Serum cortisol and thyroxine concentrations as predictors of death in critically ill puppies with parvoviral diarrhea

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Objective—To evaluate the role of adrenal and thyroid hormones in the prediction of death in a population of critically ill puppies with parvoviral diarrhea by measuring serial daily serum concentrations of cortisol and thyroxine.

Design—Prospective case-control study

Animals—57 critically ill puppies with parvoviral diarrhea admitted to the hospital and 17 clinically normal control puppies.

Procedures—Basal serum cortisol and thyroxine concentrations were measured for each dog with parvoviral diarrhea at admission (prior to treatment) and daily until death, euthanasia, or discharge.

Results—Median time between admission and death was 48 hours (ie, on day 3). Median serum cortisol concentration on day 1 (admission) in all dogs with parvoviral diarrhea (248 nmol/L) was significantly higher than in control dogs (77 nmol/L). No significant difference was found in the day 1 median serum cortisol concentration of 11 dogs that died (302 nmol/L) and 46 dogs that survived (238 nmol/L). A significantly higher median serum cortisol concentration was, however, found in nonsurvivor group dogs, compared with survivor group dogs, on days 2 and 3. Median serum thyroxine concentration on day 1 in dogs with parvoviral diarrhea was significantly lower than in control dogs (8.12 nmol/L vs 35 nmol/L, respectively). Median serum thyroxine concentration of nonsurvivor group dogs (4.4 nmol/L) was significantly lower than that of survivor group dogs (9.2 nmol/L) at admission and became even lower on days 2 and 3.

Conclusions and Clinical Relevance—High serum cortisol and low serum thyroxine concentrations at 24 and 48 hours after admission were associated with death in dogs with parvoviral diarrhea. (J Am Vet Med Assoc 2007;231:1534–1539)

The adrenal response to critical illness is essential for survival. Physical trauma varying in severity from general anesthesia and surgery to major trauma and septicemia has been shown to increase cortisol secretion and serum cortisol concentrations.1 Several studies1–10 on critically ill humans have shown a positive correlation between high serum cortisol concentration and mortality. In others, a positive association between serum cortisol concentration and degree of illness was convincingly shown.11,12 However, many other studies failed to show an association between serum cortisol concentration and mortality.13–16 One previous study17 conducted in dogs failed to show any significant difference in serum cortisol concentrations between survivors and nonsurvivors. The dearth of information on serum cortisol concentrations in critically ill dogs has been highlighted in a recent review.18

Critical illness in humans is characterized by multiple and complex alterations in the thyroid axis.19–21

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Abbreviation

IQR Interquartile range

Low concentrations of thyroxine have been shown to indicate prolonged illness and a poor prognosis in human critical care.22–25 Nonthyroidal illness in dogs is a well-known cause of the euthyroid sick syndrome, and the effects of such illness on lowering various thyroid hormone concentrations have been well described.26–29

The use of thyroxine in outcome prediction for dogs has not received much attention in veterinary medicine, and to our knowledge, no reports are found in the veterinary literature demonstrating a significant difference in thyroxine concentrations between nonsurvivor and survivor groups in the care of critically ill dogs.

In a large human study30 on several endocrine predictors, basal cortisol and thyroxine concentrations correlated best with outcome. The aim of the study reported here was to assess the usefulness of serial basal serum cortisol and thyroxine concentrations in the prediction of outcome in puppies with parvoviral diarrhea.

Materials and Methods

Animals—This prospective study was performed on 57 puppies with severe parvoviral diarrhea, admit-
studied consecutively to the high care isolation ward at the Onderstepoort Veterinary Academic Hospital of the University of Pretoria in South Africa between October 2004 and March 2005. Seventeen healthy puppies admitted to the Onderstepoort Veterinary Academic Hospital for routine vaccination were used as control dogs. They were considered healthy on the basis of a full physical examination and routine laboratory testing (ie, CBC determination and serum biochemical analysis). The study was reviewed and approved by the institutional animal use and care committee. All owners signed a written consent form allowing daily blood samples to be obtained from their dogs.

Patients with a diagnosis of parvoviral diarrhea that fulfilled the following criteria were considered eligible for inclusion in the study: their disease condition necessitated admission to the hospital, their body weights were >3 kg (6.6 lb), the diagnosis of parvoviral diarrhea was confirmed by the detection of parvoviral particles in feces by use of electron microscopy, no Babesia parasites or Ehrlichia morulae were detected on capillary and central venous blood smear evaluation, no clinical signs consistent with canine distemper such as conjunctival or nasal discharge or neurologic signs were present, and no distemper viral particles were observed on electron microscopic examination of feces.

