Serum interleukin-6 (IL-6) and IL-10 concentrations in normal and septic neonatal foals

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ABSTRACT

Previously it was reported that compared to surviving septic foals, non-surviving foals had a 35-fold increase in interleukin-10 (IL-10) and 15-fold increase in IL-6 gene expression in their peripheral blood mononuclear cells (PBMC). As gene expression profiles can be time-consuming, we sought to determine if serum IL-6 and IL-10 in foals would aid in the diagnosis and prognosis of septicemia.

A prospective study of septic neonatal foals admitted to the Cornell University Equine Hospital during 2007 and 2008 was performed. Septicemia was confirmed in 15 foals using blood culture results and sepsis scores. Blood samples for measurement of serum IL-6 and IL-10 concentrations were collected at the time of admission (T0) and again 24 (T24) and 48 (T48) hours later. Blood samples from age-matched control foals (n = 15) born at the Cornell Equine Park were obtained from foals 12–72 h after birth (T0) and again 24 (T24) and 48 (T48) hours later. IL-6 and IL-10 concentrations were determined in the serum from dams of septic foals and serum and colostrum from dams of control foals. Serum IL-6 was also measured in healthy foals prior to ingestion of colostrum. Interleukin-6 was detected using an ELISA and IL-10 was detected using a bead-based fluorescent immunoassay. Group differences were detected using a Wilcoxon rank sum test with a Bonferroni correction applied to the p value.

There were no significant differences in serum IL-10 concentration between the two groups of foals. Relative to control foals, septic foals had significantly lower serum IL-6 concentrations at all 3 time points. Relative to septic foals, control foals had significantly higher serum IL-6:IL-10 ratios. Serum IL-6 was undetectable in foals prior to ingestion of colostrum. However, colostral IL-6 concentration measured in the control mares was high (≥215 ng/mL) in all samples suggesting passive transfer of maternal IL-6 to the equine neonate. Colostral IL-10 was undetectable in 11/12 samples. Failure of passive transfer may directly influence the serum IL-6 concentration in septic foals. Neither serum IL-6 nor IL-10 alone were useful diagnostic indices of sepsis in equine neonates. Although the number of animals involved in this study was too small for the identification of a concrete value, the serum IL-6:IL-10 ratio is likely to provide a valuable prognosticator for neonatal septicemia.

1. Introduction

In systemic bacterial infections, the host response includes the production of both pro-inflammatory and anti-inflammatory cytokines. Pro-inflammatory cytokines (e.g. TNF-α, IL-1β, IL-2, IL-6 and IFN-γ) promote macro-
phage activation and phagocytosis, enhance cell-mediated immunity and up-regulate acute phase protein synthesis. Anti-inflammatory cytokines (e.g. IL-4, IL-5, IL-10, IL-13 and TGF-β) have immunoregulatory functions and support development of a humoral immune response by promoting B-cell differentiation and antibody production (Romagnani, 1996; Gogos et al., 2000). In human infants, cytokine profiles in the peripheral blood have been used for the early identification of sepsis (Ng et al., 2006, 2007; Lam and Ng, 2008). Likewise, cytokine responses in PBMC of septic equine neonates have also been examined with a view to their diagnostic and prognostic significance. Sepsis is a major cause of morbidity and mortality in the equine neonate (Paradis, 1994). Early diagnosis and prognosis are important, as treatment of the septic foal is often costly and associated with an unfavorable outcome. Pusterla et al. (2006) found that relative to healthy foals, non-surviving sick foals (septic and non-septic) exhibited marked increases in the gene expression of IL-10 in PBMC. Gene expression of several other cytokines, including IL-6, was not increased in the non-survivors relative to the survivors. In a recent prospective study completed in our laboratory, we found that compared to healthy foals, IL-4 gene expression was initially down-regulated and toll-like receptor 4 (TLR4) expression up-regulated in PBMC from septic foals (Gold et al., 2007). Furthermore, the IL-6 gene expression in PBMC isolated from septic foals that died was 15-fold greater compared with septic foals that survived (Gold et al., 2007).

