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Plasma Procalcitonin Concentration in Healthy Horses and Horses Affected by Systemic Inflammatory Response Syndrome

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Background: The diseases most frequent associated with SIRS in adult horses are those involving the gastrointestinal tract. An early diagnosis should be the goal in the management of horses with SIRS.

Objective: The objective of this study was to evaluate the plasma procalcitonin (PCT) concentration in healthy and SIRS horses to assess differences between the two groups.

Animals: Seventy-eight horses (30 healthy and 48 SIRS).

Methods: Prospective in vivo multicentric study. Horses were classified as SIRS if at least 2 of the following criteria were met: abnormal leukocyte count or distribution, hyperthermia or hypothermia, tachycardia, tachypnea. Healthy horses showed no clinical or laboratory signs of SIRS. Plasma PCT concentrations were measured with a commercial ELISA assay for equine species. Results were expressed as mean±standard deviation. T-test for unpaired data was performed between healthy and SIRS group. SIRS group was divided in 4 subgroups and *t*-test was performed between healthy versus each subgroup.

Results: PCT concentrations in healthy and SIRS horses were 18.28 ± 20.32 and 197.0 ± 117.0 pg/mL, respectively. T-test showed statistical differences between healthy versus SIRS group and between healthy versus all subgroups.

Conclusions and Clinical Importance: Results showed an increase in PCT concentration in SIRS horses as previously reported in humans and dogs. PCT could be used as a single assay in equine practice for detection of SIRS.

Key words: Biomarker; Diagnostic Test; Equine.

Endotoxins (lipopolysaccharides, LPS) are present in large quantities in the large bowel of horses, but are harmless as long as they remain within the intestinal lumen. Equine gastrointestinal diseases involving hypersecretion of fluid, motility disturbances, altered microbial flora, local stasis, ischemia, inflammation, and impaired mucosal barrier may lead to absorption of endotoxin, bacterial products or both through the compromised mucosa into the bloodstream. Endotoxemia induces release of acute phase proteins and cytokines that may result in severe organ dysfunction and death.[1] The physiologic changes associated with this inflammatory activation are alteration in heart and respiratory rate, body temperature, mucous membrane status, and capillary refill time. Recently, some authors have supposed that the term systemic inflammatory response syndrome (SIRS), rather than endotoxemia, should be used to describe the clinical status of "endotoxemic" horses. The diseases that have been associated with SIRS in adult horses are especially those involving the gastrointestinal tract, such as the inflammatory intestinal diseases and strangulating obstructions.[1] An early diagnosis should be the goal in the management of SIRS patients, allowing starting an adequate treatment in an early stage.[2,3] Due to the importance of procalcitonin (PCT) as biomarker of SIRS in human medicine,[3] the aim of the present work was to evaluate the plasma PCT concentration in healthy and SIRS horses to evaluate the differences between the two groups.

Abbreviations:

LPS	lipopolysaccharide
SIRS	systemic inflammatory response syndrome
PCT	procalcitonin
K2EDTA	potassium ethylene diamine tetraacetic
RCF	relative centrifugal force
CV	coefficient of variation
BPM	beats per minute
BPM	breaths per minute

Materials and Methods

The present in vivo multicentric experimental trial in clinical setting was approved by the Institutional Animal Care and Use Committee of the University of Pisa, University of Barcelona and Cornell

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Clinical cases were collected at the Veterinary Teaching Hospital "M. Modenato" of the Department of Veterinary Sciences, San Piero a Grado (PI), Italy, at the College of Veterinary Medicine of Cornell University, Vet Box 25, Ithaca, NY 14853 and at the Department of Animal Medicine and Surgery of the Universitat Autonoma de Barcelona, Bellaterra, Spain. All laboratory procedures were conducted in the Pharmacology and Toxicology Laboratory, Department of Veterinary Sciences, San Piero a Grado (PI), Italy.

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University. A total of 78 horses were included in the present prospective study. Thirty were healthy horses from different farms. Thirteen/30 (43%) were females, 11/30 (36%) geldings, and 6/30 (20%) stallions. Mean age was 9.8 ± 5.4 years old. Forty-eight were sick horses referred to three different veterinary teaching hospitals providing secondary health care. The following data were recorded both for healthy and sick animals, in order to classify horses in healthy and SIRS[1] groups: presence of abnormal leukocyte count or distribution as leukopenia, leukocytosis or >10% band neutrophils (reference intervals: 5.4–14.3 × 10^3 μL),[4] hyperthermia or hypothermia (reference intervals: 37.2–38.3°C),[5] tachycardia (reference intervals: 28–44 bpm),[5] tachypnea (reference intervals: 8–15 bpm).[5] All the 30 healthy horses presented a normal physical examination and clinicopathologic data within reference ranges, thus they were included in the control group. Horses with 2 or more criteria were included in the SIRS group.[1] Ten/48 (21%) sick horses were stallions, 18/48 (38%) geldings, and 20/48 (41.5%) females. Mean age was 10.3 ± 6.7 years old. The horses were of different breeds: Standardbred (n = 8), Pure Spanish Horse (n = 7), cross-breed (n = 6), Quarter Horse (n = 6), Thoroughbred (n = 6), Ponies (n = 4), Italian saddle horse (n = 3), Paint Horse (n = 3), Arabian (n = 1), Appaloosa Horse (n = 1), Belgian horse (n = 1), Hanoverian horse (n = 1), and Warmblood (n = 1). An owner's written consent was obtained for collection of plasma for all the horses included in this study. Retrospectively, SIRS horses were divided into 4 subgroups based on nature of the pathological process: 18/48 (38%) strangulated intestinal lesion, 13/48 (27%) nonstrangulated intestinal lesion, 11/48 (23%) diarrheas or colitis, and 6/48 (13%) pleuropneumonia.

