

ORAL ABSTRACTS

CORTISOL LEVELS IN SEPTIC FOALS (RESPONSIVE OR UNRESPONSIVE TO FLUID THERAPY) IN COMPARISON TO NORMAL FOALS

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Sepsis can lead to chronic release of cytokines, which may blunt the HPA axis and suppress glucocorticoid receptors. Cortisol levels are also prolonged. Relative adrenal insufficiency (RAI) is seen with SIRS/Sepsis and is partially mediated by cytokines. The use of low doses of glucocorticoids has improved outcome of septic human patients.

Objective: To determine ACTH and cortisol levels in septic foals (responsive or unresponsive to fluid therapy), and compare to normal foals of similar ages.

Study design: 15 healthy foals at Cornell's Equine Research Park and 10 septic foals hospitalized at Cornell's Hospital for Animals or Hagyard Equine Medical Institute had blood collected in EDTA tubes. Samples were frozen for batching.

Procedures: Healthy foals were randomized to age-match septic foals. Foals were selected based on sepsis score of > 11, and those either 7/10 responsive or 3/10 unresponsive to fluid therapy. Blood was collected once. ACTH and cortisol levels were measured by chemiluminescent immunoassay.

Results: Preliminary data shows 3/10 foals unresponsive to fluid therapy have high levels of ACTH with normal levels of cortisol. 6/10 foals responsive to fluid therapy had high levels of ACTH and high levels of cortisol. 0/10 foals had normal ACTH and normal cortisol levels.

Conclusions: Foals unresponsive to fluid therapy have abnormally high levels of ACTH but normal levels of cortisol showing abnormal response to the HPA axis. Foals responsive to fluid therapy had high levels of both ACTH and cortisol showing they may be responding normally to sepsis. Further data needs to be analyzed.

PROSPECTIVE EVALUATION OF COAGULATION IN CRITICALLY ILL NEONATAL FOALS

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Objective Coagulopathy is likely an under-recognized complication in critically ill neonatal foals. An objective of this study was to determine if abnormalities in common coagulation parameters of critically ill neonatal foals in the first forty eight hours of hospitalization are associated with outcome.

Procedure 63 sequentially admitted foals less than 72 hours of age at admission were included. Blood was collected at admission (Sample 1), 24 hours (Sample 2) and 48 hours after admission (Sample 3) for platelet count, prothrombin time, partial thromboplastin time, fibrin degradation products, fibrinogen and antithrombin. Reference ranges used were previously described by Barton *et al*¹. Outcome was defined as survival to discharge (S) or death prior to discharge (NS).

Results There were 15 foals with septic shock, 36 with sepsis and 12 with other conditions divided into 43 survivors and 20 non-survivors. Three or more coagulation parameters were abnormal for at least one sample period in 45 foals (71%). Septic shock and sepsis were associated with non-survival; other conditions were associated with survival. Increased concentrations of fibrinogen and antithrombin at progressive sampling periods were associated with survival while progressive decreases in partial thromboplastin time were associated with non-survival.

Conclusion Evaluation of common coagulation parameters assists in predicting outcome in critically ill neonatal foals.

¹Barton MH, Morris DD, Norton N, Prasse KW. Hemostatic and fibrinolytic indices in neonatal foals with presumed septicemia. J Vet Intern Med 1998;12:26-35.

COMPARISON OF THE CLINICIAN WITH A MATHEMATICAL MODEL TO PREDICT SURVIVAL IN HOSPITALIZED NEONATAL FOALS.

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Objective- To determine the accuracy of clinicians to predict survival in hospitalized foals ≤ 7 days of age within two hours of admission; and to compare the results to that of a previously validated mathematical model.

Procedure- Clinicians from five universities and one private referral hospital were asked to record age in days, rectal temperature, ability to stand, presence of a suckle reflex, total white blood count, serum creatinine concentration and anion gap for neonatal foals at admission. In addition, they were asked to predict whether the foal would live or die and to later record the outcome for each foal.

