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ORIGINAL RESEARCH

The time course of lactate and endocrine changes in dogs suffering from dog bite wounds

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Background: The stress response after acute trauma in humans and animals has been well-recognised in the literature. However, data on temporal changes in endocrine parameters after acute trauma in previously healthy dogs are scant.

Objective: To longitudinally track endocrine variables and lactate concentrations in dogs with canine bite wounds.

Method: Prospective study involving 20 dogs hospitalised after being bitten by another dog. Serum cortisol, thyroxine (total T4), thyrotropin (TSH), and lactate concentrations were measured on admission and every 8 hours after the recorded bite incident, for a 72-hour period.

Results: Median cortisol concentration was markedly elevated on admission (314.6 nmol/L; IQR 229.3–369.6) but returned to within the reference interval by 16 hours post-bite (99.5 nmol/L; IQR 48.7–225.4) and reached a nadir at 48 hours post-bite (38.5 nmol/L; IQR 32.1–115.9). Median total T4 concentration was within the reference interval on admission (20.6 nmol/L; IQR 12.7–27.9) but decreased below the reference interval by 8 hours (11.0 nmol/L; IQR 5.0–14.1) and reached a nadir 16 hours post-bite (7.0 nmol/L; IQR 2.9–19.7), before gradually increasing after 64 hours to reach the reference range by 72 hours. The median TSH concentrations remained within the reference interval throughout the study period. Median lactate concentration was mildly elevated on admission (3.0 mmol/L; IQR 2.0–3.9).

Conclusion: This study produced novel data on the temporal relationships of the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-thyroidal axes alterations after an acute traumatic insult in dogs.

Keywords: thyroxine, cortisol, hypothalamic-pituitary-adrenal axis, hypothalamic-pituitary-thyroidal axis, longitudinal changes, acute insult

Introduction

Endocrine derangements in canine critical illness have gained interest over recent years due to their prognostic potential across a wide spectrum of disease states. Multiple studies have shown an increase in cortisol concentration (Cortes-Puch et al. 2014; Schoeman & Herrtage 2008a) and a decrease in thyroid hormone concentrations (Schoeman et al. 2007a; Kantrowitz et al. 2001; Pashmakova et al. 2014; Giunti et al. 2017) in critically ill dogs compared to healthy controls. Furthermore, higher cortisol concentration (Schoeman et al. 2007a; Schoeman & Herrtage 2008a; Schoeman et al. 2007b; Leisewitz et al. 2019b; van Zyl et al. 2023; Eregowda et al. 2020; Swales et al. 2020; Yuki et al. 2019) and lower thyroid hormone concentrations (Schoeman et al. 2007a; Kantrowitz et al. 2001; Caldin et al. 2020; Giunti et al. 2017; Schoeman & Herrtage 2008b; van Zyl et al. 2023) have been shown to predict increased severity of disease.

These endocrine changes in canine critical care have largely focused on samples taken on admission and how these results correlate with outcome. There are very few longitudinal studies that have tracked endocrine parameters over time in order to provide further insight into the body's dynamic response to an acute insult (Cortes-Puch 2014; Schoeman et al. 2007a; Schoeman & Herrtage 2008b; van Zyl et al. 2023). Evaluating a condition where the time of insult can be clearly defined, such as a bite wound incident, allows improved interpretation of the time course of endocrine parameters after an acute insult. There is a need to gain insight into the longitudinal changes occurring during canine critical illness to aid the early detection of favourable versus unfavourable endocrine trajectories.

We hypothesised that the cortisol concentrations would increase, and the thyroxine (total T4) concentrations would decrease after a bite wound incident. These parameters would normalise over time as the patient recovered.

Materials and methods

This was a prospective study of 20 dogs treated at a university teaching hospital's small animal intensive care unit, with at least one open bite wound, after being bitten by another dog and whose clinical condition warranted the use of intravenous fluid therapy. The samples were obtained from patients participating in a study where glucose concentrations were evaluated in canine bite wounds (Schoeman et al. 2011). Seventeen healthy dogs admitted to the university for routine vaccination were used as control dogs for cortisol, total T4 and thyrotropin (TSH) concentrations. They were considered healthy on the basis of owner history, a full physical examination, complete blood count (CBC) and serum biochemistry testing.

