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# Serum cortisol concentrations in horses with colic

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ABSTRACT

Few studies have evaluated cortisol concentrations in horses with colic. In humans with septic shock, high cortisol levels are associated with an increased risk of death. The objectives of this study were to compare the serum total cortisol concentrations (STCCs) in horses with colic to those without colic, and to assess whether the STCC relates to the pathological nature or outcome of the disease.

STCCs were determined at presentation in horses with colic and in systemically healthy 'control' horses. Horses with colic were grouped based on clinical and clinico-pathological parameters at admission, treatment, lesion type and location, and outcome. Univariable and multivariable logistic regression were performed using two different outcome measures: (a) whether the horse had colic or not (yes vs. no), and (b) horse STCC (≥200 nmol/L vs. <200 nmol/L).

Horses were more likely to have colic if they presented with high STCCs (≥200 nmol/L compared with <200 nmol/L). Horses with colic and with STCCs ≥200nmol/L were more likely to have moderate or severe colic signs (compared with mild colic) and heart rates >45 beats per min (compared with ≤45 beats per min). It was concluded that colic in horses is associated with elevated STCCs, and increased STCC in horses with colic appears to relate to the severity of the disease. STCCs may provide additional decision-making and prognostic information in horses with colic but further studies are required to avoid misinterpretations associated with the wide variation in STCCs.



## Introduction

Colic in horses is associated with varying degrees of pain, and can induce endotoxaemia and cardiovascular abnormalities, all of which result in significant physiological stress. This produces stimulation of the hypothalamic-pituitary-adrenal (HPA) axis, resulting in cortisol release from the adrenal gland (Harbuz and Lightman, 1992). A limited number of studies have evaluated serum cortisol concentrations in horses with colic (Hoffsis and Murdick, 1970; Hodson et al., 1986; Santschi et al., 1991; Hinchcliff et al., 2005; Edner et al., 2007; Ayala et al., 2012). The existing studies examined serum cortisol concentrations in small numbers of horses (maximum 43) with varying colic types (Ayala et al., 2012). In one study of 35 horses with colic, the eight non-survivors had significantly higher serum total cortisol concentrations (STCCs) compared with the 27 survivors (Hinchcliff et al., 2005). In humans with septic shock, high basal cortisol concentrations have been described (Jones and Ramano, 1984), and are associated with an increased risk of death (Melby and Spink, 1958; Rothwell and Lawler, 1995; Annane et al., 2000).

The aims of the present study were to determine the STCCs in horses with different types of colic compared with a control population, and to assess whether STCCs were associated with the pathological nature of the disease or outcome. We hypothesised that STCCs increase in horses with colic and will be higher in horses requiring surgery than those requiring medical treatment. We also hypothesised that STCCs will be higher in horses with strangulating lesions than those with non-strangulating lesions and non-survivors compared with survivors.

### Materials and methods

#### Study design

A prospective observational study was performed. All horses and ponies presenting to Bell Equine Veterinary Clinic (BEVC) between January 2005 and June 2008 with acute colic (<36 h duration) were eligible for inclusion in the study as 'cases'. All systemically healthy horses and ponies transported to the clinic between January and June 2008 for elective magnetic resonance imaging for the evaluation of mild fore foot lameness were eligible for inclusion as 'controls'. 'Case' and 'control' horses were included if the following criteria were fulfilled: (1) serum was collected within 20 min of admission (venepuncture and serum collection were performed for reasons unrelated to the study and surplus serum was available for analysis); (2) client permission for inclusion in the study was obtained; (3) the STCC was determined by radioimmunoassay validated for use in horses (Dechra Specialist Laboratories); and (4) for cases, the horse's outcome was known and was not affected by financial constraints (i.e. decisions concerning treatment and/or euthanasia during the horse's hospitalisation were not determined by economic factors). The 'control' population was used for investigation of factors associated with colic (outcome [a]).

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*Data collection*

Data recorded prospectively by the admitting clinician included the date of admission, age, breed, sex, pregnancy status and distance travelled (≤32 km or >32 km). A blood sample from the jugular vein was collected in an evacuated glass tube without anticoagulant prior to administration of any medications at the clinic. The blood was allowed to clot, and then centrifuged at 1500 *g* for 10 min before removal of the serum. Serum was refrigerated and submitted to arrive within 24 h of collection at the Dechra Specialist Laboratories for measurement of STCC.

For horses with colic, the duration of colic prior to admission (h), heart rate and packed cell volume (PCV) at admission were recorded. We assessed the maximum grade of pain as follows: mild – intermittent pawing, flank watching and flehmen response; moderate – as for mild but with an increased intensity and additionally intermittently lying down; severe – as for moderate but with frequent lying down and rolling (Patipa et al., 2012).

Horses with colic were classified into those that received medical or surgical management and those that were euthanased at or shortly after admission. All horses with colic were categorised based on the lesion location and type. The outcome for all horses (survived to discharge vs. died) was recorded. Some horses that died were euthanased on humane grounds or because of a grave prognosis.

Two horses contributed two observations in the study. One horse contributed two measurements as a 'case' as it was admitted to the clinic on two occasions for colic (the first admission was a surgical colic and the second admission 9 months later was a medical colic); these were considered to be unrelated. A second horse was included once as a case (the first admission was a medical colic) and once as a control (the second admission was a healthy horse for MR imaging).

*Analysis*

The statistical analyses were performed using Stata/SE 10.1 for Windows (StataCorp LP, 2010). All continuous data (STCC, PCV, heart rate, duration of colic and age) were examined graphically to determine whether they were linear in logit and summary statistics described in Table 1. Data that did not satisfy this assumption were further categorised into polytomous or binary categorical variables.