Results- Data was collected on 202 foals. There were 123 foals for which the clinicians predicted an outcome, and all variables needed for the model were recorded. The overall survival rate was 106/123 (86%). Sensitivity and specificity of the clinician to predict survival were 92% and 23% respectively. When the clinician predicted survival, 88% of the foals survived (PPV) and when the clinician predicted non-survival only 33% of the foals died (NPV). Overall accuracy of clinicians to predict the outcome in this population was 83%. The ability of the model to predict survival and non-survival and overall accuracy was similar to that of the clinician.

Conclusion- The use of a mathematical model to predict survival is as accurate as the clinicians at the participating universities and referral hospital. This model may be useful for less experienced veterinarians or veterinarians referring cases for specialized care to give an accurate assessment to clients.

CARDIAC ISOENZYMES IN HEALTHY NORMAL CALVES AND CALVES WITH EXPERIMENTALLY INDUCED ENDOTOXEMIA

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Objective: We designed a pilot study to measure the cardiac troponins I and T (cTnI and cTnT) and CKMB mass in healthy new-born calves, and to compare values for these cardiac biomarkers as well as total CK with age matched calves undergoing experimentally induced endotoxemia.

Procedure: Colostrum fed Holstein bull calves 48-72 hours of age were administered either saline (n=9) or endotoxin (n=10; O55:B5 E.coli LPS @ 10 μ g/kg). cTnI, cTnT, CKMB and total CK were measured in experimental animals at T=0, 3 hr, 6hr, 12 hr and 24 hr post initiation of LPS or saline infusion: cTnI by the ACCESS[®] (Beckman Coulter), cTnT and CKMB using the Elecsys 2010[®]. Measured values for each parameter were analyzed using ANOVA by repeated measure design with significance set at P<0.05.

Results: cTnT was not detected at any time point in experimental or control animals (all < 0.01 ng/ml). CKMB was also undetectable in all but 5 of the 95 samples (range 0.1-0.4 ng/ml). cTnI was significantly elevated in LPS administered calves as early as 3 hours after LPS administration (mean 0.061 ng/ml) compared to controls and group specific baseline values (P<0.05). Total CK measurements were significantly elevated at T=24 hr (mean 404 U/L) in LPS calves compared to baseline and controls.

Conclusions: LPS administration is associated with rapid and statistically significant increases in cTnI in newborn calves, whilst CKMB and cTnT are not detectable in the plasma of healthy or LPS administered calves. Total CK values increase significantly 24 hours following LPS administration.

EVALUATION OF PERITONEAL FLUID LACTATE AS A MARKER OF INTESTINAL ISCHEMIA IN HORSES WITH COLIC

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Objective: To determine if blood lactate values and peritoneal fluid lactate, electrolyte, bicarbonate and glucose values are sensitive indicators of intestinal ischemia in adult horses with acute colic and to develop an equation by using logistic regression to predict the probability of a horse with colic having a strangulating obstruction.

Procedure: Venous blood and peritoneal fluid were sequentially collected from 20 clinically healthy horses and 189 horses admitted for colic to the University of California, Davis. Blood-gas, pH, electrolytes (K^+ , Na^+ , Ca^{++} , Cl^-), glucose and lactate values were determined for blood and peritoneal fluid samples; other values recorded for peritoneal fluid included colour, total protein and nucleated cell count. Information regarding diagnosis, treatment and outcome was retrieved from the medical records.

Results: Abdominal and plasmatic levels of lactate were lower in control horses compared to clinical cases. Horses with strangulating obstructions had a higher abdominal lactate (5.45 mmol/L), than horses with a non-strangulating obstruction (2.03 mmol/L). The strongest correlations with the presence of strangulating obstruction were changes in the colour of the abdominal fluid and values of abdominal fluid, chloride, pH and \log_{10} lactate.

Conclusions: Analysis of the abdominal fluid colour, pH, lactate and chloride can be used for the diagnosis of intestinal ischemia. Abdominal fluid lactate is a better predictor of intestinal ischemia than blood lactate and may aide in early detection of catastrophic abdominal lesions such as intestinal strangulation and rupture.

