration and systemic inflammation was demonstrated in the current study. Another longitudinal study on parvoviral enteritis found an negative correlation between serum TT4 concentration and the presence of SIRS, regardless of the day of hospitalisation (86). SIRS serves as a less specific, but more sensitive barometer of systemic inflammation, which thus concurs with the current findings. This negative correlation between serum TT4 and CRP concentrations is a significant finding that provides insight into the pathophysiology of NTIS, since the development and progression of NTIS, as demonstrated in this study, is correlated to the degree or severity of host inflammatory response. The relative inflammatory nature of envenomation has been established in murine studies (34). It has also been established in experimental studies that inflammation is a key element in the pathogenesis if NTIS (68, 131-133). Several studies on NTIS have found both inflammatory markers and NTIS to be associated with severity in septic patients (134-136). In human patients with systemic lupus erythematosus the development of NTIS has been associated with inflammatory activation (137). In geriatric human patients with pneumonia serum CRP concentration was found to be significantly higher in patients with NTIS (138). One paediatric study reported a significant negative correlation between free triiodothyronine and CRP (139). Puffadder envenomations are known for a greater degree of inflammatory activation (5, 16, 24). In this study, however, the increase in serum CRP concentration, as well as the decrease in serum TT4 concentration were more pronounced in the neurological cobra subgroup, considered a clinically severe subgroup. Similar patterns are seen in other studies, where sustained suppression of serum TT4 concentration seems to be present in populations with more severe disease. In parvoviral enteritis, serum TT4 concentrations in the survivors started increasing earlier, with non-survivors showing a continued decreasing trend (99).

In addition to the above discussion on the marked suppression of serum TT4 concentration in dogs with Puffadder and snouted cobra envenomation, much is to be said for the TT4-TSH dissociation.

No significant suppression of serum TSH concentration was found in this study, either in comparison to controls or to admission serum concentrations. Although serum TSH concentrations are more likely to remain within normal limits regardless of the severity of disease (83), the underlying pathophysiology remains to be explored in the veterinary field. A recent study of NTIS in parvoviral enteritis demonstrated a similar dissociation, when the association of TSH with SIRS failed in spite of SIRS being associated with serum TT4 concentration (130). NTIS is widely defined as a decrease in free serum thyroid hormones in absence of a corresponding increase in TSH (140, 141). While some studies demonstrated a suppression in TSH after interleukin injection (141-143). It is rather in the recovery of NTIS that TSH plays an instrumental role, where TSH elevation usually precedes serum TT4 concentration elevation (141). It is possible that this study lacks either the size to detect the significance of the increase in neurological subgroup serum TT4 concentration between 12 and 24 hours post envenomation, or that this study's duration was not long enough to detect a recovery from NTIS that takes place later than 36 hours post envenomation. In canine bite wounds, TSH serum concentrations remained within the reference interval throughout the study period until 56 hours post bite, whereafter an increase in TSH, that was significantly higher than admission serum concentrations, was observed (92). It is also possible that Puffadder and snouted cobra envenomation does not cause significant TSH alterations. NTIS is thought to consist of both central and peripheral alterations in the thyroid axis. This often includes peripheral adaptations such as receptor expression, deiodinase activity, hormone binding, and differential changes in tissue concentrations (68, 144, 145).

5.2 Cortisol

Only the neurological cobra subgroup at admission showed significant elevation in serum cortisol concentration at admission when compared to the control populations. The highest serum cortisol concentrations in all groups were observed at admission, with concentrations at consecutive time points being significantly lower than admission in the puffadder and neurological cobra subgroups. Although admission was limited to within 6 hours of envenomation, it is possible that the peak took place within 6 hours, and that this study lacked the temporal sensitivity to detect it. The median total serum cortisol concentration in this study at the highest point was 483 nmol/L in the neurological cobra subgroup, and 264.5 nmol/L in puffadder group. Dogs with bite wounds, experimental babesiosis, and sepsis induced by *Staphylococcus pneumoniae* had peak median serum cortisol concentrations of 314.6 nmol/L, 315 nmol/L, and >400 nmol/L respectively (92, 95, 110). Hypercortisolaemia is a consistent finding during the acute phase of other models of acute critical illness, that is proportional to severity (82, 95, 99, 110, 111, 113-115). The neurological cobra subgroup, with the highest median serum cortisol concentration, also had the highest peak in serum CRP concentration, which might serve as barometer for severity, even in the absence of a significant correlation between CRP and cortisol. Anecdotally, the patient with the highest sustained serum cortisol concentration (579 nmol/L), also had the highest CRP concentration at time point 36 hours (197.3 mg/L), and the lowest TT4 concentration (below the minimum limit of detection). In the experimental babesiosis study, the single mortality also had the highest serum cortisol concentration of 610 nmol/L (95). Although this case is an interesting example, this study had insufficient power to correlate endocrine variables with mortality or systemic inflammation. The HPA response observed in our study was also very acute, in comparison to experimental babesiosis where the peak occurred at 96 hours post infection (95),or *Staphylococcus aureus* pneumonia, where peak concentrations were reached at 24 hours post pulmonary challenge(110). As previously mentioned, the time taken for the development of parasitaemia is a significant confounder, which complicates such comparisons. In the study on dog bite wounds, the highest serum cortisol concentrations were also observed at admission (92). The longitudinal similarity between Puffadder and snouted cobra envenomation and traumatic bite wounds may group Puffadder and snouted cobra envenomation as a traumatic, potentially painful, acute phase response. This unique intersection of elevated yet rapidly normalising total serum

cortisol concentrations in neurological snake-envenomed dogs makes for an interesting endocrine model of peracute critical illness.

It must also be kept in mind that only total serum cortisol concentration was measured, and that alterations in cortisol binding, receptor activity, and metabolism, as demonstrated in human critical illness(104), might go undetected.

In humans, hypopituitarism is a rare yet established complication of snake, especially viperid, envenomations(9). Two neurological snakebite cases in the database this study was based on showed persistent hypocortisolaemia at every time point. Etomidate, an unrelated sedative-hypnotic with its concomitant risk of iatrogenic cortisol and aldosterone suppression (146-148) was not used. General anaesthesia was administered for intubation using propofol, but the other ventilated patients (n=3) had total serum cortisol concentrations that were comparable to the group median of the study population. Both these hypocortisolaemic patients survived to discharge and failed to show overt hypoglycaemia or hypotension. In order to more thoroughly investigate these cases the delta cortisol, and thus exogenous ACTH stimulation, should have been performed to rule out CIRCI(149). If CIRCI was indeed diagnosed in such cases, response to corticosteroid supplementation could have been evaluated. Given the fact that both animals survived and showed a comparable clinical picture to the broader case population, it would have been difficult to evaluate the survival benefit of such supplementation. Since the endocrine parameters were batched and assessed after completion of the study, responsiveness of this case to glucocorticoid supplementation could not be assessed, and further research is warranted to investigate the clinical management of such hypocortisolaemic cases.

5.3 CRP findings

As a positive acute phase protein, the serum CRP concentration changes over time act as indicator of inflammation. Systemic inflammation has been defined in terms of serum CRP concentrations, with a threshold of 35 mg/L(150). Although CRP was not the main aim of the study, the most important finding was that an inverse correlation between serum CRP and TT4 concentrations, elucidating an underlying inflammatory component in the suppression of serum TT4

concentrations seen in critical illness. The differing patterns in serum CRP concentration between puffadder and cobra subgroups also provided insight into their corresponding TT4 oscillations, as discussed above.