All owners signed a consent form, allowing inclusion of their dogs into the study. Patients were excluded from the study if *Babesia* parasites were detected on peripheral blood smear. All treatments were individualised based on the discretion of the attending clinician. Since all hormone analyses were performed

post hoc, no interventions were instituted based on the results of the study and no limitations were placed on the medication provided to the dogs. Ethical approval for this study was obtained from the Animal Ethics Committee of the Onderstepoort Faculty of Veterinary Science (protocol number V049/05).

Historical data, including time of the bite incident, known medical conditions, pregnancy status and the administration of any recent medication, were recorded by means of a questionnaire. Physical examinations were performed on admission and the habitus, age, sex, breed, body weight, body condition score, body temperature, pulse and respiration were recorded. The study period extended for 72 hours from the time of the bite incident, or until euthanasia or death within this 72-hour period.

Blood samples in the patient population were collected via the cephalic or jugular vein on admission and every 8 hours after the recorded bite incident, for 72 hours from the initial insult. Blood samples from the control dogs were collected once in the consulting room. Samples for cortisol, TSH and total T4 were collected into serum tubes. The serum samples were allowed to clot and the tubes were centrifuged, aliquoted and frozen thereafter. All serum samples were assayed in a single batch in order to minimise inter-assay variation. Cortisol and total T4 assays were performed in duplicate on a gamma counter with previously validated (Kemppainen & Sartin 1984; Nachreiner & Refsal 1992) radioimmunoassay kits (Coat-a-count assay, Diagnostic Products Corp, Los Angeles, California). Analytical sensitivity was 5.5 nmol/L for cortisol and 2.8 nmol/L for total T4. TSH assays were performed on a previously validated (Marca et al. 2001) immunoradiometric assay with an analytical sensitivity of 0.03 ng/mL (Coat-a-count canine TSH IRMA, Diagnostic Products Corp, Los Angeles, California). Blood L-lactate concentrations were measured immediately after collection using a handheld monitor (Accusport/Accutrend blood lactate analyser, Roche Products, Isando, South Africa) with an analytical sensitivity of 0.8 mmol/L. This monitor has not been validated for use in dogs but has been used in previous studies (Allen & Holm 2008; Karagiannis et al. 2013).

Statistical methods

All data were entered onto a Microsoft Excel® (Microsoft Corporation) spreadsheet and evaluated. Levels at each time point versus admission were compared using linear mixed models of the log-transformed values, applying the Bonferroni adjustment for multiple comparisons. For all tests, significance was set at $p < 0.05$.

Results

Patient characteristics

The study population consisted of 20 dogs with a median age of 31 months (interquartile range [IQR] 12–50) weight of 7.8 kg (IQR 6.0–8.9) and body condition score of 5 (IQR 4–6). Breeds included Jack Russell Terrier ($n = 5$), Boerboel ($n = 3$), Dachshund ($n = 3$), Miniature Pinscher ($n = 1$), German Shepherd ($n = 1$), Pitbull Terrier (n = 1), Sheepdog (n = 1), Maltese (n = 1), Yorkshire Terrier (n = 1) and Husky ($n = 1$). Two dogs were of mixed breed. Nine dogs were intact males, six were intact females, and five were neutered females.

One dog was transferred to an alternative facility after 16 hours due to financial constraints. Two dogs were euthanased within 8 hours of being bitten and one dog was euthanased after 48 hours. All euthanasias were due to a combination of financial constraints and the patients' clinical states. One dog died after 48 hours and two died after the study period (one on day 5 and the other on day 7). Three dogs were admitted 8 hours after the reported bite incident therefore, samples taken at admission were the same as the 8-hour sample. A single dog was admitted 12 hours after the reported bite incident, therefore, a sample was taken at admission but no 8-hour sample was available. Three patients had insufficient samples available to run TSH on admission whilst a single patient had insufficient sample available to run TSH over for the first 32 hours (the first TSH sample was only run at 42 hours post-bite incident). The clinical parameters on admission are recorded in Table I. The serum lactate, cortisol, total T4 and TSH concentrations at each time point are shown in Figures 1A– D.