All horses were submitted to a complete physical examination and blood collection for CBC evaluation and plasma PCT analysis. Blood samples for CBC and PCT concentrations were collected from the jugular vein using a sterile syringe and 16G needle. Each blood sample was divided in two aliquots: a 1 ml aliquot was collected in a potassium ethylene diamine tetraacetic (K_2EDTA) test tube and analyzed by a cell counter[a] within 5 minutes after the collection. A second 2.5 mL aliquot was collected in heparinized-tubes and immediately centrifuged at 2100 relative centrifugal force (RCF) for 10 min. The harvested plasma was placed in sterile tubes, frozen at −18°C and analyzed in a single batch within 3 months. There was no intravenous administration of calcium before blood collection, so as to not influence plasma PCT concentrations.

PCT concentrations were determined with a commercial kit for equine species.[b] The intra-assay coefficient of variation was determined from 10 replicates of equine plasma samples containing low and high PCT concentrations. These samples were obtained by addition of standard PCT in equine blank samples. The interassay coefficient of variation was determined from values obtained by repeating the analysis of duplicate samples with low and high PCT concentrations in 5 different assays. To establish the detection limit for equine plasma PCT, we performed repeated PCT measurements using equine samples with low PCT concentrations (<10.0 pg/mL). Samples were measured in 10 replicates in a single assay and in 5 different assays. The intra- and interassay coefficient of variations were both <15%, the limit of detection of the method was 10 pg/ml.

Descriptive data are reported as mean ± standard deviation. Kolmogorov–Smirnov test was applied to verify data distribution. T-test for unpaired data was performed to verify differences in plasma PCT concentration between healthy versus SIRS group, and between healthy versus each subgroup. Significance level was set at $P < .05$. A commercial statistical software was used.[c]

Results

All horses included in the healthy group showed no clinical and clinico-pathologic signs of SIRS, while all the 48 sick horses presented 2 or more signs of SIRS.

Table 1. Plasmatic PCT concentrations, expressed as mean ± SD, obtained in the 4 subgroups

Disease	PCT (pg/mL) (X ± SD)	P versus healthy group
Strangulated intestinal lesion	232 ± 155	<0.0001
Nonstrangulated intestinal lesion	148 ± 79	<0.0001
Diarrhea or Colitis	168 ± 91	<0.0001
Pleuropneumonia	217 ± 95	<0.0001

The plasma PCT concentration was 18.28 ± 20.32 pg/mL and 197.0 ± 117.0 pg/mL in healthy and SIRS group, respectively, while plasma PCT concentrations in various subgroups were reported in Table 1. T-test for unpaired data showed a statistical difference between healthy versus SIRS group and between healthy versus all subgroups.

Discussion

PCT appears to be an early marker of SIRS caused by bacterial infections and associated products in human medicine.[3,6] PCT originates from the CALC-I gene transcription and calcitonin-mRNA translation. In healthy patients, the transcription of the CALC-I gene is restricted to neuroendocrine cells in the thyroid gland and the lung and healthy individuals have very low serum concentrations.[3,6] During a state of infection the expression of the CALC-I gene is up regulated and PCT is released from many tissues and cell types in the body in laboratory animals, dogs and horses, as well as in human patients.[3,6–8] Moreover, plasma PCT concentration rises rapidly within 3-6 h, especially in response to bacterial infection and endotoxemia, seeming to be also an early marker of bacterial infection and endotoxemia in human patients.[3,6]

Plasma PCT concentration was lower in healthy horses than in SIRS ones. Our results are in line with earlier studies performed in equine, canine, and human species.[3,6,9–11] The mean plasma PCT concentration reported in this study, both for healthy and SIRS group, were lower than those reported in another study performed in 29 horses (24 healthy horses and 5 septic horses).[10] The dissimilarity might be due to the different PCT ELISA kit used: in our study, an equine PCT ELISA kit assay for PCT determination was used, while Rieger and colleagues (2014)[10] have used an ELISA assay in which, human antibodies were directed against equine PCT. Plasma PCT levels were similar to those reported in foals and plasmatic PCT concentrations were statistically different between healthy versus SIRS groups both in foals and in adults, confirming that PCT increases during SIRS.[11]

PCT levels in horses in this study were generally lower than levels reported in humans with SIRS.[3,6] This dissimilarity might be related to the different species studied, less severe inflammation in the studied horses, or possibly endotoxin tolerance.[12] Moreover, Yilmaz and colleagues (2008)[9] evaluated the changes in PCT concentrations in dogs after administration of endotoxin, which resulted lower than our results. This

difference could be related to the species studied (dog versus horse) and to the different assay used.

PCT concentrations obtained in all subgroups resulted statistically higher than the healthy group, confirming that plasmatic PCT levels increase during SIRS process. No statistically differences were found between subgroups; this finding might be due to the small number of animals included in the subgroups.

PCT seems to be a sensitive and specific marker of SIRS in horses. The limit of the study might be the number of cases included (n = 48). An increased study population is needed to investigate the role of PCT in being able to distinguish between septic and nonseptic SIRS in adult horses and to shortening antimicrobial treatment as already reported in humans.[3,6]

Footnotes

[a] ProCyte Dx™; IDEXX, USA
[b] Horse Procalcitonin ELISA kit; MyBiosource.com
[c] Graph Pad Prism 6, USA

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Conflict of Interest Declaration: Authors disclose no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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