The data from Pusterla et al. (2006) and Gold et al. (2007) suggest that IL-6 and IL-10 mRNA concentrations may be valuable prognosticators of survival in septic foals. However, because of the time required for processing of samples (isolation of RNA, preparation of cDNA, performing real-time PCR), a simpler and faster test that measured serum concentrations of these cytokines is required. Based upon the gene expression data, we hypothesized that serum IL-6 and IL-10 protein concentrations would be increased in septic neonatal foals and would be predictive of survival. The three objectives of this study were to: (1) develop a rapid test for quantification of equine IL-6 and IL-10 serum concentrations; (2) measure serum IL-6 and IL-10 concentrations in septic foals; and (3) determine if maternal colostral IL-6 and IL-10 concentrations potentially influence the foal serum concentrations of these two cytokines.

2. Materials and methods

2.1. Case selection

Two groups of foals were studied and consisted of neonatal septic foals admitted to the Cornell University Hospital for Animals (CUHA) during the 2007 and 2008 foaling seasons and healthy (control) foals born and raised at the Cornell University Equine Park during the spring of 2007.

All sick neonatal foals less than 14 days of age admitted to the CUHA were tentatively diagnosed as septic (based upon historical findings and initial clinical examination by the attending veterinarian) were initially enrolled. Foals were retained in the study if they had a positive blood culture or a sepsis score ≥11 (Brewer and Koterba, 1988). Following admission to the hospital, historical, physical examination, thoracic and abdominal ultrasonographic findings were recorded. Blood samples were obtained for aerobic and anaerobic blood cultures, IgG quantification (Snap Foal IgG, Idexx Laboratories, Westbrook, ME), a complete blood count (CBC), a range of common blood serum biochemical measurements and fibrinogen concentration. In addition, a 5 mL blood sample for serum analysis was obtained from each foal at the time of admission (T0), and then again 24 (T24) and 48 (T48) hours later. The serum was collected and stored at −20 °C until cytokine measurement. After initial evaluation, each hospitalized foal received at least 1 L of hyperimmune equine plasma (Foalimmune, Lake Immunogenics, Ontario, NY or HiGamm-Equi, Lake Immunogenics, Ontario, NY), broad-spectrum antimicrobials, IV fluids and other supportive therapy as needed. One foal received a plasma transfusion prior to hospital admission. A 3 mL sample of each batch of commercial plasma used to treat these septic foals was obtained for interleukin analysis.

Fifteen septic foals were enrolled, 7 females and 8 males, with a median age of 24 (range, 12–288) hours. Breeds represented included 6 Thoroughbreds, 2 Standardbreds and 1 (each) Morgan, mixed-breed, Oldenburg, Paint, Quarterhorse, Arabian and Shire. The median gestational age, as reported by owners, was 340 (range, 305–345) days. Parturition was judged by the owners to be normal in 9/15 foals, required assistance with recognized complications in 2/15 foals and was unobserved in 4/15 foals. The median sepsis score of the septic foals was 16 (8–33). Blood cultures were positive in 9/14 foals, negative in 5/14 foals and not obtained in 1 foal. These last 6 foals were classified as septic based upon their individual sepsis score of 13, 18, 22, 24, 25 and 33. Microbial isolates from blood cultures included Enterococcus faecium (2 foals), Escherichia coli (2 foals), Actinobacillus equuli (2 foals), Corynebacterium spp. (1 foal), Staphylococcus aureus (1 foal) and methicillin-resistant S. aureus (1 foal). Median duration of hospitalization was 13 (1–19) days. Five of the foals had been treated with antimicrobials prior to admission and all (15/15) foals were treated with antimicrobials during hospitalization. Two foals received non-steroidal anti-inflammatory agents (NSAIDs) prior to hospitalization and 4/15 foals were treated with NSAIDs during hospitalization. None of the foals received glucocorticoids either before or during hospitalization. Foals in the septic group were considered as survivors if they were discharged from the hospital alive. Non-survivors were foals that died or were euthanized during hospitalization. Foals that were euthanized because of financial constraints were not included in the study. Thirteen foals survived to discharge and 2/15 foals died after less than 1 day of hospitalization.

Fifteen foals, 5 females and 10 males, with a median age of 36 (12–96) hours were enrolled in the control group. The median gestational age or the age of control foals when enrolled into the study was not significantly different from that of the septic group.