HEMOPERITONEUM IN THE HORSE: 68 CASES (1989-2004)

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Objective: To identify the clinical findings, etiologies, and outcome associated with hemoperitoneum in the horse and to evaluate factors associated with outcome.

Procedure: The medical records of horses diagnosed with hemoperitoneum were analyzed, excluding cases of post-operative abdominal hemorrhage. Information obtained included history, signalment, physical examination findings, diagnostic test results, and outcome.

Results: Sixty-eight horses were diagnosed with hemoperitoneum. Breed distribution was 28 Thoroughbred, 14 Arabian, 10 Quarterhorse, 5 Warmblood, 3 Appaloosa and 1 each of 8 other breeds. There were 40 mares, 24 geldings, and 4 stallions, with a mean age of 13.8 years (range 1 month to 40 years). Colic was the presenting complaint in 78% of cases. Clinical findings included shock (66%) and pale mucous membranes (58%). Mean HR was 79 bpm (range 30-216), mean RR was 35 bpm (range 8-92), mean PCV was 27% (range 9.4-73) and TP was 5.7 g/dL (range 3.0-9.3). Cause of hemoperitoneum was attributed to trauma in 30.6%, neoplasia in 16.7%, uterine artery rupture in 12.5%, mesenteric injury in 11.1%, DIC in 5.6%, other causes in 2.8%, and undetermined in 20.8%. Fifty percent of the horses survived, 37% were euthanized, and 13% died. Outcome was significantly associated with the clinical finding of shock, respiratory rate, PCV, and diagnosis.

Conclusions: Hemoperitoneum is an infrequent but significant cause of colic in the equine patient. The predominant etiologies for hemoperitoneum in this case series were trauma, neoplasia, and undetermined. Identification of the underlying cause is important because of its impact on outcome.

CHANGES IN CENTRAL VENOUS PRESSURE AND BLOOD LACTATE CONCENTRATION DURING ACUTE BLOOD LOSS IN HORSES

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Objective: To describe the changes in central venous pressure (CVP), central venous oxygen pressure ($P_{CV}O_2$), blood lactate concentration, heart rate, and indirect blood pressure in a model of acute hemorrhage in horses.

Procedure: A volume of 16 mL/kg of blood was removed from seven mares to simulate acute blood loss. Heart rate, indirect blood pressure, $P_{CV}O_2$, CVP, and blood lactate were monitored throughout the study, and during replacement of blood.

Results: A significant decrease in CVP occurred between baseline (8.2 ± 3.5 cm H₂O) and measurements obtained after blood loss (-0.1 ± 3.8 cm H₂O) ($p < 0.01$). The mean blood lactate concentration at baseline (0.7 ± 0.2 mmol/L) and at maximal blood loss (2.2 ± 1.0 mmol/L) was also different ($p < 0.05$). The mean $P_{CV}O_2$ at baseline (32.3 ± 1.2 mmHg) dropped following blood loss (28.9 ± 1.4 mmHg), but this difference was not statistically significant. There was no statistically significant change in heart rate or indirect blood pressure pre- and post-blood loss. CVP returned to baseline values (7.9 cm H₂O, $p < 0.05$), while lactate and $P_{cv}O_2$ approached pre-hemorrhage values, immediately after whole blood replacement.

Conclusion: This study demonstrates alterations in CVP and blood lactate concentration associated with acute blood loss in horses. Changes in heart rate and indirect blood pressure may not be as sensitive at detecting moderate (20% of blood volume) hemorrhagic hypovolemia. Values for CVP, lactate, and $P_{cv}O_2$ improved with administration of whole blood and may therefore be useful in directing replacement therapy.

POLYNEURITIS EQUI: 7 CASES ON 1 FARM

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Objective: Six horses and 1 spinal cord from 1 farm in the western USA were presented to The Ohio State University College of Veterinary Medicine for diagnostic and post mortem evaluation for suspected polyneuritis equi.

