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Plasma procalcitonin concentration in healthy calves and those with septic systemic inflammatory response syndrome

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ABSTRACT

The diagnosis of sepsis in calves is challenging. Blood culture and clinical signs combined with a complete blood count have been used for the diagnosis of sepsis. Recent literature in humans and animal species has been focused on sepsis-specific biomarkers, such as procalcitonin (PCT), that may more accurately and efficiently diagnose sepsis. The aim of this study was to evaluate plasma PCT concentrations in healthy and septic calves. Twenty healthy control calves and 58 sick calves with septic systemic inflammatory response syndrome (SIRS) based on SIRS score and clinical findings were included. Calves with septic SIRS were further divided in septic SIRS survivors (SSS) and non-survivors (SSNS). Plasma PCT concentrations were measured with a commercial ELISA assay for cattle. A receiver operating characteristic curve was used to determine cut-off values and corresponding sensitivity and specificity for the diagnosis of sepsis. Differences in plasma PCT concentration between groups (control vs. SSS vs. SSNS) were evaluated.

Plasma PCT concentrations in healthy calves and those with septic SIRS were 33.3 pg/mL (0–44.3 pg/mL) and 166.5 pg/mL (85.9–233.0 pg/mL), respectively ($P < 0.001$). The optimal cut-off value to predict septic SIRS was 67.39 pg/mL (81.0% sensitivity, 95.0% specificity). Plasma PCT concentrations were 127.4 pg/mL (72.2–216.0 pg/mL) and 234.3 pg/mL (204.5–309.4 pg/mL) in the SSS and SSNS subgroups, respectively. Statistically significant differences were found among groups (control vs. SSS and SSNS, $P < 0.0001$; SSS vs. SSNS, $P > 0.05$). These results confirmed an increase in plasma PCT concentrations in calves with septic SIRS, as previously reported in humans and other species.

Introduction

Since the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference in 1991, the term sepsis has been defined as the 'systemic inflammatory response to infection' (Bone et al., 1992). The expression 'systemic inflammatory response syndrome' (SIRS) describes a clinical condition that represents the culmination of the activation of a complex network of acute endogenous mediators, which lead to uncontrolled and widespread inflammation (Alberti et al., 2005). SIRS can be associated with many different factors, including hypoxia, burns, trauma, immunologic reactions, bacterial and viral infections (Bone et al., 1992; Alberti et al., 2005). Confirmation of microbial infection in the presence of SIRS is required for a diagnosis of sepsis (Zabrecky et al., 2015).

Sepsis in calves is either sporadic or epidemic, reaching a prevalence of 30% if predisposing factors for the development of neonatal septicemia are present (Aldrige et al., 1993; Fecteau et al., 2009). Many factors predispose calves to sepsis, such as failure of passive transfer, management deficits, adverse environmental conditions, cold stress, protein-energy malnutrition, micronutrient deficiencies, or bacterial colonization of a local site, such as the umbilicus, gastro-intestinal tract or respiratory system (House et al., 2015). The pathophysiologic changes associated with inflammatory activation in sepsis are dehydration and alterations in heart rate, respiratory rate, body temperature, mucous membrane status and capillary refill time, as well as leukopenia, hypotension and generalized weakness (House et al., 2015). The

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definitive diagnosis of sepsis is based on a blood culture. However, the sensitivity of blood culture can be low and a negative result must be interpreted with caution (Fecteau et al., 2009). In veterinary medicine, clinical signs combined with a complete blood count (CBC) and scores are considered useful in the diagnosis of sepsis. However, sepsis-specific biomarkers have become a recent focus of research in both humans and veterinary species because they could potentially increase diagnostic accuracy and efficiency (Fecteau et al., 2009; Fielding and Magdesian, 2015; Ercan et al., 2016).

Procalcitonin (PCT) has been investigated as a biomarker of sepsis in humans (Riedel, 2012; Deliberato et al., 2013; Afsar and Sener, 2015), and in horses (Toribio et al., 2003; Pusterla et al., 2006; Rieger et al., 2014; Bonelli et al., 2015a and b; Bonelli et al., 2017; Barton et al., 2016), cattle (Ercan et al., 2016) and dogs (Giunti et al., 2006; Yilmaz et al., 2008). Healthy individuals have very low serum PCT concentrations due to the restriction of the CALC-I gene transcription by the neuroendocrine cells in the thyroid gland and in the lungs (Riedel, 2012; Afsar and Senar, 2015). The expression of the CALC-I gene is up-regulated in many cell types in septic humans (Riedel, 2012; Afsar and Senar, 2015) and in septic animals (Toribio et al., 2003; Giunti et al., 2010); thus, PCT is released into the circulation from many cell types. In septic human patients, PCT rises rapidly (within 3–6 h) and decreases by half within 24 h when the infection and/or host immune response is controlled (Riedel, 2012; Afsar and Senar, 2015). PCT also remains stable in blood specimens at room temperature, unlike other markers of sepsis (Carrol et al., 2002). In veterinary medicine, studies have reported plasma PCT concentrations in septic and non-septic foals (Bonelli et al., 2015a), adult horses (Rieger et al., 2014; Bonelli et al., 2015b; Bonelli et al., 2017), and dogs (Giunti et al., 2006; Yilmaz et al., 2008). The aim of this study was to evaluate and compare plasma PCT concentrations in healthy and septic calves.