To determine if a history of previous administration of corticosteroids to dogs or known malignancies that might have involved the adrenal glands was an exclusion criterion for the study. Dogs were treated according to a standard hospital protocol comprising rehydration by IV administration of crystalloid fluid or colloid fluid (when in severe shock or when severely hypoproteinemic) and IV administration of amoxicillin with gentamicin added once patients were rehydrated. Buprenorphine was administered as necessary for abdominal pain. Fluids for IV administration were spiked with potassium chloride or 50% dextrose when indicated. Metoclopramide or ondansetron was administered to control vomiting. Dogs were also started on enteral feeding soon after rehydration.

**Study design**—Blood samples in the patient population were collected prior to treatment on admission (day 1) and daily thereafter between 8:00 AM and 11:00 AM, until death, euthanasia, or hospital discharge. Day 2 blood sample collection took place at approximately 20 to 24 hours after that of day 1 and at 24 hour intervals thereafter. Blood samples from control dogs were collected once in the consulting room after they had waited a similar period as the study dogs in the reception area of the same hospital. Blood was collected from the jugular vein by needle venipuncture in all dogs and placed into plastic tubes for serum collection. Blood samples were allowed to clot and tubes were centrifuged within 1 hour of collection. The serum was harvested and stored at −80°C until analyzed.

**Assays**—All samples from the patient population were assayed in a single batch; samples from control dogs were assayed in a second batch. Serum cortisol and thyroxine concentrations were determined in duplicate on a gamma counter with a previously validated radioimmunoassay kit. Sensitivities of the cortisol and thyroxine assays were 5.5 nmol/L and 2.8 nmol/L, respectively. For statistical analysis, values <5.4 nmol/L and 2.7 nmol/L, respectively, were considered below the limit of detection.

**Data analysis**—The usefulness of high serum cortisol concentration (ie, >224 mmol/L) as a predictor of death in puppies with parvovirus diarrhea was determined. Sensitivity, specificity, positive predictive values, and negative predictive values for high serum cortisol concentrations on day 3 (approx 48 hours after admission) were calculated by use of the following equations:

\[
\text{Sensitivity} = \frac{TP}{(TP + FN)}
\]

\[
\text{Specificity} = \frac{TN}{(FP + TN)}
\]

\[
\text{Positive predictive value} = \frac{TP}{(TP + FP)}
\]

\[
\text{Negative predictive value} = \frac{TN}{(FN + TN)}
\]

where TP, FN, TN, and FP are the number of true-positive results, false-negative results, true-negative results, and false-positive results, respectively.

Data were tested for normality by the Kolmogorov-Smirnov test. Within-group serial daily differences were measured by use of the Friedman test for related samples. Differences in median serum cortisol and thyroxine concentrations between nonsurvivor and survivor groups were analyzed by use of the Mann-Whitney U test for nonparametric data. Comparisons between nonsurvivor and survivor groups were only made up to day 3, after which only 2 dogs in the nonsurvivor group remained, precluding any further reliable comparisons between the outcome groups. Correlation between variables was assessed by the Spearman rank correlation coefficient (r_s). For all comparisons and correlations, values of P < 0.05 were considered significant. Values are reported as median and range or IQR, as indicated. Statistical analysis was performed on a personal computer by use of a commercial software package.

**Results**

**Patient population**—Fifty-seven dogs with parvoviral diarrhea were included in the study. Eleven dogs were in the nonsurvivor group and 46 in the survivor group. Of nonsurvivor group dogs, 2 died on admission (day 1), and another dog died on day 2. Six dogs died during day 3, and of the remaining 2 dogs, 1 was euthanatized on day 4 and the other on day 6. Euthaniasias were performed because of a poor prognosis; these dogs were included for analysis in the nonsurvivor group. Of the 11 dogs that died, 10 were male and 1 was female. Overall, 38 of 57 (67%) dogs with parvoviral diarrhea were male and 19 (33%) were female. Median patient age was 3.5 months (range, 2 to 12 months). Median body weight was 5.7 kg (12.6 lb) with a range of 3 to 19 kg (6.6 to 41.9 lb). Median time between hospital admission and death was 48 hours (range, 2 to 120 hours). Median length of hospital stay for all dogs was 4 days (range, 1 to 13 days). No patient received adrenal suppressive agents such as etomidate or corticosteroids at any time during the study period. Severe dehydration resulted in scant serum harvest, and as such serum cortisol and thyroxine concentrations were not determined for 3 dogs (2 dogs in the survivor group and 1
dog in the nonsurvivor group) on day 1 and for 1 dog (nonsurvivor group) on day 2.