Procedure: A history was obtained from the manager and referring veterinarians. Six horses had a complete physical examination and EMG performed. Samples were collected for hematology, serum biochemistries, serum vitamin E, CSF analysis, and serology for EHV 1 and 4, Equine Influenza virus, and EPM. Post mortem examination was performed on all 6 horses and on the isolated spinal cord.

Results: History included several horse demonstrating clinical signs of polyneuritis equi in the broodmare band and over time the disease moved to the show horses. There was no evidence of toxic exposure. Clinical signs included urinary and fecal incontinence, hind limb muscle atrophy, and hind limb weakness. EMG demonstrated prolonged insertional activity, positive sharp waves, and fibrillation. Mild hyperfibrinogenemia, mild hyperproteinemia, mild leucocytosis and mild pleocytosis were detected in most horses. Serum vitamin E concentrations were within normal limits. Serology for the respiratory viruses was positive in most horses. Post mortem changes included chronic lymphoplasmacytic myelitis and radiculoganglioneuritis with axon dropout and demyelination in the cauda equina.

Conclusion: In all cases clinical signs and post mortem findings were consistent with polyneuritis equi. The primary lesions were found in the cauda equina and were inflammatory in nature. The lesions were similar in all cases, although they varied in severity. No cause or etiologic agent was identified.

COMMON VARIABLE IMMUNODEFICIENCY IN FOUR ADULT HORSES

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Objective Common variable immunodeficiency is a rare disorder in adult horses caused by impaired antibody production. Hallmarks of this disease include recurrent bacterial infection, hypogammaglobulinemia and impaired humoral response to vaccination.

Procedure Four adult horses (6 year old Thoroughbred gelding, 14 year old Thoroughbred mare, 8 year old Arabian mare, 12 year old Thoroughbred gelding) were hospitalized between 2001 and 2004 for treatment of presumed acute bacterial meningitis (3) and pneumonia (1). None of the horses were related and all had significant weight loss. Three horses had persistent hypoproteinemia due to hypoglobulinemia; all had lymphopenia. All of the horses responded successfully to treatment and were discharged.

Results Immunologic testing was performed for each horse during hospitalization and every few months upon discharge. All had abnormal lymphocyte distribution, hypogammaglobulinemia and decreased *in vitro* response to mitogens using lymphocyte proliferation assays. Serum immunoglobulin levels were measured using radial immunodiffusion and each horse had persistent IgM deficiency; three of four horses had persistent IgG deficiency. Flow cytometric analysis of peripheral blood lymphocyte subpopulations demonstrated persistent, severe B cell lymphopenia with relative increase in T cells for each horse. Three horses were tested and lacked proper antibody response to vaccination with tetanus toxoid using the comparative toxin-antitoxin neutralization test.

Conclusion Immunologic testing should be considered in adult horses with history of recurrent infections, presumed bacterial meningitis, lymphopenia and hypogammaglobulinemia.

EFFECTS OF OPIOID AGONIST (MORPHINE) AND OPIOID ANTAGONIST (N-METHYLNALTREXONE) ON INTESTINAL MOTILITY IN NORMAL ADULT HORSES

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Although a potent analgesic, morphine may reduce gastrointestinal (GI) function and predispose horses to colic. Methylnaltrexone (MNTX), an opioid receptor antagonist does not cross the blood-brain-barrier. We reported that MNTX directly stimulates isolated smooth muscle strips from equine jejunum and colon (Van Hoogmoed et al. 2004).

Objective: Determine morphine effect on GI transit and the potential reversal with MNTX.

Procedure: 5 horses were randomized and divided in: Group-1, control received saline 10 mL BID; Group-2, received morphine 0.5 mg/kg BID; Group-3, received MNTX+morphine 0.75 mg/kg + 0.5 mg/kg BID. GI function recorded over 3 days included defecation frequency, amount feces produced, GI transit time (barium filled spheres), fecal water content, and borborygmus score. Treatments were compared with ANOVA.