Materials and methods

Animals

This in vivo multicentric experimental trial was conducted in a clinical setting and was approved by the Italian Animal Care (DL 116/92) and by the Institutional Animal Care and Use Committees of the University of Pisa (Approval Number 2825; Approval Date 28 January 2014) and the University of Milan (Approval Number 2; Approval Date 15 February 2016). At Cornell University, residual blood from specimens routinely collected from calves on hospital admission was used. Written consent was obtained from the owners of all calves included.

During the research period, a total of 260 calves were admitted to the veterinary teaching hospitals involved in the study. However, the authors excluded cases that did not fit the inclusion criteria, resulting in the inclusion of 78 calves aged 9.6 ± 4.3 days. Twenty of 78 were healthy Holstein calves (n = 11/20 females and n = 9/20 males) aged 8.4 ± 3.9 days and were housed at the university dairy farm of the University of Pisa. Fifty-eight of 78 (n = 35/58 females and n = 23/58 males), aged 10.6 ± 4.2 days, were sick, client-owned calves referred to three participating veterinary teaching hospitals providing secondary health care (the Veterinary Teaching Hospital 'M. Modenato', University of Pisa, Italy; the 'Nemo' Farm Animal Hospital, Cornell University, Ithaca, NY, USA; the Clinic for Ruminants and Swine, University of Milan, Italy). Sick calves were Holstein Friesian (n = 50/58) or mixed dairy breeds (n = 8/58).

Collection of clinical and clinical pathological data

A complete history was recorded, when possible, for each calf at admission time, especially information concerning previous antimicrobial treatment. All calves were subjected to a complete physical examination and blood was collected for CBC with differential white cell count and blood culture at admission time. Calves needed only manual restraint for all procedures. A 1 mL blood sample for CBC was collected from the jugular vein in an EDTA tube (FL Medical) and analyzed by a cell counter (ProCyte Dx, IDEXX) within 5 min of collection. The EDTA blood was also used for blood smears, which were air-dried and stained using an automatic stainer (Aerospray 7150 Hematology Slide Stain-Cytocentriguge). The differential white cell count was performed by microscopic examination at 400× and 1000× magnification, counting 100 cells. A sample for blood culture was collected aseptically and a commercial culture system (OXOID SIGNAL Blood Culture System, Oxoid) was used as previously reported (Daley et al., 1990; Rohner et al., 1995). The outcome was recorded for all the sick client-owned calves as 'discharged' or 'died/euthanased'.

Inclusion criteria

Calves < 3 weeks old were enrolled. Each calf was scored according to a SIRS scale as previously described (Trefz et al., 2016). Only calves with a positive SIRS score were included in the septic SIRS group. SIRS positivity was based on the presence of two or more of the following criteria (Trefz et al., 2016): (1) presence of an abnormal leucocyte count, i.e. leukopenia or leukocytosis (reference interval, 5–12 × 10^9/L); (2) hyperthermia or hypothermia (reference interval, 38.5– 39.5 °C); (3) tachycardia (> 120 beats/min); and (4) tachypnea (> 36 breaths/min). Calves with SIRS were considered septic if there was clinical or necropsy evidence of septicemia (Lofstedt et al., 1999). In particular, the ante-mortem criteria were as follows: (1) positive blood culture; (2) culture of the same bacterial agent from at least two body fluids; or (3) culture of a bacterial agent from a single joint in a calf with joint effusion involving multiple joints. The post-mortem criteria were as follows: (1) morphologic changes, such as multiple disseminated abscesses of similar size, purulent vasculitis and intravascular identification of bacteria, or fibrin in multiple body cavities; (2) bacterial isolation from heart blood; or (3) recovery of the same bacterial isolate from at least two tissues (excluding intestine). No calves included in the control group had any signs of SIRS (SIRS score, 0), or clinical signs of septicemia.

Evaluation of plasma PCT concentration

A 2.5 mL aliquot was collected at admission time in heparinized tubes (FL Medical) and immediately centrifuged at 2.100 g for 10 min. The harvested plasma was placed in sterile tubes, frozen at −18 °C, and analyzed for PCT in a single batch within 3 months. The concentrations of PCT were determined using a commercial kit for cattle (Bovine Procalcitonin ELISA kit, MyBiosource.com). The intra-assay coefficient of variation was determined from 10 replicates of calf plasma samples with known low and high PCT concentrations. These samples were obtained by adding standard amounts of PCT provided with the ELISA kit in blank samples of calf blood. The inter-assay coefficient of variation was determined by repeating the analysis of duplicate samples with low and high PCT concentrations in five different assays. Samples were measured in 10 replicates in a single assay and in five different assays. The intra- and inter-assay coefficient of variations were both <15%; the limit of detection of the method was 10 pg/mL. To establish the detection limit for bovine plasma PCT, we performed repeated PCT measurement using bovine samples with low PCT concentrations (<10.0 pg/mL). Results less than the limit of detection were expressed as 0.