Control population—Healthy dogs consisted of 10 sexually intact males and 7 sexually intact females. Median age was 3 months (range, 2 to 13 months). Median body weight was 7 kg (15.4 lb) with a range of 2 to 24 kg (4.4 to 52.9 lb).

Serum cortisol concentrations on admission—Median serum cortisol concentrations on admission (day 1) in all dogs with parvoviral diarrhea was significantly higher than in control dogs (248 nmol/L [IQR, 115 to 451 nmol/L] vs 77 nmol/L [IQR, 43 to 94 nmol/L], respectively). Although the median serum cortisol concentration was higher in nonsurvivor group dogs (302 nmol/L [IQR, 106 to 392 nmol/L]) than in survivor group dogs (238 nmol/L [IQR, 119 to 384 nmol/L]), the difference was not significant (P = 0.361; Figure 1). Of the 54 serum cortisol concentrations determined for dogs with parvoviral diarrhea at the time of admission, 34 (63%) were greater than the established reference range limit for dogs (ie, > 160 nmol/L).31

Subsequent daily serum cortisol concentrations—On days 2 and 3, the proportions of patients with serum cortisol concentrations of > 160 nmol/L were 28% and 17%, respectively. On day 2, the remaining 9 nonsurvivor group dogs had significantly (P < 0.001) higher median serum cortisol concentrations (266 nmol/L [IQR, 202 to 365 nmol/L]) than survivor group dogs (n = 45; 96 nmol/L [IQR, 43 to 127 nmol/L; Figure 1]). Median serum cortisol concentrations were significantly (P < 0.001) different on day 3 between 8 nonsurvivor group dogs and 46 survivor group dogs (279 nmol/L [IQR, 209 to 529 nmol/L] vs 62 nmol/L [IQR, 33 to 118 nmol/L], respectively). Six of 8 nonsurvivor group dogs died later on day 3. Median serum cortisol concentrations remained high in the nonsurvivor group and did not change significantly (P = 0.867) in this group from days 1 to 3, whereas the survivor group had a significant (P < 0.001) decrease in median serum cortisol concentrations from days 1 to 3.

None of the surviving dogs had a cortisol concentration of > 224 nmol/L at 48 hours after admission (day 3), whereas 6 of 8 nonsurvivor group dogs had serum cortisol concentrations above this cutoff point at this juncture. These data indicate that a cortisol concentration of > 224 nmol/L at 48 hours after admission in a dog with parvoviral diarrhea has a sensitivity of 75%, specificity of 100%, positive predictive value for nonsurvival of 1 (ie, 100% certainty that dogs with cortisol concentrations > 224 nmol/L will die), and negative predictive value of 0.96 (ie, 96% certainty that dogs with cortisol concentrations of ≤ 224 nmol/L will not die).

Serum thyroxine concentrations on admission—Median serum thyroxine concentrations on admission (day 1) in all dogs with parvoviral diarrhea was significantly lower than in control dogs (8.12 nmol/L [IQR, 3.07 to 14.7 nmol/L] vs 35 nmol/L [IQR, 30 to 37 nmol/L], respectively; Figure 2). Median serum thyroxine concentrations on admission of dogs with parvoviral diarrhea were significantly (P = 0.037) lower in nonsurvivor group dogs (4.41 nmol/L [IQR, 2.7 to

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**Figure 1**—Box plots of the daily serum cortisol concentrations in 57 puppies with parvoviral diarrhea from days 1 to 3 of hospitalization and 17 control puppies on day 1 only. For each box plot, T-bars represent the main body of data, which is equal to the range in most instances (circles indicate outliers). The box represents the IQR, and the horizontal bar within the box is the median. Eleven dogs died or were euthanatized as a result of poor prognosis, and 46 dogs survived. *Significantly (P < 0.05) higher than survivor group dogs on days 2 and 3.