Lactate

The median lactate concentration was mildly elevated upon admission (3.0 mmol/L; IQR 2.0–3.9) with 60% of the patients having a lactate concentration > 2.5 mmol/L. The median lactate concentration returned within the reference interval by 8 hours post-bite incident (2.4 mmol/L; IQR 1.7–3.2) and reached a nadir at 32 hours (1.3 mmol/L; IQR 1.1–1.78). When compared to admission samples, a significantly lower lactate concentration was seen at 24 hours ($p < 0.001$), 32 hours ($p < 0.001$), 40 hours

Table I: Table showing the clinical parameters on hospital admission

F	Temperature (°C)	Pulse	Respiration	Mucous membrane colour
1	38.6	140	Panting	Pink
2	36.2	104	52	Pink
3	37.7	100	28	Pale
4	38.5	108	36	Pink
5	39.7	120	Panting	Pink
6	38.6	100	48	Pink
7	38.7	160	72	Pink
8	37.1	140	28	Pink
9	38	128	20	Pink
10	37.3	140	40	Pale
11	38.7	120	24	Pink
12	39.4	80	24	Pink
13	38.6	120	Panting	Pink
14	38.2	92	36	Pink
15	39.2	88	24	Pink
16	36.7	144	48	Pale
17	37.7	150	24	Pale
18	37.9	132	30	Pink
19	38.9	160	28	Pink
20	38.6	104	56	Pink

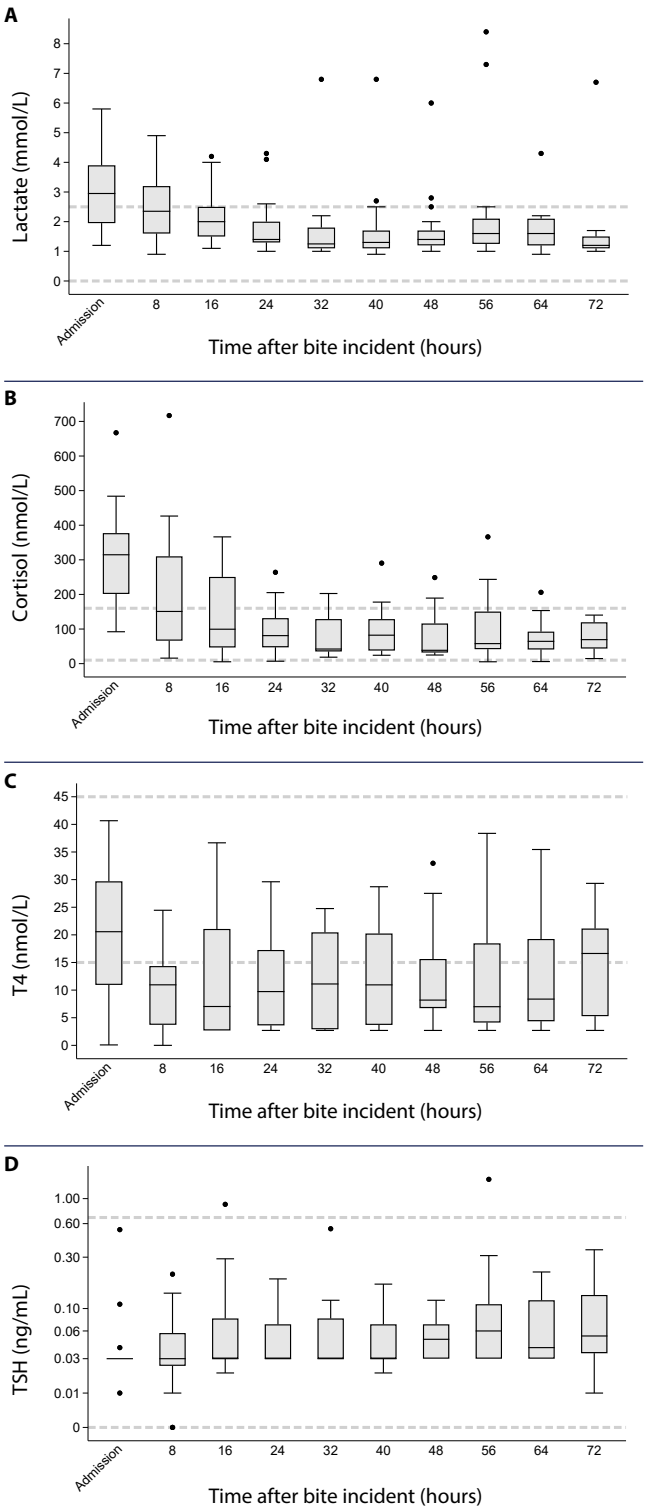

Figure 1: Changes in serum lactate (A), cortisol (B), total T4 (C) and TSH (D) concentrations, with upon admission into hospital and at 8, 16, 24, 32, 40, 48, 56, 64 and 72 hours after a canine bite wound. Boxes indicate IQR with median as horizontal line, whiskers are upper and lower fence values and dots are outliers. Normal reference interval (RI) represented by area between two dashed lines.