5.5 Correlation of variables

A clear, yet weak, negative correlation was observed between serum CRP and TT4 concentrations. This correlates with the group differences seen above, where puffadder envenomations induced a suppression of serum TT4 concentration that outlasted the suppression induced by cobra envenomation. The relative inflammatory nature of envenomation has been established in murine studies (34). Puffadder envenomations, as discussed in the literature review, are known for a greater degree of inflammatory activation(5, 16, 24). This correlation of serum CRP concentration as barometer of systemic inflammation, and TT4 concentrations thus imply an inflammatory component in the development of NTIS. It is established in experimental studies that inflammation is a key element in the pathogenesis if NTIS(68, 131-133). Several studies on NTIS have found both inflammatory markers and NTIS to be associated with severity in septic patients(134-136). In human patients with systemic lupus erythematosus the development of NTIS has been associated with inflammatory activation (137). In geriatric human patients with pneumonia serum CRP concentration was found to be significantly higher in patients with NTIS ($P<0.05$)(138). One paediatric study found a significant negative correlation between free triiodothyronine and CRP serum concentrations (139), and another found a clear correlation of both values with mortality(151). This study thus aligns the findings in dogs with the sparse research available in human NTIS and provides insight into the inflammatory nature of the endocrine changes associated with critical illness.

In the correlation of total serum cortisol and serum TT4 concentrations, the Spearman's rho only reached -0.19($P >0.05$), This is contrasted with another study on dogs with systemic inflammatory response syndrome, where CRP serum concentrations and critical illness related corticosteroid insufficiency were found to be significantly correlated(114). Serum TT4 and TSH concentrations were also not shown to be significantly correlated, with a Spearman's rho of 0.042. although an established inverse correlation between these variables are to be expected in endocrinopathies(152,

153). This highlights the need for larger studies on these correlations, due to the high possibility of type II error. Interpreted in the context of greater total serum cortisol elevation in the puffadder group, it is to be expected that the serum cortisol concentration elevations should have similar correlation patterns to serum TT4 concentration changes.

5.6 Limitations

There were several limitations in the current study, mostly owed to its clinical, non-experimental nature. The population size of the study is thought to be the most significant limitation. Especially in studies where the differences in effect are expected to be small, a larger sample size is necessary to detect it (154). The study was also limited by owner finances and consent that limited the standardisation of the treatment protocol. The control groups of the study were also limited in their health status, as ideally one would not only be able to compare snake envenomed animals to healthy controls, but also to sick controls with an unrelated illness. In that way, the homogeneity of the endocrine response to illness in general could be contrasted with disease-specific changes in Puffadder and snouted cobra envenomation. Control groups were also limited in comparison to more objective and robust reference intervals, but control groups allowed for interpretive statistics to be performed, for example correlations between endocrine concentrations and CRP within the control population.

The age of the data is a significant limitation. However, there are no other studies available on the changes in serum cortisol, TT4 and TSH concentrations in snake envenomation, and the decreased incidence of snake envenomation in recent years, owing to urbanisation, would hinder the emulation of such a dataset. Moreover, Immulite 1000 assay used in this study shows very good correlation with the newer Immulite 2000 chemiluminescent assays. Being a post-facto analysis of prospectively collected data, most constraints of the age of the data could be mitigated. Inclusion criteria and standardised protocols were part of the original study design, which prevented major confounders in endocrine analysis. The minimum limit of detection on the TT4, although lower than more modern assays, still resulted in many patients having serum TT4 concentrations below the lower limit of detection. The second generation TSH assay used is known to lack the sensitivity

required to detect fine oscillations in the HPT during critical illness. In humans, it is recommended that TSH assays use in critical illness should have a detectable limit of 0.01 mU/L (141).

Another significant limitation that is especially relevant in such longitudinal studies is the variable time periods that lapsed between envenomation and presentation. The median interval between envenomation and presentation in the puffadder and cobra group was 1.5 (1-2) and 3 (1-4.5) hours respectively. After admission, all time points were calculated from the time of observed envenomation, and not from admission. This enabled the variance to be limited to the first time point and not repeated at and between time points 12-, 24-, and 36-hours post envenomation.

Missing data was also a limitation present in this study due to its clinical nature. Mortalities earlier in the study resulted in missing data at the later time point, and CRP values were missing for many individuals at the first time point. Statistical methods that are proven robust in clinical settings with missing data was used to minimize the impact of missing datapoints on the dataset in general.

The relatively short duration of the study period limited the longitudinal insight to be gained, since the normalisation of TT4 could not be observed, or the timing thereof compared with other longitudinal studies. A longer study period may have provided more insight into the longitudinal endocrine changes associated with Puffadder and snouted cobra envenomation in dogs.

Chapter 6: Conclusion

Puffadder (*Bitis arietans*) and snouted cobra (*Naja annulifera*) envenomations cause significant endocrine disturbances in dogs. Furthermore, the most severe perturbations in both the HPA and the HPT axes were seen in a subgroup of snouted cobra envenomed dogs showing neurological signs. Significant suppression of serum TT4 concentrations, proportional to serum CRP concentration elevations, were observed in the combined study population at every time point. Non-neurological cobra envenomed dogs reached a milder and earlier TT4 nadir at 12 hours post-envenomation and recovered by 24 hours post-envenomation. A complete lack of significance was seen in TSH changes over time, which is discordant with the more defined serum TT4 concentration pattern observed. Only the neurological cobra subgroup at admission showed a significant hypercortisolaemia when compared to controls.

Clinical implications, although not the main aim of the current study, include the prevalence of isolated hypocortisolaemic dogs envenomed by snouted cobras. Clinicians might consider testing HPA parameters in neurological snouted cobra-envenomed patients to screen for hypocortisolaemia, although the supplementation of corticosteroids in cases of hypocortisolaemia would be controversial. Another definitive clinical implication of this study would be the ubiquitous presence of NTIS in snake envenomation, which should prevent the clinician from making diagnoses of thyroid disorders in these dogs.

Puffadder and snouted cobra envenomation has been characterised as a model of peracute critical illness characterised by distinct suppression in serum TT4 concentration , proportional to the degree of systemic inflammatory activation that could contribute to the broader understanding of endocrine allostasis in critical illness.

Future studies on the endocrine changes associated with snake envenomation would benefit from interventional experimental designs, that would not only allow dynamic testing of HPA status, but also evaluation of response to treatment in hypocortisolaemic animals. Larger studies performed over a longer period would also be able to gain valuable longitudinal insight into the recovery of snake envenomed dogs from the perturbations described in the current study. Similar to findings in humans, snake envenomations make promising models for endocrine research, not only in the

unique manifestations in minority patient groups, but also in the contribution to the broader understanding of critical illness endocrinology.

Bibliography

1. World-Health-Organization(WHO). Snakebite envenoming: a strategy for prevention and control Geneva: World Health Organization; 2019 [Available from: https://apps.who.int/iris/bitstream/handle/10665/324838/9789241515641-eng.pdf?ua=1.

2. Warrell DA. Snake bite. The Lancet. 2010;375(9708):77-88.

3. Kasturiratne A, Wickremasinghe AR, de Silva N, Gunawardena NK, Pathmeswaran A, Premaratna R, et al. The global burden of snakebite: a literature analysis and modelling based on regional estimates of envenoming and deaths. PLoS medicine. 2008;5(11):e218.

4. Eckersley G, Hohn E, Reyers F, Turner G, Wolmarans L. A Comparison between the disease status of a hospitalized dog from a developing community and one from a developed community. Journal of the South African Veterinary Association. 1992;63(1):2-6.

5. Lobetti R, Joubert K. Retrospective study of snake envenomation in 155 dogs from the Onderstepoort area of South Africa. Journal of the South African Veterinary Association. 2004;75(4):169-72.