Results: Compared to controls, morphine delayed GI transit for 6 hours. Morphine decreased: defecation frequency from 3.1 ± 1 to 0.9 ± 0.5 defecations, weight of feces produced from 4.1 ± 0.7 to 1.1 ± 0.7 kg, water content from 76 ± 2.7 to 73.5 ± 2.9 % and borborygmus score from 13.2 ± 2.9 to 6.3 ± 3.9 ($p < 0.01$). The GI transit time was delayed by 20-30 hours. MNTX partially prevented the morphine deleterious effect by increasing defecation frequency to 1.5 ± 0.6 , weight of feces 2.6 ± 1.1 kg, water content 75.9 ± 2.5 %, borborygmus score 12.4 ± 3.2 and GI transit delay was partially reversed.

Clinical Relevance: At the dose used, morphine delayed GI transit. MNTX is a potential treatment to morphine therapy in order to prevent GI dysfunction.

VASOPRESSIN FOR THE TREATMENT OF REFRACTORY HYPOTENSION IN EQUINE COLIC

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Objective: To retrospectively evaluate the efficacy of vasopressin (VP) in managing hypotension unresponsive to sympathomimetics during exploratory laparotomy in equine colic patients.

Procedure: From April 2000 through March 2005, 12 adult horses admitted for colic received VP during exploratory laparotomy. These records were reviewed for pre-operative laboratory abnormalities, sympathomimetic therapy, VP dose, heart rate and blood pressure response to VP therapy, intra-operative laboratory abnormalities, and outcome.

Results: The most common pre-operative laboratory abnormalities included hemoconcentration (75%), azotemia (75%), metabolic acidosis (67%), hyperglycemia (83%), and increased serum lactate (92%). Hypotension in all horses was initially treated with sympathomimetics. Two horses received dobutamine and phenylephrine, one received dobutamine and norepinephrine, and nine were given dobutamine, phenylephrine and norepinephrine. The mean VP dose was 0.003 U/kg/min (0.0005 – 0.01 U/kg/min) with a duration of treatment from 25 to 270 minutes. Heart rate decreased in all horses following VP and mean arterial pressure increased in 11 horses. These changes were sustained throughout VP administration. Intra-operative arterial blood samples demonstrated hypoxemia in one horse and metabolic acidosis and increased serum lactate in 12 horses. Six horses were euthanized at surgery, and one died in the recovery stall. Five horses recovered, but three were euthanized within 3 days. Two horses were discharged, but one was euthanized 24 hours after discharge.

Conclusion: VP was effective in the treatment of hypotension refractory to sympathomimetics. However, the need for VP intraoperatively to manage refractory hypotension in the equine colic patient suggests a grave prognosis.

LPS-INDUCED TOLERANCE IN EQUINE PERIPHERAL BLOOD MONONUCLEAR CELLS

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Objective: Endotoxemia is one of the leading causes of morbidity/mortality in equine patients. These effects are primarily due to a profound inflammatory response of the host to LPS released from bacteria. In other species, prior exposure to low concentrations of LPS can render the patient "tolerant" to subsequent lethal LPS challenge. This phenomenon is called endotoxin tolerance. Little is known about this phenomenon in horses. This project examined LPS-induced tolerance in equine whole blood.

Procedure: Heparinized blood was collected from 4 healthy, fit, adult Thoroughbred geldings. Horses were determined to be healthy by physical examination and CBC and fibrinogen concentrations. Whole blood was incubated for 22 hours with (TOLERANT) or without (CONTROL) *E. coli* O55:B5 LPS (10 ng/ml). Cells were washed and equal volumes stimulated with LPS (0, 100, or 1000 ng/ml) for 3 hours. Cells were lysed using ABI Tempus blood stabilization tubes and RNA isolated from each of the groups. cDNA was synthesized from the RNA, and quantitative RT-PCR performed for the genes TNF α , IL-1 β , IL-4, IL-6, IL-10, and iNOS. Gene expression was compared to the calibrator gene, equine GAPDH. Results were analyzed by ANOVA with significance $p < 0.05$.