($p < 0.001$), 48 hours ($p < 0.001$), 56 hours ($p = 0.003$) and 64 hours ($p < 0.001$) post-bite incident. Median lactate concentration remained within the reference interval (< 2.5 mmol/L) throughout the study period, except for the lactate concentration on admission.

Cortisol

Median cortisol concentration was markedly elevated on admission when compared to control dogs (314.6 nmol/L [IQR 229.3–369.6] vs. 77 nmol/L [IQR 43–94], respectively) with 89% of the patients having a cortisol concentration above the reference interval (10–160 nmol/L). The cortisol concentration decreased within the reference interval by 16 hours post-bite (99.5 nmol/L; IQR 48.7–225.4). The median cortisol concentration remained between 38.5 and 81 nmol/L for the remainder of the study period, with the nadir seen at 48 hours (38.5 nmol/L; IQR 32.1–115.9). When compared to admission samples, a significantly lower cortisol concentration was seen at 8 hours ($p = 0.001$), 16 hours ($p < 0.001$), 24 hours ($p < 0.001$), 32 hours ($p < 0.001$), 40 hours ($p < 0.001$), 48 hours ($p < 0.001$), 56 hours ($p < 0.001$), 64 hours ($p < 0.001$) and 72 hours ($p < 0.001$) post-bite incident.

Thyroid hormones

Median total T4 concentration was within the reference interval (15–45 nmol/L) upon admission and only mildly lower than that of the control dogs (20.6 nmol/L [IQR 12.7–27.9] vs. 35 nmol/L [IQR 30–37 nmol/L], respectively) although 32% of the patients had total T4 concentrations below the reference interval of 15 nmol/L. The median total T4 concentration decreased below the reference interval by 8 hours post-bite incident (11 nmol/L; IQR 5–14.1) with 78% of the patients possessing a total T4 concentration below 15 nmol/L. The median total T4 concentration remained below the reference interval over the subsequent hours with the nadir seen at 16 hours (7 nmol/L; IQR 2.9–19.7). Total T4 concentrations gradually increased and returned to the reference interval at 72 hours post-bite (16.6 nmol/L; IQR 5.3–21.1). Although a decrease in total T4 concentration was seen from admission, these values did not reach significance.

Median TSH concentration remained stable during the initial study period and then started to rise at 40–48 hours (0.05 ng/mL; IQR 0.01–0.07) with a peak in median TSH concentration seen at 56 hrs post-bite incident (0.06 ng/mL; IQR 0.04–0.10) which was significantly higher than the TSH concentration on admission ($p = 0.001$). The median TSH concentrations remained within the reference interval (< 0.68 ng/mL) throughout the study period.

Discussion

This study is the first to illustrate the temporal changes in endocrine parameters in dogs that were hospitalised for canine bite wounds. The median cortisol concentration was markedly elevated on presentation (with a median time since the bite incident of 80 minutes) but decreased to within the reference interval by 16 hours post-bite incident. Similar changes in cortisol concentration have been demonstrated in experimental canine staphylococcal pneumonia (Cortes-Puch et al. 2014), experimental *Babesia rossi* infection (van Zyl et al. 2023) and in puppies that survived natural parvovirus infection (Schoeman et al. 2007a). The peak in median cortisol concentration occurred later in the experimental *Babesia* and experimental pneumonia studies when compared to the current study. It is reasonable to expect that the traumatic event of a dog fight would cause an immediate stress response with an earlier surge in cortisol