6. Bolon I, Finat M, Herrera M, Nickerson A, Grace D, Schütte S, et al. Snakebite in domestic animals: First global scoping review. Preventive Veterinary Medicine. 2019;170(1):104729.

7. Silva A, Maduwage K, Sedgwick M, Pilapitiya S, Weerawansa P, Dahanayaka NJ, et al. Neurotoxicity in Russell's viper (Daboia russelii) envenoming in Sri Lanka: A clinical and neurophysiological study. Clinical toxicology. 2016;54(5):411-9.

8. World-Health-Organization(WHO). WHO guidelines for the production, control and regulation of snake antivenom immunoglobulins: World Health Organisation; 2010 [Available from: http://www.who.int/bloodproducts.

9. Bhattacharya S, Krishnamurthy A, Gopalakrishnan M, Kalra S, Kantroo V, Aggarwal S, et al. Endocrine and metabolic manifestations of snakebite envenoming. The American Journal of Tropical Medicine and Hygiene. 2020;103(4):1388.

10. Gutiérrez JM, Calvete JJ, Habib AG, Harrison RA, Williams DJ, Warrell DA. Snakebite envenoming. Nature reviews Disease primers. 2017;3(1):1-21.

11. Ansari MA, Hussain A, Shah NA, Shah SZA, Baloch ZAQ. Snake Bite: Vasculotoxic effects in patients The Professional Medical Journal. 2015;22(03):299-305.

12. Warrell D, Ormerod L, Davidson NM. Bites by puff-adder (Bitis arietans) in Nigeria, and value of antivenom. British Medical Journal. 1975;4(5998):697-700.

13. Leisewitz A, Blaylock R, Kettner F, Goodhead A, Goddard A, Schoeman J. The diagnosis and management of snakebite in dogs-a southern African perspective. Journal of the South African Veterinary Association. 2004;75(1):7-13.

14. Brink S, Steytler J. Effects of puff-adder venom on coagulation, fibrinolysis and platelet aggregation in the baboon. South African Medical Journal. 1973;47(6).

15. Morita T. Structures and functions of snake venom CLPs (C-type lectin-like proteins) with anticoagulant-, procoagulant-, and platelet-modulating activities. Toxicon. 2005;45(8):1099-114.

16. Nagel SS, Schoeman JP, Thompson PN, Wiinberg B, Goddard A. Hemostatic analysis of dogs naturally envenomed by the African puffadder (Bitis arietans) and snouted cobra (Naja annulifera). Journal of Veterinary Emergency and Critical Care. 2014;24(6):662-71.

17. Megale AAA, Portaro FC, Da Silva WD. Bitis arietans snake venom induces an inflammatory response which Is partially dependent on lipid mediators. Toxins. 2020;12(9):594.

18. Aroch I, Yas-Natan E, Kuzi S, Segev G. Haemostatic abnormalities and clinical findings in Vipera palaestinae-envenomed dogs. The Veterinary Journal. 2010;185(2):180-7.

19. Langhorn R, Persson F, Åblad B, Goddard A, Schoeman JP, Willesen JL, et al. Myocardial injury in dogs with snake envenomation and its relation to systemic inflammation. Journal of Veterinary Emergency and Critical Care. 2014;24(2):174-81.

20. Villafañe J, Pedersini P, Bertozzi L, Drago L, Fernandez-Carnero J, Bishop M, et al. Exploring the relationship between chronic pain and cortisol levels in subjects with osteoarthritis: results from a systematic review of the literature. Osteoarthritis and cartilage. 2020;28(5):572-80.

21. Skovira EJ, Behrend EN, Martin LG, Palmer LE, Kemppainen RJ, Lee HP. Effect of laparotomy on the pituitary-adrenal axis in dogs. American Journal of Veterinary Research. 2017;78(8):919-25.

22. Michelsen J, Heller J, Wills F, Noble G. Effect of surgeon experience on postoperative plasma cortisol and C-reactive protein concentrations after ovariohysterectomy in the dog: a randomised trial. Australian Veterinary Journal. 2012;90(12):474a-8.

23. Silva-de-Franca F, Villas-Boas IM, Serrano SMdT, Cogliati B, Chudzinski SAdA, Lopes PH, et al. Naja annulifera Snake: New insights into the venom components and pathogenesis of envenomation. PLoS Neglected Tropical Diseases. 2019;13(1):e0007017.

24. Goddard A, Schoeman JP, Leisewitz AL, Nagel SS, Aroch I. Clinicopathologic abnormalities associated with snake envenomation in domestic animals. Veterinary Clinical Pathology. 2011;40(3):282-92.

25. Takacs Z, Wilhelmsen KC, Sorota S. Snake α-neurotoxin binding site on the Egyptian cobra (Naja haje) nicotinic acetylcholine receptor is conserved. Molecular biology and evolution. 2001;18(9):1800-9.

26. Takacs Z, Wilhelmsen KC, Sorota S. Cobra (Naja spp.) nicotinic acetylcholine receptor exhibits resistance to erabu sea snake (Laticauda semifasciata) short-chain α-neurotoxin. Journal of molecular evolution. 2004;58(1):516-26.

27. Tan N-H, Tan C-S. A comparative study of cobra (Naja) venom enzymes. Comparative Biochemistry and physiology B, Comparative Biochemistry. 1988;90(4):745-50.

28. Mohamed A, Fouad S, Abbas F, Abdel-Aal A, Abdel-Baset A, Hassan A, et al. Metabolic studies of the Egyptian and allied African snake venoms. Toxicon. 1980;18(3):381-3.

29. Karthik S, Phadke KD. Snakebite-induced acute renal failure: A case report and review of the literature. Pediatric Nephrology. 2004;19(1):1053-4.

30. Perera TR, Skerrett-Byrne DA, Gibb Z, Nixon B, Swegen A. The future of biomarkers in veterinary medicine: emerging approaches and associated challenges. Animals. 2022;12(17):2194.

31. Goodsaid FM, Mendrick DL. Translational medicine and the value of biomarker qualification. Science Translational Medicine. 2010;2(47):47ps4.

32. Schoeman JP. The Endocrine Response to critical illness In: Ettinger SJ, Feldman EC, Cote E, editors. Textbook of Veterinary Internal Medicine 8ed: Elsevier health sciences; 2016. p. 560-1.

33. Stone SF, Isbister GK, Shahmy S, Mohamed F, Abeysinghe C, Karunathilake H, et al. Immune response to snake envenoming and treatment with antivenom; complement activation, cytokine production and mast cell degranulation. PLoS neglected tropical diseases. 2013;7(7):e2326.

34. Alsolaiss J, Evans CA, Oluoch GO, Casewell NR, Harrison RA. Profiling the murine acute phase and inflammatory responses to African snake venom: an approach to inform acute snakebite pathology. Toxins. 2022;14(4):229.

35. Vick JA, Shipman WH. Effects of whole bee venom and its fractions (apamin and melittin) on plasma cortisol levels in the dog. Toxicon. 1972;10(4):377-80.

36. Balozet L. Scorpionism in the old world. . In: Bücherl W, Buckley E, editors. Venomous animals and their venoms Venomous intervertebrates. 3. New York: Academic Press; 1971. p. 349-68.

37. Huang H-C. Effects of phospholipases A2 from Vipera russelli snake venom on blood pressure, plasma prostacyclin level and renin activity in rats. Toxicon. 1984;22(2):253-64.

38. Mohamed A, Nawar N, Mohamed FA. Effects of hydrocortisone and polyvalent antivenin on the histological changes in the lung after Naja nigricollis envenomation. Toxicon. 1973;11(2):181-3.