Univariable and multivariable logistic regression were performed (Hosmer and Lemeshow, 2000). All *P*-values reported in boldface in the univariable and multivariable tables were based on likelihood ratio (LR) tests. All reported *P*-values (not in boldface) that corresponded to individual levels of categorical variables were based on Wald tests. A *P*-value of <0.05 denoted statistical significance.

Two models were considered. In model (a), colic (yes or no) was the outcome. Variables for horses with and without colic were considered. These variables included: age, breed, sex, distance travelled and STCC. For model (b), cortisol concentration was modelled initially as a continuous variable, and subsequently as a binary outcome using a threshold cut-off point of 200 nmol/L, i.e. greater than the upper end of the normal laboratory value (155 nmol/L). The optimum cortisol cut-point for distinguishing between animals with colic and no colic (Dohoo et al., 2010) was based on a receiver operating characteristic (ROC) curve and cut-point specific likelihood ratios (LR+ and LR–). The ROC curve plots sensitivity vs. false positive rate (1-Specificity) of the 'test' (i.e. the variable 'cortisol') at a number of different cut-points. The LR+ is the probability of a positive test result from a horse with colic relative to a horse without colic. The LR– is the probability of a negative result from a horse with colic relative to a horse without colic (Dohoo et al., 2010). This model only included horses admitted to the clinic with colic and considered horse-level variables such as heart rate, sex, pregnancy status, breed, PCV, duration of colic, severity of colic signs, distance travelled, location of the lesion, type of lesion, treatment at admission and outcome.

Both multivariable models were built using a forward selection process using variables that were significant at the univariable level ($P < 0.20$). After reaching a preliminary main-effects model, the significance of all previously excluded variables was re-evaluated by adding each one back to the model, and all possible 2-way interactions between significant main-effects were considered. Several variables were considered a priori to be confounders and as such, were forced into the final model regardless of whether they were statistically significant. These included: horse age, sex, breed, pregnancy status, distance travelled and duration of colic.

A measure of the fit of the final multivariable model was assessed using the Hosmer–Lemeshow goodness-of-fit test. The predictive ability of the model was determined by generating a ROC curve. If the model had no predictive ability, the area under the ROC curve would be 0.5 and the curve would approximate a diagonal line; if the sensitivity and specificity of the model were both 100%, the area under the curve would be 1.0. A model with an area under the ROC curve of >0.7 was considered to provide reasonable discrimination (Hosmer and Lemeshow, 2000).

## Results

There were 483 horses referred to BEVC for colic during the study period, of which 179 met the inclusion criteria. There were 84 horses referred for elective magnetic resonance imaging of the feet, of which 30 met the inclusion criteria. Of the 30 'control' horses included in the study, there were seven mares, 22 geldings and one stallion. There was 1 pony, 11 Thoroughbreds/Thoroughbred crosses and 12 Warmbloods/Warmblood crosses; the remaining breeds ($n = 6$)

**Table 1**
Distribution of cortisol concentrations (nmol/L) with respect to each horse level variable. Mean, standard deviation (SD) and range are recorded. The normal laboratory reference range of serum cortisol concentration is 25–155 nmol/L.

| Variables | Number of observations | Mean ± SD cortisol | Range cortisol |
|---|---|---|---|
| Treatment | | | |
| Medical | 62 | 271.60 ± 120.13 | 77–590 |
| Surgical | 109 | 427.68 ± 225.64 | 131–1314 |
| Euthanased | 8 | 481.13 ± 228.13 | 183–900 |
| Control | 30 | 218.70 ± 79.61 | 20–350 |
| Outcome | | | |
| Survived | 129 | 340.86 ± 189.29 | 77–1314 |
| Died | 50 | 466.68 ± 232.19 | 142–1070 |
| Control | 30 | 218.70 ± 79.61 | 20–350 |
| Breed | | | |
| TB, TBx, WB, WBx, Hunter, Arabs, Cobs and Heavy horses | 151 | 353.81 ± 215.59 | 20–1314 |
| Ponies, Shetlands, miniature horses and other breeds | 58 | 352.41 ± 169.93 | 120–1045 |
| Duration of colic | | | |
| Duration of colic less than 24 h | 165 | 384.73 ± 212.78 | 77–1314 |
| Duration of colic 24 h or more | 14 | 273.21 ± 128.01 | 104–541 |
| Severity of clinical signs | | | |
| Mild clinical signs | 48 | 258.56 ± 144.53 | 77–900 |
| Moderate clinical signs | 78 | 332.90 ± 114.57 | 138–648 |
| Severe clinical signs | 53 | 545.81 ± 258.35 | 104–1314 |
| Distance travelled | | | |
| Distance travelled < 32 km | 139 | 376.89 ± 214.47 | 20–1314 |
| Distance travelled > 32 km | 70 | 306.83 ± 172.01 | 77–916 |
| PCV (L/L) | | | |
| PCV ≤ 46 | 151 | 327.80 ± 140.16 | 77–714 |
| PCV > 46 | 28 | 635.96 ± 310.85 | 146–1314 |
| Heart rate | | | |
| Heart rate ≤ 45 | 73 | 282.75 ± 140.56 | 77–908 |
| Heart rate > 45 | 106 | 440.23 ± 224.75 | 131–1314 |

TB, Thoroughbred; TBx, Thoroughbred cross; WB, Warmblood; WBx, Warmblood cross; CI, confidence interval.