**Figure 2**—Box plots of the daily serum thyroxine (T4) concentrations in 57 puppies with parvoviral diarrhea from days 1 to 3 of hospitalization and 17 control puppies on day 1 only. For each box plot, T-bars represent the main body of data, which is equal to the range in most instances (circles indicate outliers). The box represents the IQR, and the horizontal bar within the box is the median. Eleven dogs died or were euthanatized as a result of poor prognosis, and 46 dogs survived. *Significantly (P < 0.05) lower than control group dogs on day 1. #Significantly (P < 0.05) lower than survivor group dogs on days 2 and 3.
8.73 nmol/L) than in survivor group dogs (9.2 nmol/L [IQR, 4 to 15.8 nmol/L]).

Subsequent daily serum thyroxine concentrations—On day 2, the remaining 9 nonsurvivor group dogs had significantly (P < 0.001) lower median serum thyroxine concentrations (2.7 nmol/L [IQR, 2.7 to 2.7 nmol/L]) than 45 survivor group dogs (10.5 nmol/L [IQR, 4.96 to 17.05 nmol/L]; Figure 2). A significant difference in serum thyroxine concentration was also apparent on day 3 between 8 nonsurvivor group dogs (2.7 nmol/L [IQR, 2.7 to 2.74 nmol/L]) and 46 survivor group dogs (10.78 nmol/L [IQR, 5.28 to 17.39 nmol/L]). Serum thyroxine concentrations in survivor group dogs had a moderate but significant (P = 0.042) increase from days 1 to 3. The nonsurvivor group, however, had a significant (P = 0.009) decrease in serum thyroxine concentrations from days 1 to 3; these values especially decreased from days 1 to 2.

Serum cortisol versus serum thyroxine concentrations—Daily serum cortisol concentrations correlated negatively with daily serum thyroxine concentrations throughout the study period. Correlation coefficients for day 1, 2, and 3 were r = -0.673 (P < 0.001), r = -0.549 (P < 0.001), and r = -0.628 (P < 0.001), respectively.

Discussion

In this study, basal serum cortisol concentrations were increased above the normal reference range during the acute stage of infection (ie, hospital admission) in 63% of puppies with parvoviral diarrhea. Dogs with more severe illness leading to death had significantly higher median serum cortisol concentrations on days 2 and 3 of hospitalization than dogs that survived. Results of this study indicate that a dog with parvoviral diarrhea has a worse prognosis if its serum cortisol concentrations fail to normalize. An important finding in this study was the striking decrease in median serum cortisol concentrations in survivor group dogs from day 2 onwards, when serum cortisol concentration became indistinguishable from that of control dogs.

Median basal serum thyroxine concentration was lower in nonsurvivor group dogs than survivor group dogs on admission (day 1) and decreased further, whereas the median concentrations in dogs that survived showed a marginal increase. As expected, an inverse correlation was found between basal serum cortisol and thyroxine concentrations during critical illness. Although the current radioimmunoassay for canine thyroxine is unable to read thyroxine values with accuracy at values < 2.8 nmol/L, which is the lower end of the linear portion of the graph, it is worth mentioning that all nonsurvivor group dogs had serum thyroxine concentrations below this limit of detection at 24 hours after admission (day 2). These readings, which were all adjusted to 2.7 nmol/L for statistical purposes, were indeed low, and this upward adjustment thus belied the true P value for the difference between the survivor and nonsurvivor groups.

Our data, although concuring with findings of many human studies,3–10,22–25 seem to be at variance with a previous veterinary study17 in which no correlation was found between basal serum cortisol and death. That study involved only 20 patients. These patients had disparate illnesses, consisting of acute and chronic conditions. The different neuroendocrine paradigms of acute and chronic illnesses have been highlighted for critically ill humans.32 The serial nature of the sample collections in our study supported this important concept, vindicating the difference between acute and chronic illness, as far as both serum cortisol and thyroxine concentrations are concerned. The dramatic decrease in serum cortisol concentrations in the survivor group, occurring beyond the day of admission, on the one hand indicates the acute nature of the adrenocortical response and on the other demonstrates its near normal adjustment in more chronic illness. It is thus not entirely unexpected that serum cortisol concentrations would have failed to predict death in nonacute illness. Finally, the mean hospital stay in the former study was only 2 days, and only 37% had basal serum cortisol concentrations above the range regarded as normal for healthy animals, which, in retrospect, confirms the more chronic nature of their illnesses.17 The mean hospital stay of dogs with parvoviral diarrhea in our study was 4.7 days, all patients were admitted with the same acute illness, and 63% had admission basal serum cortisol concentrations above 160 nmol/L.