39. Ezzat A, El-Aal AA. Effect of cobra Naja haje venom on the adrenal activity in rabbits. Qatar University Scientific Bulletin. 1989;9(1):169-76.

40. Schechter N, Handy IC, Pezzementi L, Schmidt J. Distribution of α-bungarotoxin binding sites in the central nervous system and peripheral organs of the rat. Toxicon. 1978;16(3):245-51.

41. Edery H, Ishay J, Lass I, Gitter S. Pharmacological activity of oriental hornet (Vespa orientalis) venom. Toxicon. 1972;10(1):13-23.

42. Rothschild A, Rothschild Z. Liberation of pharmacologically active substances by snake venoms. Snake venoms. 1979:591-628.

43. Mohamed A, El-Serougi M, Kamel A. Effects of Walterinessia ageypta venom on blood sodium, potassium and catecholamines, and urine 17-ketosteroids. Toxicon. 1964;2(2):103-7.

44. Nagel SS. Haemostatic function of dogs naturally envenomed by the African puffadder (Bitis arietans) or snouted cobra (Naja annulifera): University of Pretoria; 2012.

45. Christensen MB, Langhorn R, Goddard A, Andreasen EB, Moldal E, Tvarijonaviciute A, et al. Canine serum amyloid A (SAA) measured by automated latex agglutination turbidimetry is useful for routine sensitive and specific detection of systemic inflammation in a general clinical setting. Journal of Veterinary Medical Science. 2013;75(4):459-66.

46. Wolff H. Is it time to denominate hypopituitarism after snake bite? QJM: An International Journal of Medicine. 2013;106(4):390-.

47. Warrell D, Phillips R, Moore R, Burke C. Acute and chronic pituitary failure resembling Sheehan's syndrome following bites by Russell's viper in Burma. The Lancet. 1987;330(8562):763-7.

48. Wolff H. Insuficiência hipofisária anterior por picada de ofídio. Arq Bras Endocrinol Metab. 1958;7:25-47.

49. Burke C. The anterior pituitary, snakebite and Sheehan’s syndrome. Quarterly Journal of Medicine. 1990;75(276):331-3.

50. Eapen C, Chandy N, Kochuvarkey K, Zacharia P, Thomas P, Ipe T. Unusual complication of snake bite: hypopituitarism after viper bites. In: Ohsaka A, Hayashi K, Sawai Y, editors. Animal, Plant, and Microbial Toxins. 2. New York: Plenum Press; 1976. p. 467-73.

51. Golay V, Roychowdhary A, Dasgupta S, Pandey R. Hypopituitarism in patients with vasculotoxic snake bite envenomation related acute kidney injury: a prospective study on the prevalence and outcomes of this complication. Pituitary. 2014;17(1):125-31.

52. Naik BN, Bhalla A, Sharma N, Mokta J, Singh S, Gupta P, et al. Pituitary dysfunction in survivors of Russell's viper snake bite envenomation: A prospective study. Neurology India. 2018;66(5):1351.

53. Warrell DA, Williams DJ. Clinical aspects of snakebite envenoming and its treatment in low-resource settings. The Lancet. 2023;401(10385):1382 - 98.

54. Sheehan HL. Post-partum necrosis of the anterior pituitary. The Journal of Pathology and Bacteriology. 1937;45(1):189-214.

55. Gopalakrishnan M, Vinod KV, Dutta TK, Shaha KK, Sridhar MG, Saurabh S. Exploring circulatory shock and mortality in viper envenomation: a prospective observational study from India. QJM: An International Journal of Medicine. 2018;111(11):799-806.

56. Hart G, Proby C, Dedhia G, Yeo T, Joplin G, Burrin J. Burmese Russell's viper venom causes hormone release from rat pituitary cells in vitro. Journal of endocrinology. 1989;122(2):489-94.

57. Diri H, Karaca Z, Tanriverdi F, Unluhizarci K, Kelestimur F. Sheehan's syndrome: new insights into an old disease. Endocrine. 2016;51:22-31.

58. Francis N, Phillips RE, Warrell DA. Contribution of focal haemorrhage and microvascular fibrin deposition to fatal envenoming by Russell's viper (Vipera russelli siamensis) in Burma. Acta tropica. 1989;46(1):23-38.

59. Bhat S, Mukhopadhyay P, Raychaudhury A, Chowdhury S, Ghosh S. Predictors of hypopituitarism due to vasculotoxic snake bite with acute kidney injury. Pituitary. 2019;22:594-600.

60. Phillips R, Warrell D. Bites by Russell's viper (Vipera russelli siamensis) in Burma: haemostatic, vascular, and renal disturbances and response to treatment. The Lancet. 1985;326(8467):1259-64.

61. Goswami R, Kochupillai N, Crock PA, Jaleel A, Gupta N. Pituitary autoimmunity in patients with Sheehan's syndrome. The Journal of Clinical Endocrinology & Metabolism. 2002;87(9):4137-41.

62. Thein CM, Byard RW. Characteristics and relative numbers of lethal snake bite cases in medicolegal practice in central Myanmar–A five year study. Journal of forensic and legal medicine. 2019;63(1):52-5.

63. Szabo S, Hüttner I, Kovacs K, Horvath E, Szabo D, Horner H. Pathogenesis of experimental adrenal hemorrhagic necrosis (" apoplexy"): ultrastructural, biochemical, neuropharmacologic, and blood coagulation studies with acrylonitrile in the rat. Laboratory Investigation; a Journal of Technical Methods and Pathology. 1980;42(5):533-46.

64. Warrell DA. Snake venoms in science and clinical medicine 1. Russell's viper: biology, venom and treatment of bites. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1989;83(6):732-40.

65. Karki BR, Sedhai YR, Bokhari SRA. Waterhouse-Friderichsen Syndrome. In: La Rosa S, editor. Endocrine Pathology Encyclopedia of Pathology. Cham: Springer International Publishing; 2022. p. 849-50.

66. McIver B, Gorman C. Euthyroid sick syndrome: an overview. Thyroid. 1997;7(1):125-32.

67. Wartofsky L, Burman KD. Alterations in Thyroid Function in Patients with Systemic Illness: The “Euthyroid Sick Syndrome”. Endocrine Reviews. 1982;3(2):164-217.

68. Fliers E, Boelen A. An update on non-thyroidal illness syndrome. Journal of Endocrinological Investigation. 2021;44(8):1597-607.

69. Wenzek C, Boelen A, Westendorf A, Engel D, Moeller L, Führer D. The interplay of thyroid hormones and the immune system – where we stand and why we need to know about it. European Journal of Endocrinology. 2022;186(1):R65-77.

70. Boonen E, Van den Berghe G. Endocrine responses to critical illness: novel insights and therapeutic implications. The Journal of Clinical Endocrinology & Metabolism. 2014;99(5):1569-82.

71. Angelousi AG, Karageorgopoulos DE, Kapaskelis AM, Falagas ME. Association between thyroid function tests at baseline and the outcome of patients with sepsis or septic shock: a systematic review. European Journal of Endocrinology. 2011;164(2):147-55.

72. Slag MF, Morley JE, Elson MK, Crowson TW, Nuttall FQ, Shafer RB. Hypothyroxinemia in critically iii patients as a predictor of high mortality. Journal of the American Medical Association. 1981;245(1):43-5.

73. Plikat K, Langgartner J, Buettner R, Bollheimer LC, Woenckhaus U, Schölmerich J, et al. Frequency and outcome of patients with nonthyroidal illness syndrome in a medical intensive care unit. Metabolism. 2007;56(2):239-44.

74. Rothwell P, Udwadia Z, Lawler P. Thyrotropin concentration predicts outcome in critical illness. Anaesthesia. 1993;48(5):373-6.