concentration compared to experimental infections which create a more insidious onset of systemic inflammation. In parvovirus survivors, an initial peak in cortisol concentration was seen on admission that decreased to reference interval by days 2 and 3. The median cortisol concentration in parvoviral puppies and dogs diagnosed with babesiosis on admission to the same hospital (248 nmol/L [IQR 115–451] and 156 nmol/L [IQR 71–268], respectively) was lower than that seen in the current study (314.6 nmol/L; IQR 229.3–369.6 nmol/L) (Schoeman et al. 2007a; Schoeman & Herrtage 2008a). Moreover, the percentage of patients with parvoviral diarrhoea and canine babesiosis that had serum cortisol concentrations above the reference interval (> 160 nmol/L) on admission was also lower (63% and 42% respectively) than in our study (89%) (Schoeman et al. 2007a; Schoeman et al. 2007b). This may be because sampling commenced closer to the onset of the initial insult as owners may be more adept at identifying a bite wound incident requiring veterinary intervention, whilst the onset of parvoviral and babesiosis infection will invariably go undetected. Earlier sampling may have allowed monitoring closer to the true cortisol peak.

The median total T4 concentration was within the reference interval on admission but decreased below normal within the first 8 hours of the bite incident. The median total T4 concentration remained below normal over the subsequent hours with the nadir in total T4 concentration seen at 16 hours. Total T4 concentration started to rise at 64 hours and returned to the low-normal range at 72 hours post-bite incident; 16 hours after the peak in TSH concentration. Similarly, total T4 concentrations were significantly depressed at 8 and 12 hours after administration of intravenous endotoxin to healthy dogs and gradually started to rise again thereafter (Panciera et al. 2003). These findings were again mirrored in an experimental *Babesia rossi* infection where the median total T4 concentration decreased post-infection with a return to baseline post-treatment. In that study, the dogs that received a higher dose of parasite inoculum developed clinically more severe disease, with a lower total T4 concentration and earlier development of the total T4 nadir at 108 hours post-infection. The dogs that received a lower dose of parasite inoculum showed a milder decline in total T4 concentrations with a slower decline to the total T4 nadir at 144 hours post-infection (van Zyl et al. 2023). In parvovirus-infected puppies, the median total T4 concentration upon admission (35 nmol/L; IQR 30–37 nmol/L) was higher than that seen in our study (20.6 nmol/L; IQR 12.7–27.9 nmol/L) (Schoeman et al. 2007a). Again, this may be because the traumatic event of a dog bite wound may cause more immediate changes in the hypothalamic-pituitary-thyroidal axis than the progressive development of disease seen with parvoviral disease. Alternatively, total T4 concentrations may be higher in puppies infected with parvoviral diarrhoea due to the younger age in this population group (Scott-Moncrieff 2012; Taszkun et al. 2021). When total T4 was monitored in dogs presenting with acute illness, 100% of dogs had total T4 concentrations below the reference interval on admission, which improved to 50% by day 3, and 0% by day 6 (Bolton & Panciera 2023). Only 31% of dogs had a total T4 concentration below the reference interval on admission in our study. This increased to 78% eight hours after the reported bite wound incident and, similar to the Bolton study, decreased to 46% by the third day (72 hours) after the reported bite wound. Part of the inclusion criteria in the Bolton study required patients on admission to all have total T4 concentrations below the reference interval, which could explain the initial differences seen when assessing the total T4 concentrations on admission.

Previous studies have found an inverse relationship between cortisol and total T4 during critical illness (Schoeman et al. 2007a; Schoeman et al. 2007b) with chronic cortisol exposure known to inhibit TSH release by the hypothalamus (Rubello et al. 1992). Pro-inflammatory cytokines such as tumour necrosis factor alpha (TNF-α), interleukin (IL)-1 and IL-6 are believed to alter pituitary and thyroid gland functions, resulting in increased cortisol and decreased total T4 concentrations (Leisewitz et al. 2019a; Karga et al. 2000). In the current study, the reduction in total T4 concentration lagged behind the increased cortisol concentration. It is impossible to determine whether the peak in cortisol concentration truly occurred on admission or whether this peak took place before sampling commenced. Nevertheless, the total T4 nadir occurred at least 16 hours after the peak in cortisol concentration. There are additional studies evaluating the role of cortisol and inflammation in the development of euthyroid sick syndrome (ESS) (Karga et al. 2000; Schoeman et al. 2007a). When pro-inflammatory cytokines and endocrine parameters were measured in healthy individuals undergoing elective abdominal surgery, a rapid increase in cortisol was seen followed by a rapid decline in T3 concentration, without any changes in IL-6 or TNF-α during this period. The authors concluded that the rise in cortisol could provide some explanation for the early decrease in serum T3 concentration seen in ESS (Michalaki et al. 2001).