Results: LPS stimulation of CONTROL cells resulted in a significant, dose-dependant increase in gene transcription of each of the target genes. This response was almost completely abolished in the TOLERANT cells for all genes.

Conclusion: These data suggest that equine blood cells can become tolerized to LPS in a manner similar to that observed in other species.

POSTER PRESENTATIONS

CLINICAL PROGNOSTIC INDICATORS FOR SURVIVAL OF CRITICALLY ILL NEONATAL CRIAS

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Objective: The goal of this study was to identify historical, clinical and laboratory variables, as objective prognostic indicators for survival of critically ill neonatal crias.

Procedure: A retrospective cohort analysis was completed of 78 critically ill neonatal crias (60 alpacas, 18 llamas \leq 4 weeks old), which presented to the CSVM Neonatal-Intensive-Care-Unit at Tufts University (5/1999–3/2005). Neonates presenting for failure of passive transfer (FPT) or trauma without systemic disease were excluded. Statistical outcome analyses included descriptive, univariate and multivariate models, based on the normality of data distribution.

Results: Thirty-six female and 42 male neonatal crias were hospitalized for 4.8 (+/-4) days at a median admission age of 1 day. The most common diagnoses included FPT (63%), neonatal encephalopathy [NE] (33%), lower airway disease (27%), congenital defects (23%), clinical signs of prematurity (22%), confirmed sepsis (15%) and diarrhea (12%). The overall survival was 74% (58/78), whereas 9 crias died and 9 were euthanized due to terminal illness (death unspecified in 2/20). Univariate outcome analyses demonstrated that leukopenia upon presentation ($<6 \times 10^3$ cell/uL, odds ratio [OR]=13.1) and the presence of lower airway disease (OR=4.3) were significantly associated with non-survival ($P<0.01$). Multivariate analysis further confirmed that leukopenic crias were 10.7 times more likely to die, after adjustment for all other covariates. Diarrhea, prematurity, sepsis as well as laboratory result of creatinine, anion gap and fibrinogen upon presentation, did not affect outcome ($P>0.05$).

Conclusions: Early clinical and selected laboratory analyses appear crucial for prediction of survival chances in critically ill neonatal crias.

POST MORTEM EVALUATION OF FATAL ILLNESS IN NEONATAL FOALS

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Objective: The objectives of this study were to evaluate the ante and post-mortem data of critically ill neonatal foals, in order to characterize fatal clinical disease and subsequently provide prognostic value.

Procedure: A retrospective cohort analysis of 511 neonatal foals (<28 days of age) presented to Cummings School of Veterinary Medicine at Tufts University between 1990-2003, and the post-mortem reports of 87/113 non-survival patients was performed. Statistical methods included descriptive, paired samples T-test and Mann Whitney U analyses, based on the normality of data distribution.

Results: Multi-system (>1 body system) changes were observed in 90% of necropsied foals (mean incidence: 3 systems/animal). Pathologic conditions within the respiratory tract (67/87; 77%) and gastro-intestinal system (66/87; 76%) were most common, although the incidence of gastric ulceration was low (15/87; 17%). Immunoglobulin data was available for 409 admitted foals. IgG levels >800 mg/dL were associated with a significantly lower mortality rate (14.7%) compared to foals with failure of passive transfer (FPT) upon presentation (34.5% fatality if IgG <800 mg/dL; $P<0.0001$). A high sepsis score (7 vs. 13; $P<0.001$) was also related to poor patient outcome. Univariate analysis further revealed that non-survivors were slightly, but significantly older upon presentation than surviving neonatal foals (2 vs. 1 day of age; $P<0.001$).

Conclusions: Prompt identification and referral of critically ill equine neonates, early recognition of FPT and sepsis as risk factors for non-survival, as well as aggressive medical management of multi-system disease may enable the clinician to improve survival of critically ill neonatal foals.