Short Communication

Cortisol Circadian Rhythm Ratio: A Simple Method to Detect Stressed Horses at Higher Risk of Colic?

Baity B. Leal DVM, MSc\textsuperscript{a,b}, Geraldo E.S. Alves DVM, PhD\textsuperscript{a}, Robert H. Douglas DVM, PhD\textsuperscript{c}, Beatriz Bringel DVM\textsuperscript{c}, Robert J. Young BSc (Hons), PhD\textsuperscript{e}, João Paulo A. Haddad DMV, PhD\textsuperscript{d}, Walmir S. Viana DVM\textsuperscript{f}, Rafael R. Faleiros DVM, PhD\textsuperscript{a}

\textsuperscript{a}Departamento de Clínica e Cirurgia Veterinárias, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
\textsuperscript{b}Departamento de Medicina Veterinária at UniViçosa, UniViçosa, Brazil
\textsuperscript{c}BET Labs, Lexington, KY
\textsuperscript{d}Departamento de Medicina Veterinária Preventiva, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
\textsuperscript{e}Conservation, Ecology and Applied Behavior Group, Pontifícia Universidade Católica de Minas Gerais, Minas Gerais, Brazil
\textsuperscript{f}Regimento de Cavalaria Alferes Tiradentes, Minas Gerais, Brazil

Keywords:
Cortisol circadian rhythm
Stress
Confinement
Horse

\textbf{A B S T R A C T}

With the hypothesis that police horses with abnormal cortisol circadian rhythm (CCR) had more chance to develop colic, the aim of this study was to determine the CCR ratio in horses subjected to different housing and work conditions and to associate abnormal CCR ratio with incidence of colic. A total of 116 police horses belonging to four different groups were studied. In all, 31 were full-time stabled and performed urban patrolling activity, 27 were full-time stabled and performed equine therapy and sports activities, 25 were part-time stabled and performed urban patrolling, and 33 animals were kept full-time on pasture and did not perform any kind of work activity. Venous blood samples were collected between 6 and 7 AM and also 8 hours later, and cortisol concentrations were determined by radioimmunoassay to calculate the CCR ratio. Annual colic incidences (6 months before and after the day on which CCR ratio was determined) were assessed by examining individual medical files. The overall incidence of abnormal CCR ratio (<0.30) was 63.3%, and 107 cases of colic were reported for this population with a mean incidence of 0.92 colic/horse/yr. Horses with abnormal CCR had 2.3 times more chance to have colic (95% CI: 1.07 to 5.28, \(P = .03\)) when compared with those with normal CCR. In conclusion, high incidence of abnormal CCR ratio indicates that police horses were under stressful conditions. Horses with abnormal CCR ratios were more prone to suffer colic episodes, indicating that CCR ratio determination could perhaps be useful in detecting horses at risk of colic.

© 2011 Elsevier Inc. All rights reserved.

1. Introduction

Colic is still responsible for more deaths in horses than any other disease \cite{1}. Many factors have been associated with the incidence of colic. Feeding types, feeding practices, and the occurrence of gastrointestinal parasites have been described as main direct risk factors \cite{1}. Moreover, the indirect association between chronic stress and colic has been postulated because studies have shown that horses subjected to stressful conditions (such as restricted access to pasture and intense exercise) may be at an increased risk of colic as compared with undisturbed ones \cite{1}.

The determination of serum cortisol values has been used to estimate the level of animal stress and welfare in several species, including horses \cite{2,3}. The absence of
a normal cortisol circadian rhythm (CCR) is a reliable indicator of chronic stress and poor welfare [4]. Horses in natural undisturbed habitats and trained horses, which have been adapted to their environment, show a normal oscillation in blood cortisol concentrations, which usually is higher in the morning and decreases throughout the day [5].

Experimental studies in horses have used daily multiple blood samples to detect the CCR in horses [5]. However, Douglas [6] proposed a simple method to detect abnormal CCR in horses using two blood samples. By this method, the CCR was considered abnormal when the ratio between values (difference/higher value) was < 0.30 [6]. The aim of this study was to determine the CCR ratio of police horses subjected to different housing and work conditions, and to associate the CCR ratio results with the incidence of colic.

2. Materials and Methods

2.1. Animals

A total of 116 horses belonging to four different groups based on their type of work activity and housing conditions were used. Group FSW (full-time stabled horses subjected to urban patrol) comprised 31 horses (age: 10 ± 4 years; corporal score: 2.9 ± 0.2 [1-5 scale]; 13 females and 18 geldings) that were stabled full-time in individual 6.25-m² stalls without any kind of bedding, and performed urban patrolling activity (8 h/d, 4 d/wk). Group FSS (full-time stabled horses subjected to sports activities) comprised 27 horses (age: 14 ± 6 years; corporal score: 3.4 ± 0.7; 10 females; 15 geldings and two stallions) that were stabled full-time in individual 16-m² stalls with shavings bedding, and performed equine therapy and sports activities (3 h/d, 6 d/wk). Horses from FSW and FSS groups were located at the same urban facility. Group PSW (part-time stabled horses subjected to urban patrol activities) comprised 25 horses (age: 11 ± 4 years; corporal score: 3.1 ± 0.5; 15 females, 15 geldings, and one stallion) that were stabled part-time in 6.25-m² stalls without bedding with 12-hour daily access to a common pasture, and performed urban patrolling (8 h/d, 4 d/wk). These horses were housed in a distinct urban facility. Group FRE (horses kept exclusively on pastures without working activities) comprised 33 horses (age: 10 ± 7 years; corporal score: 2.6 ± 0.5; 29 females and four geldings) that were kept full-time outdoors on pasture in a farm distant from urban centers, and did not perform any kind of activity except free walking. All horses were fed chopped grass (Pennisetum purpureum) and a commercial concentrate food (15% protein, 3,100 Kcal/kg) in a mean ratio of 1:1 based on dry matter.