75. Ilias I, Stamoulis K, Armaganidis A, Lyberopoulos P, Tzanela M, Orfanos S, et al. Contribution of endocrine parameters in predicting outcome of multiple trauma patients in an intensive care unit. Hormones - Athens. 2007;6(3):218.

76. Memiş D, Gursoy O, Tasdogan M, Süt N, Kurt İ, Türe M, et al. High C-reactive protein and low cholesterol levels are prognostic markers of survival in severe sepsis. Journal of Clinical Anesthesia. 2007;19(3):186-91.

77. MALDONADO LS, MURATA GH, HERSHMAN JM, BRAUNSTEIN GD. Do thyroid function tests independently predict survival in the critically ill? Thyroid. 1992;2(2):119-23.

78. Ray DC, Macduff A, Drummond GB, Wilkinson E, Adams B, Beckett GJ. Endocrine measurements in survivors and non-survivors from critical illness. Intensive care medicine. 2002;28(1):1301-8.

79. Ray D, Drummond G, Wilkinson E, Beckett G. Relationship of admission thyroid function tests to outcome in critical illness. Anaesthesia. 1995;50(12):1022-5.

80. Mebis L, Langouche L, Van den Berghe G. Changes within the thyroid axis during the course of critical illness. In: Van den Berghe G, editor. Acute Endocrinology. Totowa: Humana Press; 2008. p. 199-213.

81. Giunti M, Troia R, Battilani M, Giardino L, Dondi F, Andreani G, et al. Retrospective evaluation of circulating thyroid hormones in critically ill dogs with systemic inflammatory response syndrome. Journal of veterinary science. 2017;18(4):471-7.

82. Yuki M, Aoyama R, Hirano T, Tawada R, Ogawa M, Naitoh E, et al. Investigation of serum cortisol concentration as a potential prognostic marker in hospitalized dogs: a prospective observational study in a primary care animal hospital. BMC veterinary research. 2019;15(1):1-9.

83. Kantrowitz LB, Peterson ME, Melián C, Nichols R. Serum total thyroxine, total triiodothyronine, free thyroxine, and thyrotropin concentrations in dogs with nonthyroidal disease. Journal of the American Veterinary Medical Association. 2001;219(6):765-9.

84. Mooney CT, Shiel RE, Dixon RM. Thyroid hormone abnormalities and outcome in dogs with non-thyroidal illness. Journal of Small Animal Practice. 2008;49(1):11-6.

85. Nishii N, Okada R, Matsuba M, Takashima S, Kobatake Y, Kitagawa H. Risk factors for low plasma thyroxine and high plasma thyroid-stimulating hormone concentrations in dogs with non-thyroidal diseases. Journal of Veterinary Medical Science. 2019;81(8):1097-103.

86. Oikonomidis I, Theodorou K, Papaioannou E, Xenoulis P, Adamama-Moraitou K, Steiner J, et al. Serial measurement of thyroid hormones in hospitalised dogs with canine parvoviral enteritis: Incidence of non-thyroidal illness syndrome and its association with outcome and systemic inflammatory response syndrome. The Veterinary Journal. 2021;274:105715.

87. Panciera DL, Ritchey JW, Ward DL. Endotoxn-induced nonthyroidal illness in dogs. American Journal of Veterinary Research. 2003;64(2):229-34.

88. Bolton TA, Panciera DL, Voudren CD, Crawford-Jennings MI. Thyroid function tests during nonthyroidal illness syndrome and recovery in acutely ill dogs. Journal of Veterinary Internal Medicine. 2024;38(1):111-22.

89. Corsini A, Del Baldo F, Lunetta F, Ribichini S, Giunti M, Fidanzio F, et al. Total thyroxine, triiodothyronine, and thyrotropin concentrations during acute nonthyroidal illness and recovery in dogs. Journal of Veterinary Internal Medicine. 2024;38(3):1345-52.

90. Pashmakova MB, Bishop MA, Steiner JM, Suchodolski JS, Barr JW. Evaluation of serum thyroid hormones in dogs with systemic inflammatory response syndrome or sepsis. Journal of Veterinary Emergency and Critical Care. 2014;24(3):264-71.

91. Schoeman JP, Herrtage ME. Serum thyrotropin, thyroxine and free thyroxine concentrations as predictors of mortality in critically ill puppies with parvovirus infection: a model for human paediatric critical illness? Microbes and infection. 2008;10(2):203-7.

92. van Zyl E, Thompson P, Schoeman J. The time course of lactate and endocrine changes in dogs suffering from dog bite wounds. Journal of the South African Veterinary Association. 2024;95(1):131-6.

93. Elliott DA, King LG, Zerbe CA. Thyroid hormone concentrations in critically III canine intensive care patients. Journal of Veterinary Emergency and Critical Care. 1995;5(1):17-23.

94. Torres SM, Feeney DA, Lekcharoensuk C, Fletcher TF, Clarkson CE, Nash NL, et al. Comparison of colloid, thyroid follicular epithelium, and thyroid hormone concentrations in healthy and severely sick dogs. Journal of the American Veterinary Medical Association. 2003;222(8):1079-85.

95. Van Zyl E, Leisewitz AL, Atkinson BK, Goddard A, Rautenbach Y, Thompson PN, et al. Serial changes in the concentrations of cortisol and thyroid hormones in Beagle dogs infected with Babesia rossi. Ticks and Tick-borne Diseases. 2023;14(2):102-7.

96. Schoeman JP, Rees P, Herrtage ME. Endocrine predictors of mortality in canine babesiosis caused by Babesia canis rossi. Veterinary Parasitology. 2007;148(2):75-82.

97. Jeong Y, Choi S, Ahn J-O, Chung J-Y, Jung H-S. Serum thyroxine and thyrotropin level according to the severity of acute pancreatitis in dogs with hypothyroidism. BMC Veterinary Research. 2024:Preprint.

98. Caldin M, Rocchi P, Zoia A, Botto A, Bertolini G, Zappa G, et al. Thyroid axis and adrenal activity in 28 day survivor and nonsurvivor dogs involved in recent road traffic accidents: A cohort study of 420 dogs. Research in Veterinary Science. 2020;132(1):243-9.

99. Schoeman JP, Goddard A, Herrtage ME. Serum cortisol and thyroxine concentrations as predictors of death in critically ill puppies with parvoviral diarrhea. Journal of the American Veterinary Medical Association. 2007;231(10):1534-9.

100. Hamblin PS, Dyer SA, Mohr VS, Le Grand BA, Lim C-F, Tuxen DV, et al. Relationship between Thyrotropin and Thyroxine Changes during Recovery from Severe Hypothyroxinemia of Critical Illness. The Journal of Clinical Endocrinology & Metabolism. 1986;62(4):717-22.

101. Schoeman JP, editor Metabolic and Endocrine Changes in Critical Illness World Small Animal Veterinary Association World Congress Proceedings; 2014.

102. Heming N, Sivanandamoorthy S, Meng P, Annane D. The Endocrine System in Sepsis. In: Wiersinga WJ, Seymour CW, editors. Handbook of Sepsis. Cham: Springer International Publishing; 2018. p. 61-79.

103. Rothwell PM, Lawler PG. Prediction of outcome in intensive care patients using endocrine parameters. Critical care medicine. 1995;23(1):78-83.

104. Langouche L, Téblick A, Gunst J, Van den Berghe G. The Hypothalamus-Pituitary-Adrenocortical response to critical illness: a concept in need of revision. Endocrine Reviews. 2023;44(6):1096-106.

105. Annane D, Sébille V, Troché G, Raphaël J-C, Gajdos P, Bellissant E. A 3-Level Prognostic Classification in Septic Shock Based on Cortisol Levels and Cortisol Response to Corticotropin. Journal of the American Medical Association. 2000;283(8):1038-45.