The study reported here documented the range of admission basal serum cortisol concentrations in critically ill dogs with parvoviral diarrhea as 19 to 825 nmol/L and the median as 248 nmol/L (IQR, 113 to 451 nmol/L) and helps to answer the question of an appropriate serum cortisol concentration range in acute critical illness in dogs, as posed in a recent review.33 Low serum cortisol concentrations in many of these sick dogs with parvoviral diarrhea could potentially be explained by their antecedent anorexia, as cortisol concentrations have been shown to decrease in puppies after 24 to 36 hours of fasting.33 It is noteworthy that serum cortisol concentrations in control dogs ranged from 15 to 226 nmol/L, with an IQR of 43 to 94 nmol/L. Only 2 control dogs had values > 160 nmol/L, which is surprising considering the excitement and stress associated with a dog’s visit to a veterinary hospital. It was not the aim of this study to establish a reference range for this age group of puppies, but their serum cortisol concentrations are probably not different than the reference range of 10 to 160 nmol/L established for mature dogs.

It is well known that thyroxine concentrations are suppressed by critical illness in general and high corticosteroid concentrations in particular.34,35 The fact that the basal thyroxine concentrations were already lower in nonsurvivor group dogs than in survivor group dogs on day 1 (while the respective cortisol concentrations of the groups were similar) seems to indicate that the pituitary-thyroid axis is suppressed by factors other than the corticosteroid stress response. It is known in humans that cytokines such as interleukin-6 exert a stimulatory role in the hypothalamo-pituitary-thyroid axis, whereas interleukin-6 has an inhibitory effect on the hypothalamo-pituitary-adrenal axis.36 Results of our study, when seen in combination with a recently performed pilot study3 showing a positive correlation of interleukin-6 with death in dogs ad-

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mitted with systemic inflammatory response syndrome, may lend credence to this cytokine as being instrumental in the endocrine perturbations seen in our dogs.

The relatively small number of dogs in our patient population precludes generalizations on the sex ratio in parvoviral diarrhea or the predisposition of males to die, as was ostensibly shown in this group of dogs. This aspect would need larger populations to verify its importance, although it is well known in human critical care medicine that male babies have a higher mortality rate than female babies.37–39

One might also speculate that the initial stress of hospitalization could have been responsible for the high basal serum cortisol concentrations on day 1. However, control dogs, with blood samples collected at the same hospital and after waiting similar periods in the same waiting room, did not bear this assertion out. Furthermore, even the less severely ill surviving dogs had markedly higher basal serum cortisol and lower thyroxine concentrations than control dogs, which would seem to vindicate their acute illness as a cause of these perturbations. The clinical nature of this study meant that the researchers had no control over when, in the course of its disease process, a dog with parvoviral diarrhea was admitted for treatment. This, as well as the differing degree of illness, the relatively small sample size, and other factors such as patient genotype, would partly explain the lack of uniformity in basal serum hormone concentrations seen in this study.

Survival depends on the maintenance of homeostasis. By measuring loss of homeostasis, physiologic and endocrine scoring systems predict survival with high sensitivity. Prediction of death, however, is less sensitive because the causes of death in patients in the intensive care unit (eg, pulmonary edema, gastrointestinal hemorrhage, shock, and dehydration), although induced by illness, may not be closely related to illness severity, as death depends not only on physiologic developments but also on our management of patients.9 This lack of sensitivity of basal serum cortisol concentrations in the prediction of death is illustrated in our study by the 2 dogs that died, although their cortisol concentrations were < 224 nmol/L 48 hours after admission.

As a model of critical illness, there appears to be a close association between the degree of cortisol and thyroxine perturbations and death in dogs with parvovirus diarrhea, confirming the predictive capacity of basal serum cortisol and thyroxine concentrations. This would not necessarily be expected to be the same in studies in which combinations of acute and chronically ill animals with disparate illnesses are used. We advise caution in overinterpreting the clinical importance of findings of our study because the data generated refer to a particular critical illness (ie, parvovirus diarrhea) at a particular institution. The study reported here does, however, provide insight into the endocrine perturbations in critical illness.

References


### New Veterinary Biologic Products

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<th>Species and indications for use</th>
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<td>For the detection of antibodies to <em>Mycobacterium tuberculosis</em> and <em>M. bovis</em> in elephant serum, plasma, and whole blood</td>
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