2.2. Sample and Data Acquisition

In a single day, venous blood samples were collected from the horses in the morning (6 to 7 AM) and in the afternoon (8 hours after the first sample). All blood collection procedures were performed quickly and in a quiet environment to minimize acute stress, and consequently to avoid any possible immediate induced changes in the blood cortisol levels [2]. Cortisol concentrations in serum were determined by a radioimmunoassay technique (BET Laboratories, Lexington, KY). CCR ratios for each horse were calculated by dividing the difference of the two sample values by the higher one. The CCR ratio was considered abnormal when the result was < 0.30 [6]. The clinical records from those horses were analyzed and the overall and individual incidence of colic (pain of gastrointestinal origin that demanded veterinary assistance) during the year of the experiment (6 months before and after the day on which CCR ratio was determined) was determined.

2.3. Statistical Analysis

The difference in CCR ratio values between groups was assessed by analysis of variance followed by a post hoc Student–Newman–Keuls test. The difference between the incidence of abnormal CCR ratio and colic among groups was analyzed by the χ² test. To assess a possible association, the incidence of colic in horses with or without abnormal CCR ratios was analyzed by the χ² test and the odds ratio was obtained. P < .05 was used to identify statistical significance for all statistical analyses.

3. Results

Mean cortisol serum concentrations (±SD) from samples collected in the morning and afternoon were 66.9 ± 30.0 and 58.6 ± 29.7 ng/mL, respectively. The overall CCR ratio mean was 0.26 ± 0.16. The FRE group had higher CCR ratios and lower incidence of abnormal CCR as compared with all other groups (Table 1). During the experimental period, 107 cases of colic were reported for this population with a mean incidence of 0.92 colic/horse/yr. The FRE group had the lowest incidence of horses with colic (12.1%), which was lower when compared with the other groups (Table 2). Horses with CCR ratios lower than 0.30 had 2.3 times more chance to have colic (95% CI: 1.07 to 5.28, P = .03) when compared with those with normal CCR. Considering just the stabled horses (FSW, FSS, and PSW groups), the odds ratio for that association was 3.1 (95% CI: 1.37 to 7.12, P = .006).

Table 1

Mean CCR ratio and incidence of abnormal CCR ratio in FSW, FSS, PSW, and FRE

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>CCR Ratio (ng/mL)</th>
<th>Incidence of CCR Ratio &lt;0.30</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Horses %</td>
</tr>
<tr>
<td>FSW</td>
<td>31</td>
<td>0.22±1.04</td>
<td>24* 77.4</td>
</tr>
<tr>
<td>FSS</td>
<td>27</td>
<td>0.26±0.16</td>
<td>19* 70.4</td>
</tr>
<tr>
<td>PSW</td>
<td>25</td>
<td>0.20±0.13</td>
<td>18* 72.0</td>
</tr>
<tr>
<td>FRE</td>
<td>33</td>
<td>0.35±0.18</td>
<td>12* 36.4</td>
</tr>
<tr>
<td>Total</td>
<td>116</td>
<td>0.26±0.16</td>
<td>73 63.6</td>
</tr>
</tbody>
</table>

CCR, cortisol circadian rhythm; FSW, full-time stabled horses subjected to urban patrol; FSS, full-time stabled horses subjected to sports activities; PSW, part-time stabled horses subjected to urban patrol activities; FRE, horses kept exclusively in pasture without working activities; SD, standard deviation.

In the columns, values followed by different letters a,b in the same column are statistically different, P < .05.
4. Discussion and Conclusion

The measurement of cortisol blood levels has been used in horses with acute colic to assess the severity of disease [7] and to predict survival [7,8]. However, to our knowledge, there are no studies testing the hypothesis that horses with abnormal CCR have more chance to develop colic.

The FRE group had lower incidence of abnormal CCR ratio and a higher CCR ratio when compared with others groups, which did not differ within the group. Disturbances of the circadian rhythm in cortisol are observed during situations of chronic stress in many species. Flattened circadian rhythms in cortisol are observed in stressed pigs and during several human psychological disorders, such as during certain types of depression, chronic fatigue syndrome, and post-traumatic stress disorder [8,9]. These findings indicate that stabled horses in urban environment were in a state of stress and that the type of confinement (partial × full time), the type of stall and bedding (large stall with shavings × small stall without bedding), or the type of work (sports × patrolling) did not change the capability of the horses in coping with such housing conditions.

The overall colic incidence (0.92 colic/horse/yr) was higher than previously published ranges of 0.035 to 0.106/ horse/yr [1,10]. However, the FRE group presented lower rate than the others. High levels of concentrate, restricted forage intake, forage quality, meal schedule, stall confinement, and type of work have been reported as main risk factors for colic in horses [1] and may explain such difference among groups.

The significant association between abnormal CCR ratio and colic incidence showed, in the studied population, that horses with values < 30% were more prone to suffer colic episodes. Although our findings are based on a single CCR ratio determination from a specific horse population, we speculate that CCR ratio determination can be a simple and useful technique to detect horses at risk of colic. Further prospective studies considering serial CCR ratios and investigating other horse populations will clarify this assumption.

Acknowledgments

The authors acknowledge the help provided by the RCAT—Alferes Tiradentes Cavalry Regiment. Funding for this study was provided by Fapemig and Capes, Brazil.

References