106. Bollaert P-E, Fieux F, Charpentier C, Lévy B. Baseline Cortisol Levels, Cortisol Response to Corticotropin, and Prognosis in Late Septic Shock. Shock. 2003;19(1):13-5.

107. Sam S, Corbridge TC, Mokhlesi B, Comellas AP, Molitch ME. Cortisol levels and mortality in severe sepsis. Clinical Endocrinology. 2004;60(1):29-35.

108. Henley D, Lightman S, Carrell R. Cortisol and CBG—Getting cortisol to the right place at the right time. Pharmacology & therapeutics. 2016;166:128-35.

109. Sobolewska J, Dzialach L, Kuca P, Witek P. Critical illness-related corticosteroid insufficiency (CIRCI) - an overview of pathogenesis, clinical presentation and management. Frontiers in Endocrinology. 2024;15(1):01-10.

110. Cortés-Puch I, Hicks CW, Sun J, Solomon SB, Eichacker PQ, Sweeney DA, et al. Hypothalamic-pituitary-adrenal axis in lethal canine Staphylococcus aureus pneumonia. American Journal of Physiology-Endocrinology and Metabolism. 2014;307(11):E994-E1008.

111. Swales H, Batchelor DJ, O'Connell EM. Evaluation of serum cortisol concentration as a prognostic indicator for nonsurvival to hospital discharge in critically ill dogs. Journal of the American Veterinary Medical Association. 2020;256(9):1034-40.

112. Eregowda CG, De UK, Singh M, Prasad H, Akhilesh, Sarma K, et al. Assessment of certain biomarkers for predicting survival in response to treatment in dogs naturally infected with canine parvovirus. Microbial Pathogenesis. 2020;149:104485.

113. Leisewitz AL, Goddard A, Clift S, Thompson PN, De Gier J, Van Engelshoven JM, et al. A clinical and pathological description of 320 cases of naturally acquired Babesia rossi infection in dogs. Veterinary parasitology. 2019;271(1):22-30.

114. Marchetti M, Pierini A, Favilla G, Marchetti V. Critical illness-related corticosteroid insufficiency in dogs with systemic inflammatory response syndrome: A pilot study in 21 dogs. The Veterinary Journal. 2021;273(1):105677.

115. Csöndes J, Fábián I, Szabó B, Máthé Á, Vajdovich P. Assessment of adrenocortical reserve capacity and inflammatory parameters in critically ill dogs. Acta Veterinaria Hungarica. 2017;65(4):475-86.

116. Melby JC, Spink WW. Comparative studies on adrenal cortical function and cortisol metabolism in healthy adults and in patients with shock due to infection. The Journal of clinical investigation. 1958;37(12):1791-8.

117. Resiere D, Mehdaoui H, Neviere R. Inflammation and oxidative stress in snakebite envenomation: a brief descriptive review and clinical implications. Toxins. 2022;14(11):802.

118. Libby P. Inflammatory mechanisms: the molecular basis of inflammation and disease. Nutrition reviews. 2007;65(suppl_3):S140-S6.

119. Bickler PE. Amplification of snake venom toxicity by endogenous signaling pathways. Toxins. 2020;12(2):68.

120. Rucavado A, Nicolau CA, Escalante T, Kim J, Herrera C, Gutiérrez JM, et al. Viperid envenomation wound exudate contributes to increased vascular permeability via a DAMPs/TLR-4 mediated pathway. Toxins. 2016;8(12):349.

121. Cray C, Zaias J, Altman NH. Acute phase response in animals: a review. Comparative medicine. 2009;59(6):517-26.

122. Cuesta JM, Singer M. The stress response and critical illness: a review. Critical care medicine. 2012;40(12):3283-9.

123. Martin LG. Critical illness–related corticosteroid insufficiency in small animals. Veterinary Clinics: Small Animal Practice. 2011;41(4):767-82.

124. Burkitt JM, Haskins SC, Nelson RW, Kass PH. Relative adrenal insufficiency in dogs with sepsis. Journal of veterinary internal medicine. 2007;21(2):226-31.

125. Chippaux J-P, Massougbodji A, Habib AG. The WHO strategy for prevention and control of snakebite envenoming: a sub-Saharan Africa plan. Scielo Brasil; 2019. p. 1678-9199.

126. Goddard A, University of Pretoria. Department of Companion Animal Clinical S, Christopher MMs, Leisewitz ALc-s. A study of the prognostic usefulness of blood leukocyte changes in canine parvoviral enteritis 2006.

127. Singh AK, Jiang Y, White T, Spassova D. Validation of nonradioactive chemiluminescent immunoassay methods for the analysis of thyroxine and cortisol in blood samples obtained from dogs, cats, and horses. Journal of Veterinary Diagnostic Investigation. 1997;9(3):261-8.

128. Kjelgaard-Hansen M, Jensen AL, Kristensen AT. Evaluation of a commercially available human C-reactive protein (CRP) turbidometric immunoassay for determination of canine serum CRP concentration. Veterinary Clinical Pathology. 2003;32(2):81-7.

129. Duricki DA, Soleman S, Moon LD. Analysis of longitudinal data from animals with missing values using SPSS. Nature protocols. 2016;11(6):1112-29.

130. Oikonomidis IL, Theodorou K, Papaioannou E, Xenoulis PG, Adamama-Moraitou KK, Steiner JM, et al. Serial measurement of thyroid hormones in hospitalised dogs with canine parvoviral enteritis: Incidence of non-thyroidal illness syndrome and its association with outcome and systemic inflammatory response syndrome. The Veterinary Journal. 2021;274(1):105715.

131. Boelen A, Platvoet-ter Schiphorst M, Bakker O, Wiersinga W. The role of cytokines in the lipopolysaccharide-induced sick euthyroid syndrome in mice. Journal of endocrinology. 1995;146(3):475-83.

132. Boelen A, Maas M, Lowik C, Platvoet M, Wiersinga W. Induced illness in interleukin-6 (IL-6) knock-out mice: a causal role of IL-6 in the development of the low 3, 5, 3'-triiodothyronine syndrome. Endocrinology. 1996;137(12):5250-4.

133. Stouthard J, Van der Poll T, Endert E, Bakker P, Veenhof C, Sauerwein H, et al. Effects of acute and chronic interleukin-6 administration on thyroid hormone metabolism in humans. The Journal of Clinical Endocrinology & Metabolism. 1994;79(5):1342-6.

134. Mönig H, Arendt T, Meyer M, Kloehn S, Bewig B. Activation of the hypothalamo-pituitary-adrenal axis in response to septic or non-septic diseases–implications for the euthyroid sick syndrome. Intensive care medicine. 1999;25(1):1402-6.

135. Boelen A, Kwakkel J, Fliers E. Beyond low plasma T3: local thyroid hormone metabolism during inflammation and infection. Endocrine reviews. 2011;32(5):670-93.

136. Boelen A, Platvoet-Ter Schiphorst M, Wiersinga W. Association between serum interleukin-6 and serum 3, 5, 3'-triiodothyronine in nonthyroidal illness. The Journal of Clinical Endocrinology & Metabolism. 1993;77(6):1695-9.

137. Meng X, Lv A, Tang M, Liu X, Wang X, Li Y, et al. Non-thyroidal disease syndrome in patients with systemic lupus erythematosus: relation to disease inflammatory activity. Clinical Rheumatology. 2024;43(5):1551-8.

138. Okoye C, Niccolai F, Rogani S, Lemmi B, Peta U, Del Vecchio S, et al. Is non-thyroidal illness syndrome (NTIS) a clinical predictor of COVID-19 mortality in critically ill oldest old patients? Journal of Endocrinological Investigation. 2022;45(9):1689-92.