Breeds represented were Warmblood (10), Thoroughbred (2) and 1 (each) Thoroughbred cross, Irish Draft.
and Canadian Sport Horse. Each of these foals had an uneventful parturition, had a gestational age ≥334 days and lacked detectable abnormalities on physical examination. Blood samples were obtained from each foal and used for all tests described above for the septic group. A 5 mL blood sample for serum analysis was obtained from each foal at the time of enrolment into the study (T0) and then again at 24 (T24) and 48 (T48) hours later. Serum samples were also obtained from 5 control foals before they ingested colostrum. Based upon initial physical examination and clinical pathology findings, the median sepsis score of this group was 0 (range, 0–2). All of the control foals were maintained at the Cornell University Equine Park under the daily supervision of the farm manager and remained healthy for at least 6 months following their initial evaluations.

Serum samples were obtained within the T0–T48 time frame for measurement of IL-10 and IL-6 in 5/15 of the control foal mares and 11/15 of the septic foal mares. Colostrum samples, collected shortly after foaling, were obtained from 12/15 of the control foal mares. No colostrum samples were available from the septic foal mares.

All experimental procedures were approved by the Animal Care Committee of Cornell University and were in accordance with guidelines established by the National Institutes of Health. For the foals admitted to the Cornell University Hospital for Animals, owner consent authorizing the investigators to obtain blood samples for the study was obtained.

2.2. Determination of IL-6 and IL-10 in serum and colostrum

Interleukin-6 was measured in an enzyme linked immunosorbent assay (ELISA) as previously described for other equine cytokines (Wagner et al., 2006, 2008). Here, a polyclonal goat anti-horse IL-6 antibody (AF1886, R&D Systems, Inc., Minneapolis, MN) was used for coating the ELISA plates (ImmunoPro Maxisorp, Nalge Nunc Int., Rochester, NY). The antibody was diluted to a final concentration of 1 μg/mL in carbonate buffer (15 mmol Na₂CO₃, 35 mmol NaHCO₃, pH 9.6) and incubated overnight at 4°C. Afterwards, the coating solution was discarded and empty spaces on the plates were blocked for 30 min at room temperature by addition of phosphate buffered saline (PBS, pH 7.2) containing 0.5% (w/v) bovine serum albumin. Plates were washed five times with phosphate buffer (2.5 mmol NaH₂PO₄, 7.5 mmol Na₂HPO₄, 145 mmol NaCl, 0.1% (v/v) Tween 20, pH 7.2). A recombinant equine IL-6 (1886-EL, R&D Systems, Inc., Minneapolis, MN) diluted in 2-fold serial dilutions ranging from 50 to 0.78 ng/mL was used as standard to determine IL-6 concentrations in the samples. The serum and colostrum samples were diluted from 1:10 to 1:100,000 in phosphate buffer. All samples were applied to the ELISA plates and incubated for 90 min at room temperature. After five washes (see above) plates were filled with biotinylated goat anti-horse IL-6 (AF1886, R&D Systems, Inc., Minneapolis, MN) diluted 1:100 in phosphate buffer, incubated for 60 min and washed again. Then, a streptavidin–horse-radish peroxidase solution (Jackson ImmunoResearch Lab., West Grove, PA) was added to the plates for another 30 min. After a final wash, the plates were filled with substrate buffer (33.3 mmol citric acid, 66.7 mmol NaH₂PO₄, pH 5.0), supplemented with 130 μg/mL 3′,5′-tetramethylbenzidine (TMB, Sigma, St. Louis, MO) and 0.01% (v/v) hydrogen peroxide. Substrate solution was incubated for 20 min in the dark and the reaction was stopped by adding one volume of 0.5 mol H₂SO₄. Plates were evaluated in a Synergy HT ELISA reader (Bio-Tek, Winooski, VT) at 450 and 630 nm absorbance. The IL-6 ELISA had an analytical sensitivity of 780 pg/mL.

Interleukin-10 concentrations in serum and colostrum were determined using a fluorescent bead-based assay (Luminex Corp., http://www.luminexcorp.com) and a pair of monoclonal anti-equine IL-10 antibodies (Wagner et al., 2008). The assay was performed and analyzed in a Luminex IS 100 instrument (Luminex Corp., http://www.luminexcorp.com) as described previously (Wagner and Freer, 2009). Serum samples were used undiluted. Colostrum samples were diluted 1:3 and 1:10. The analytical sensitivity of the equine IL-10 singleplex assay was 4 pg/mL.