Median TSH concentrations remained stable during the initial study period, which mirrors the changes in endocrine parameters in patients with acute myocardial infarcts (Karga et al. 2000). Interestingly, serum TSH concentrations also remained stable after administration of intravenous endotoxin to healthy dogs (Panciera et al. 2003) and remained within reference intervals during experimental *Babesia* infection (van Zyl et al. 2023) and dogs presenting with acute illness of varying aetiologies (Bolton & Panciera 2023). In the current study, the median TSH concentration rose at 40 hours and peaked at 56 hours post-bite incident. Twenty-four hours after the peak in TSH, total T4 concentrations also started to rise. This preceding rise in TSH has been consistently described in multiple conditions and suggests that TSH plays a vital role in increasing total T4 concentrations during the recovery phase from critical illness (Karga et al. 2000; Feelders et al. 1999). The median TSH concentration appeared to rise as the median cortisol concentration declined. Some reports have identified an indirect relationship between cortisol and TSH (Rothwell et al. 1993; Re et al. 1976). Pharmacological doses of dexamethasone and physiological concentrations of cortisol both had a suppressive effect on TSH secretion (Re et al. 1976). Furthermore, when patients were injected with IL-6, a rise in cortisol concentration preceded a decline in TSH (Torpy et al. 1998).

There were several limitations to our study. The sample size of 20 dogs was small. No grading system was used to categorise the severity of the bite wounds. It is therefore possible that endocrine kinetics may have differed between dogs that had more severe bite wounds and those that had milder wounds. Attempting to determine the true severity of a canine bite wound can become extremely difficult because bite wounds are notorious for their "tip of the iceberg" effect where mild overlying skin injury may hide severe crushing and avulsion injury.

In order to provide optimum treatment to each patient, limitations were not placed on medications provided to the dogs and these medications were not assessed during the study period. There is evidence to suggest that analgesics such as morphine, ketamine and fentanyl may result in iatrogenic changes in cortisol concentration in people (Franchimont et al. 2002; D'Elia et al. 2003; Hall et al. 1990; Franchi et al. 2007; Broadbear et al. 2004; Pascoe et al. 2008; Švob Štrac et al. 2012; Khalili-Mahani et al. 2015; Esmaeili Mahani et al. 2005; Lesage et al.; 2001; Mellon & Bayer 1998) whilst carprofen and trimethoprim sulpha antibiotics have been shown to alter total T4 concentrations in dogs (Bolton & Panciera 2023). Based on the pain inflicted with bite wound injuries, it was impossible to avoid using these analgesics whilst simultaneously providing adequate pain relief. The study measured total cortisol concentrations and did not differentiate between free and protein-bound cortisol. Critical illness often results in changes in albumin and cortisol-binding globulin concentrations, which may have resulted in an inaccurate estimation of the biologically active cortisol component (Venkatesh et al. 2015; Beishuizen et al. 2001; Cohen et al. 2012; Christ-Crain et al. 2007).

Conclusion

In conclusion, bite wounds produced an initial increase in cortisol concentrations, followed by a reduction in total T4 concentrations that remained below reference intervals between 8 and 64 hours post-bite incident. Median TSH concentrations remained stable during the initial study period and then started to rise at 40 hours and peaked at 56 hours post-bite. Twenty-four hours after the rise in TSH, a gradual rise in total T4 concentration was seen at 64 hours post bite. The initial increased cortisol concentration decreased to reference intervals by 16 hours and reached a nadir by 48 hours after the bite incident. This study produced novel data on the temporal relationships of the alterations in the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-thyroidal axes after canine bite wounds. This can aid future studies by providing expected trajectories in endocrine parameters after an acute insult.

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Conflict of interest

The authors declare they have no conflicts of interest that are directly or indirectly related to the research.

Ethical approval

Ethical approval for this study was obtained from the Animal Ethics Committee of the Onderstepoort Faculty of Veterinary Science (protocol number V049/05).

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