139. Sharma S, Dabla PK, Kumar S, Dublis S. Thyroid hormone dysfunction and CRP levels in neonates with sepsis. Journal of Endocrinology and Metabolism. 2013;3(3):62-6.

140. Krug N, Bercker S, Busch T, Friese S, Jahn N, Voelker MT. Non-thyroidal Illness Syndrome (NTIS) is no independent predictor for mortality in ICU patients. BMC anesthesiology. 2023;23(1):103.

141. Economidou F, Douka E, Tzanela M, Nanas S, Kotanidou A. Thyroid function during critical illness. Hormones. 2011;10(2):117-24.

142. Torpy D, Tsigos C, Lotsikas A, Defensor R, Chrousos G, Papanicolaou D. Acute and delayed effects of a single-dose injection of interleukin-6 on thyroid function in healthy humans. Metabolism. 1998;47(10):1289-93.

143. Tsigos C, Papanicolaou DA, Defensor R, Mitsiadis CS, Kyrou I, Chrousos GP. Dose effects of recombinant human lnterleukin-6 on pituitary hormone secretion and energy expenditure. Neuroendocrinology. 1997;66(1):54-62.

144. Kaptein EM, Kaptein JS, Chang EI, Egodage PM, Nicoloff JT, MAssry SG. Thyroxine transfer and distribution in critical nonthyroidal illnesses, chronic renal failure, and chronic ethanol abuse. The Journal of Clinical Endocrinology & Metabolism. 1987;65(4):606-16.

145. Mebis L, Van den Berghe G. Thyroid axis function and dysfunction in critical illness. Best Practice & Research Clinical Endocrinology & Metabolism. 2011;25(5):745-57.

146. Keating SC, Sage AM, Ambrisko TD, Somrak A, Carroll MQ, Oba PM, et al. The effect of midazolam or lidocaine administration prior to etomidate induction of anesthesia on heart rate, arterial pressure, intraocular pressure and serum cortisol concentration in healthy dogs. Veterinary Anaesthesia and Analgesia. 2020;47(2):160-7.

147. Qin B, Hu H, Cao B, Zhu Z. Effects of continuous infusion of etomidate at various dose rates on adrenal function in dogs. BMC anesthesiology. 2015;16(2):1-6.

148. Yeung JK, Zed PJ. A review of etomidate for rapid sequence intubation in the emergency department. Canadian Journal of Emergency Medicine. 2002;4(3):194-8.

149. Creedon JMB. Controversies surrounding critical illness-related corticosteroid insufficiency in animals. Journal of Veterinary Emergency and Critical Care. 2015;25(1):107-12.

150. Langhorn R, Oyama M, King L, Machen M, Trafny D, Thawley V, et al. Prognostic importance of myocardial injury in critically ill dogs with systemic inflammation. Journal of veterinary internal medicine. 2013;27(4):895-903.

151. Agarwal A, Khanna A, Nanda S, Sonika P, Kumari A. Correlation of Thyroid Hormones and CRP Level in Neonatal Sepsis. Indian Journal of Public Health Research & Development. 2016;7(3):188.

152. Yu P, Yuan H, Chen H, Li X. Thyroid function spectrum in Cushing's syndrome. BMC Endocrine Disorders. 2024;24(1):80.

153. Bianco AC, Nunes MT, Hell NS, Maciel RM. The role of glucocorticoids in the stress-induced reduction of extrathyroidal 3, 5, 3′-triiodothyronine generation in rats. Endocrinology. 1987;120(3):1033-8.

154. Serdar CC, Cihan M, Yücel D, Serdar MA. Sample size, power and effect size revisited: simplified and practical approaches in pre-clinical, clinical and laboratory studies. Biochemia medica. 2021;31(1):27-53.

Annexures

Annexure A: Ethics Approval

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Research Ethics Committee 10 August 2023

LETTER OF APPROVAL

Ethics Reference No	**REC089-23**
Protocol Title	**Endocrine Allostasis in Snake Envenomation: changes in the Cortisol, Thyroxine and Thyroid Stimulating Hormone levels of dogs envenomed by the snouted cobra and African puffadder**
Principal Investigator	**Dr N Viljoen**
Supervisors	**Prof JP Schoeman**

Dear Dr N Viljoen,

We are pleased to inform you that your submission conforms to the requirements of the Faculty of Veterinary Sciences Research Ethics committee.

Please note the following about your ethics approval:

1. Please use your reference number (REC089-23) on any documents or correspondence with the Research Ethics Committee regarding your research.
2. Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
3. Please note that ethical approval is granted for the duration of the research as stipulated in the original application (for Post graduate studies e.g. Honours studies: 1 year, Masters studies: two years, and PhD studies: three years) and should be extended when the approval period lapses.
4. The digital archiving of data is a requirement of the University of Pretoria. The data should be accessible in the event of an enquiry or further analysis of the data.

Ethics approval is subject to the following:

1. The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.
2. Note: All FVS animal research applications for ethical clearance will be automatically rerouted to the Animal Ethics committee (AEC) once the applications meet the requirements for FVS ethical clearance. As such, all FVS REC applications for ethical clearance related to human health research will be automatically rerouted to the Health Sciences Research Ethics Committee, and all FVS applications involving a questionnaire will be automatically rerouted to the Humanities Research Ethics Committee. Also take note that, should the study involve questionnaires aimed at UP staff or students, permission must also be obtained from the relevant Dean and the UP Survey Committee. Research may not proceed until all approvals are granted.

Recommended for approval

We wish you the best with your research.

Yours sincerely

PROF M. OOSTHUIZEN
Chairperson: Research Ethics Committee

100 YEARS OF VETERINARY EDUCATION

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Annexure B: Client consent forms

Client consent forms, as reported in (44)

I,…… Owner/representative of the owner (please delete where not applicable) of ……(name of animal) a …… old (age) ……. (breed) hereby give permission for information (presenting abnormalities, test results, response to treatment) about my dog to be used in research that will enable veterinarians to better understand and treat snakebites in dogs.

I understand that a volume of blood (9 ml) will be collected from my dog in addition to what would normally be collected from him/her for the usual tests done at the Outpatient's clinic. I give permission for this to be done.

I understand that data about myself (name, suburb of residence) and my dog (name, patient number, age, sex, breed and clinical data) will be stored on a computer database. Personal information that could identify my dog or me will not be divulged to persons uninvolved in the research without my express consent.

Signature:……..
Place:……...
Date:……..

Annexure C: Examination recording sheets

Examination Finding recording sheets, as reported in (44).