2.3. Statistical analysis

The distribution of the data was non-Gaussian. Differences between the two groups of foals in selected demographic variables, clinical pathology data and serum IL-10 and IL-6 concentration at the 3 time points (T0, T24, T48 hours) were analyzed using the Wilcoxon rank sum test. IL-6 and IL-10 ratios were compared between foals at T0 using the Wilcoxon rank sum test. Because multiple comparisons were made during the analysis, a Bonferroni correction was calculated and applied. For statistical significance, a p value ≤ 0.017 (≈0.05/3) was used. All computations were performed by use of a statistical software program (Statistix 8, Analytical Software, Tallahassee, FL).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Median (range) clinicopathological data of control and septic foals.</th>
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<tbody>
<tr>
<td></td>
<td>Control (n = 15)</td>
</tr>
<tr>
<td>Seg. neutrophils (cells/μL)</td>
<td>6600 (3100–10,900)</td>
</tr>
<tr>
<td>Band neutrophils (cells/μL)</td>
<td>0 (0–200)</td>
</tr>
<tr>
<td>Lymphocytes (cells/μL)</td>
<td>1300 (600–2900)</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>200 (100–600)</td>
</tr>
<tr>
<td>IgG (mg/dL)</td>
<td>&gt;800</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>5.9 (5.1–6.8)</td>
</tr>
<tr>
<td>Iron (μg/dL)</td>
<td>175 (42–371)</td>
</tr>
</tbody>
</table>
3. Results

3.1. Demographic and clinicopathologic data

Median values for selected clinicopathologic data from the two groups of foals are displayed in Table 1. There was no significant difference between the two groups in lymphocyte counts. Statistical analysis was not performed on other physical and clinicopathologic data that were included in the sepsis score calculation, as sepsis scores were significantly different between the two groups \((p < .0001)\).

3.2. Cytokine concentrations in serum and colostrum

3.2.1. Interleukin-6

Shown in Fig. 1 is the serum IL-6 concentrations of the control and septic foals measured at the 3 time points evaluated. Relative to control foals, septic foals had significantly lower median serum IL-6 concentrations at each time point: T0 = 30.5 ng/mL \((p = .0003)\); T24 = 36.1 ng/mL \((p = .0007)\); T48 = 35.0 ng/mL \((p = .0016)\). In contrast, the median serum IL-6 concentrations of the control foals at T0, T24 and T48 were 1883 ng/mL, 1955 ng/mL and 1659 ng/mL, respectively. Serum samples with undetectable IL-6 concentrations were set as 0.1 ng/mL for statistical evaluation.

3.2.2. Interleukin-10

Shown in Fig. 2 is the serum IL-10 concentrations of the control and septic foals measured at the 3 time points evaluated. There were no significant differences in serum IL-10 concentrations between the two groups of foals at any of the three sampling times \((all \ p \ values > .19)\). For the statistical analysis and in this figure all samples with undetectable IL-10 concentrations were set as 0.1 pg/mL.

3.2.3. Interleukin-6 and interleukin-10 ratios

We then examined the combination of IL-6 and IL-10 levels together in the control and septic foals. Fig. 3 indicates that healthy control foals have significantly higher IL-6:IL-10 ratios compared to septic foals. The analysis of IL-6:IL-10 ratios was performed using cytokine

<table>
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<tr>
<th>Table 2</th>
<th>Median (range) IL-10 and IL-6 concentrations in serum and colostrum of mares of control and septic foals.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mares of control foals</td>
</tr>
<tr>
<td>IL-6 (ng/mL) (serum, T = 0–48)</td>
<td>3242 (1016–28,183) ((n = 5))</td>
</tr>
<tr>
<td>IL-6 (ng/mL) (colostrum)</td>
<td>11,429 (215–100,973) ((n = 12))</td>
</tr>
<tr>
<td>IL-10 (pg/mL) (serum, T = 0–48)</td>
<td>&lt;4 (&lt;4–172) ((n = 5))</td>
</tr>
<tr>
<td>IL-10 (pg/mL) (colostrum)</td>
<td>&lt;4 (&lt;4–5173) ((n = 12))</td>
</tr>
</tbody>
</table>

The lower limit of detection is 4 pg/mL for the IL-10 assay and 0.78 ng/mL for the IL-6 ELISA.
concentrations as the unit of comparison ($p = .0025$) and also by using ranks as the unit of comparison ($p = .0011$; data not shown). Both comparisons suggested that the IL-6:IL-10 ratio in serum is a valuable prognosticator for neonatal sepsis in foals.

4. Discussion

Although the gene expression of IL-10 (Pusterla et al., 2006) and IL-6 (Gold et al., 2007) in PBMC has been examined in healthy and sick (septic and non-septic) equine neonates, to the authors' knowledge, this is the first time that serum IL-6 and IL-10 protein concentrations in neonatal foals have been measured directly via rapid ELISA (IL-6) and fluorescent bead-based assays (IL-10).

As has been applied in the human medical field, we investigated the usefulness of these cytokine assays in the very early detection of sepsis in neonatal foals. To classify foals as septic, we used a combination of the sepsis score, which is routinely used in equine neonatal medicine (Brewer and Koterba, 1988), as well as the results of blood cultures, considered to be the 'gold standard'. Although sepsis score and positive blood cultures generally agree well, some discrepancies may arise. For example, in this study 5 foals had high sepsis scores but negative blood culture results. The most likely reason for this divergence was deemed prior antibiotic administration that inhibited bacterial growth. Conversely, 1 foal had a sepsis score of 8 but a positive blood culture (Corynebacterium spp.), which was judged not to be a contaminant. These two situations illustrate the usefulness of using both methods to define the septic foal. Nevertheless, the moderate sensitivity and specificity of the sepsis score (Peek et al., 2006) and the time delay for blood culture results (average 2–3 days) highlights the need for a more sensitive and specific rapid test that can identify early, the septic neonate.

Interleukin-6 is a pro-inflammatory cytokine predominantly produced by leukocytes and hepatocytes in response to infection and trauma. In human adults and infants, an increased serum IL-6 concentration has been found to be a sensitive indicator of sepsis. Furthermore, persistent increases in IL-6 are associated with increased duration of hospitalization and increased risk of dying (Buck et al., 1994; Reyes et al., 2003; Goepfert et al., 2004; Latif et al., 2004; Pavcnik-Arnol et al., 2004; Harris et al., 2005). In contrast we found that median serum IL-6 concentrations were highest in the control foals and exceeded median serum IL-6 values in the septic foals by 62-fold at T0, 54-fold at T24 and 47-fold at T48. These results appeared to conflict with the previously reported gene expression that demonstrated no difference in IL-6 mRNA levels in PBMC from healthy foals and from hospitalized septic (Gold et al., 2007) or sick (septic and non-septic) foals (Pusterla et al., 2006) on admission. Can these differences be reconciled? One explanation could be that mRNA expression and secreted protein concentrations are not always linked. However, in human PBMC stimulated with LPS and C3a, IL-6 protein secretion into the culture media was associated with increases in mRNA expression (Fischer et al., 1999). IL-6 protein and mRNA expression levels were also both increased after stimulation of human cord blood mononuclear cells with Streptococcus agalactiae, the major pathogen of neonatal sepsis in humans (Berner et al., 2001). Likewise, Jorgensen et al. (2001) examined effects of sirolimus, a potent immunosuppressive and antiproliferative agent that inhibited cytokine production in human blood and in human PBMC stimulated with various bacterial products, including LPS. A good correlation between IL-10 protein in serum and mRNA expression in CD14+ cells was found (Jorgensen et al., 2001). In equine neonates, the situation seemed to be more complex due to the sometimes massive transfer of IL-6 with the colostrum. Our data demonstrate (1) that prior to ingestion of colostrum, serum IL-6 concentrations are undetectable in healthy foals and (2) that equine colostrum, like colostrum of other animal species, contains IL-6 (Böttcher et al., 2000; Hagiwara et al., 2000; Nguyen et al., 2007; Zanardo et al., 2007). Thus, the healthy control foals—those that most likely ingested adequate quantities of colostrum at birth (as reflected by serum IgG concentrations exceeding 800 mg/dL)—also ingested higher amounts of colostral IL-6. Strong evidence for the transfer of cytokines, including IL-6, from colostrum to serum in the neonate has already been demonstrated in calves (Yamanaka et al., 2003) and piglets (Nguyen et al., 2007). Although maternally derived IL-6 is likely to influence the immune response of the neonate, its exact function on the foal's immune system is still unknown.