Case no: Date:

Patient sticker

Identification of snake:

Method of identification:

Time and date of bite:

Time elapsed between envenomation and presentation:

Site of envenomation:

Number of fang marks:

Description of envenomation site (with emphasis on degree of swelling as mild, moderate or severe, bleeding/oozing, painful):

Temperature:		Arrhythmia present:	
Pulse:		Bleeding evident:	
Respiratory rate + depth:		Loss of swallowing reflex or any evidence of a neurological abnormality (explain):	
Mucous membrane colour: Capillary refill time: <1s 1s 2s >2s			

Patient detail		**Date of collection:**
Name:	Breed:	
Age:	Sex:	
Patient no:		**Trial Number:**
Owner:		
Owner hosp no:		
Tel no:		

Sample	**Collection procedure**	**Tests and Results**			
1. Serum	1x 3ml vacutainer				
	Store remaining sample at -80□C				
2. Citrate	2x 3ml vacutainer	TEG tracing			
		PT			
	TEG tracing performed at 30 min	aPTT			
		D-Dimers			
	All coagulation tests:	Fibrinogen			
	Samples to be batched	Antithrombin			
	Citrated plasma stored at -80 □C				
3. EDTA	1x 3ml vacutainer	Ht		Mono	
		WCC		Eos	
	PCR & RLB – samples to be batched	Nmat		Ret %	
	Whole blood stored at -80□C	Bands		Platelets	
		Lymph			
4. Urine		**Urinalysis**			
	5ml sample via cystocenthesis	pH		Colour	
		SG		Appearance	
	Store remaining sample at -80□C	Proteins		Smell	
		Bilirubin			
		Urobilino			
		Haem/bld			
5. In saline agglut.	Collected at admission	ISA			
6. Faecal float	Collected at admission	Float			

Annexure D: Treatment Protocol

Standardised Treatment Protocol for snake-envenomed dogs, based on a review article by Leisewitz et. al.(13). As described in (44)

Treating cytotoxic envenomation:

Most cases require no treatment, no antivenom administration and recover well. Antibiotics are unnecessary; unless necrosis of tissues has occurred (e.g. spitting cobra bites). Analgesics aren't usually indicated, as pain seems to be minimal in most cases with swelling. Cases showing progressive swelling or deterioration however should be admitted for observation. The following treatment regimens may apply:

1. Start on maintenance rates of crystalloid fluid therapy (Shock rates may be necessary in patients showing hypovolaemic shock).
2. Synthetic colloids can be used (e.g. hetastarch).
3. Whole blood transfusions may be necessary if the haematocrit continues to fall. Decreased haematocrit may be due to haemorrhage at the bite site, or secondary immune-mediated haemolysis with evidence of a +ve ISA and haemoglobinaemia or haemoglobinuria.
4. Intravenous administration of as much antivenom as the owner can afford (as many as 8 vials may be given).
5. If upper airway obstruction occurs due to progressive swelling a tracheostomy tube must be placed.
6. Critically ill dogs should be placed on intravenous broad-spectrum antibiotic cover.

Treating neurotoxic envenomation:

Dogs suspected to have suffered a neurotoxic envenomation should be observed very closely and no treatment given until it is obvious that signs are present:

1. If signs of severe weakness or shallow breathing are noticed, an intravenous general anaesthetic needs to be administered and an ET tube placed. The patient should be

ventilated manually with an AMBU bag or via a closed circuit anaesthetic machine while the ventilator is set up.

2. A mechanical ventilator needs to be set up immediately. Ventilation will be required for at least 6-12hrs while the antivenom is reversing the paralysis. General anaesthesia is maintained for at least 6-12hrs with pentobarbitone or a constant rate infusion of propofol before attempts should be made to wean the dog off the ventilator.
3. Administer as much polyvalent antivenom as the owner can afford slowly intravenously over half an hour.
4. Adequate nursing care of a patient on a ventilator includes regular turning, maintaining fluid balance, ET tube cleaning via suction and good urinary catheter management.
5. The use of prophylactic antibiotics is controversial; however human ventilated patients are very susceptible to developing ventilator-induced pneumonia and are always placed on broad-spectrum antibiotics.
6. The venom of the snouted cobra may cause neurotoxic as well as local cytotoxic effects. These cases therefore need to be managed appropriately and effectively.

Treating haemotoxic envenomation:

The most important treatment is administering appropriate antivenom. A limited number of polyvalent antivenom is kept in the outpatient's pharmacy. Monovalent antivenom is not kept routinely and will need to be obtained directly from the supplier (Edenvale, Johannesburg) via courier. Fresh whole blood or fresh plasma transfusion may also be necessary. Monitor urine output if renal failure is suspected. Thrombolytics are contraindicated (esp. heparin) as venom-induced thrombin is resistant to its action. Indications include:

- Any patient with an active bleed (internal or external).
- With laboratory evidence of significant coagulopathy (prothrombin time and partial thromboplastin time more than double the control).
- Where blood fails to clot in a test tube

Annexure E: Complete Dataset

Complete Dataset used for the current study:

Time	Case Number	CRP (mg/L)	TT4 (nmol/L)	TSH (ng/mL)	Cortisol(nmol/L)
0	1	5.1	25	0.137	193
0	5	13.29	6.4	2.16	262
0	6	5.1	32.7	0.049	267
0	9	5.1	16.7	0.216	153
0	10	5.1	23.8	0.228	315
0	15		6.4	0.11	555
0	16	50.64	22.7	0.066	90.2
0	17	12.75	17.9	0.105	361
0	18	5.1	14.2	0.03	28
0	2	5.1	7.1	0.092	549
0	7		20.8	0.435	30.6
0	8	5.1	15.2	0.332	153
0	11	5.1	16.1	0.062	1380
0	12		22.9	0.065	136
0	13	5.1	25.2	0.049	483
0	14		30	0.156	157

0	20		20.8	0.339	328
0	21		6.8	0.153	169
12	1	64.09	6.4	0.049	102
12	5	5.1	6.4	1.66	249
12	6	89.64	28.2	0.03	49.1
12	9	113.7	21.2	0.167	51.3
12	10	117.65	23	0.155	164
12	15	86.07	6.4	0.064	204
12	16	59.19	15.4	0.092	84.7
12	17	60.59	6.4	0.046	127
12	2	69.43	7.1	0.043	472
12	7	90.71	6.4	0.088	28
12	8	56.53	19.7	0.676	30.9
12	11		6.4	0.069	191
12	12	163.75	11.4	0.148	114
12	13	54.11	6.4	0.03	206
12	14	26.08	25.1	0.05	34.2
12	20	60.09	17.8	0.22	61.2
12	21	65.27	7.39	0.194	117

24	1	57.53	6.4	0.101	193
24	5	5.1	6.4	1.41	568
24	6	57.08	20.2	0.038	136
24	9	54.96	11.9	0.191	59.6
24	10	49.56	12.3	0.104	117
24	15	65.93	6.4	0.104	121
24	16	33.43	17	0.077	42.2
24	17	84	6.4	0.037	197
24	2		6.4	0.039	469
24	7	108.19	6.4	0.273	28
24	8	78.34	20.2	0.333	66.8
24	11	121.86	6.4	0.122	552
24	12	68.38	18.5	0.077	105
24	13	77.53	14.5	0.212	117
24	14	8.56	34.1	0.051	46.9
24	20	53.6	24.6	0.245	72.8
24	21	73.55	11.1	0.151	54.4
36	1	76.54	12.3	0.081	80.3
36	5	5.1	6.4	1.22	134

36	6	58.36	20.1	0.057	153
36	9	55.16	10.3	0.211	85.8
36	10	45.92	12.8	0.171	28
36	15	60.25	6.4	0.126	63.7
36	16	58.17	10	0.059	67.3
36	7	60.96	6.4	0.293	28
36	8	5.1	16.6	0.33	58.2
36	11	197.3	6.4	0.03	579
36	12	100.6	10	0.062	135
36	13	5.1	15.6	0.103	38.4
36	14	8.65	32.8	0.113	131
36	20	5.1	28.4	0.208	372
36	21	61.52	14.9	0.122	162

Control Dataset

Time	Case Number	TT4	TSH	Cortisol
Control	300	17.9	0.458	325
Control	600	31.5	0.123	153
Control	700	18.4	0.245	188

Control	800	25.2	0.318	95.7
Control	900	33.5	0.178	46.9
Control	1400	19.9	0.24	306
Control	1500	28.4	0.116	49.7
Control	1900	22.7	0.138	178
Control	2000	39.5	0.081	57.7
Control	2100	40.4	0.044	25
Control	2200	24.5	0.108	133
Control	2300	19.2	0.318	137