Since the leading cause of equine neonatal septicemia is failure of passive transfer (i.e. inadequate colostral intake) this would explain in part why IL-6 concentrations would be higher in healthy foals compared to the septic foals. Thus, it is possible that the relatively low serum IL-6 concentrations detected in the septic foals (30–36 ng/mL) represented at least partially endogenous cytokine production from PBMC responding to the bacteremia. Even so, the response of the two non-surviving septic foals (IL-6 = 30 and 179 ng/mL) was unremarkable, given that IL-6 expression in PBMC of non-surviving septic foals is 15-fold greater than that of surviving septic foals (Gold et al., 2007). Furthermore, the reason why the neonatal foal fails to mount a vigorous IL-6 protein response, as opposed to the septic human infant (Lam and Ng, 2008), is unknown but makes this cytokine less useful as a single marker in the early diagnosis of sepsis.

Fig. 3. Ratio of serum IL-6:IL-10 concentrations of the control and septic foals measured at T0. The ratio of serum IL-6:IL-10 concentration is significantly higher in the control foals versus the septic foals.

Since the leading cause of equine neonatal septicemia is failure of passive transfer (i.e. inadequate colostral intake) this would explain in part why IL-6 concentrations would be higher in healthy foals compared to the septic foals. Thus, it is possible that the relatively low serum IL-6 concentrations detected in the septic foals (30–36 ng/mL) represented at least partially endogenous cytokine production from PBMC responding to the bacteremia. Even so, the response of the two non-surviving septic foals (IL-6 = 30 and 179 ng/mL) was unremarkable, given that IL-6 expression in PBMC of non-surviving septic foals is 15-fold greater than that of surviving septic foals (Gold et al., 2007). Furthermore, the reason why the neonatal foal fails to mount a vigorous IL-6 protein response, as opposed to the septic human infant (Lam and Ng, 2008), is unknown but makes this cytokine less useful as a single marker in the early diagnosis of sepsis.
Interleukin-10 is an anti-inflammatory cytokine that inhibits the production of pro-inflammatory cytokines. In human patients with sepsis, increases in serum IL-10 are associated with a poorer prognosis, a higher risk of multi-organ failure and death (Monneret et al., 2004; Sherry et al., 1996; Giannoudis et al., 2000). Relative to the control group, our septic foal group did not exhibit a significantly higher serum IL-10 concentration at any of the time points. These findings differ from those by Pusterla et al. (2006) who found that gene expression of IL-10 was significantly higher in non-surviving septic foals. Our study was limited in that only 2 septic foals did not survive making it difficult to assess the prognostic usefulness of IL-10 in this population of equine neonates. Interestingly, by far the highest serum IL-10 concentration recorded (857,260 pg/mL) in our study was that of a septic foal that died a few hours after the sample was taken. Although, the other non-surviving septic foal that died shortly after admission, had an IL-10 concentration of only 518 pg/mL, a value that was lower than in the surviving and 3 of the control foals. Serum IL-10 concentrations in equine neonates are not confounded by colostral ingestion (IL-10 in the dams colostrum was generally undetectable). Nevertheless, serum concentrations of this cytokine altered widely in sera of control and septic equine neonates and are thus unlikely to provide useful diagnostic or prognostic indicators of sepsis when used alone.

In human medicine, analysis of multiple biochemical markers and calculation of ratios between 2 or more of these markers have been frequently employed to enhance the diagnostic utilities of cytokine measurement (Lam and Ng, 2008). When we examined IL-6 and IL-10 together, we found that the ratio of IL-6:IL-10 at T0 was significantly higher in healthy control foals compared to septic foals.

5. Conclusion

In conclusion, our study demonstrated that a rapid and reliable method of measuring serum IL-6 and IL-10 concentrations in neonatal foals could be developed. However, based upon the small sample size (30 foals) and low number of fatalities (2 foals) neither serum IL-6 nor IL-10 concentrations alone in neonatal foals enable the early detection of sepsis or reliable prognoses to be generated for clients. However, IL-6:IL-10 ratio appears to have the most promise for use as a prognostic indicator, with significantly higher IL-6:IL-10 ratios in healthy control foals compared to septic foals. A future study with a larger septic foal populations is needed to determine prognostic significance as too few non-surviving septic foals were included in this study. Maternal factors are likely important and should be examined where possible by measuring cytokines concurrently in mare serum and colostrum. Development of serum testing for other biochemical markers along with IL-6 and IL-10 such as C-reactive protein and TNF-α would enable us to build a broader picture of the inflammatory cascade within a septic foal. From there, combinations of diagnostic markers can be used to better assess severity of condition and likely outcome.

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