An Empty Colon is the Devil's Playground

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Colic, defined as abdominal pain, is a common gastrointestinal disease among horses. While that five-letter word traditionally comes with a stigma of dire circumstance, recent studies have painted a more optimistic picture. According to a recent study by the Morris Animal Foundation, 74.9% of horses survived colic surgery and had a median survival time of 79.2 months post-operatively. The most common surgical finding in this study was non-strangulating displacement of the large intestine, which accounted for 37.3% of intraoperative diagnoses (Immonen, 2017). The large intestine of the horse is a nearly 100-foot vat containing a lethal population of bacteria, that even in instances of relatively benign causes of colic, can lead to increased morbidity and mortality.

A right dorsal displacement of the large colon occurs when the left dorsal and ventral colon becomes trapped between the cecum and the right body wall and can be associated with a large colon impaction. While medical management is an option for treating this type of colic, surgical correction is necessary with persistent or severe abdominal pain and worsening abdominal distension. Statistical prognosis for horses with right dorsal displacement can exceed 90% (Southwood, 2014). However, the post-operative period can be associated with high morbidity and mortality even after the primary cause of colic is resolved. Endotoxemia is one complicating factor with particularly deadly consequences that can be difficult to prevent and control.

On the evening of January 5, 2018, Angel Martin, a 13-year-old Spotted Saddlehorse mare, presented to MSU-CVM Equine Services for a two-day history of colic. She was seen by two different veterinarians prior to presentation; one on the night of the 4th where she was initially tachycardic with absent borborygmi and was treated with sedation, flunixin meglumine, and 6 liters of water via a nasogastric tube. The following morning, she had passed two small

fecal piles and eaten some hay but continued to be painful. She was transported to the second referring veterinarian who administered flunixin meglumine orally and mineral oil via nasogastric tube. Diagnostic bloodwork was also performed there, which showed a lymphopenia, markedly elevated glucose and total bilirubin as well as a mildly elevated CK. Rectal palpation revealed a suspected impaction on the left side, so she was referred to MSU-CVM Equine Service for further evaluation.

Coming off the trailer, Angel was bright, alert and responsive and remained quiet in the stall. She weighed 1,335lbs with a body condition score of 7/9 and her abdomen appeared distended. She was tachycardic with a heart rate of 60 beats per minute but normothermic with a rectal temperature of 100.3F and a normal respiration rate of 12 breaths per minute. Her mucous membranes were pink and she had a capillary refill time of 2 seconds. A nasogastric tube was present in the left nostril as well as a left jugular catheter. Gastric lavage retrieved no reflux of stomach contents. Once Angel was deemed stable, further diagnostics were performed including an abdominal ultrasound exam, rectal palpation and screening bloodwork. Bloodwork results were not remarkedly changed from those at the referring veterinarian; CBC results showed a neutrophilia and lymphopenia, chemistry revealed a markedly elevated glucose, total bilirubin and mildly elevated CK as well as a decreased sodium, phosphorous and magnesium. Stall side lactate was 2g/dL, normal of which is considered less than 2g/dL. Please include numbers and normal reference ranges for all lab values for tests performed at MSU

On abdominal ultrasound, the intestines appeared to be displaced to the right caudal quadrant. The small intestine visualized was dilated, had good motility and mixing, but lacked propulsive movement. There was a large distended viscus in the right caudal aspect where the cecum should have been and the cecal band could not be imaged. Nearly the entirety of the left

side was occupied by a large distended viscus, which had a taenial band consistent with the ventral colon. Angel was then sedated with 1mg/kg of Xylazine intravenously and given 0.3mg/kg of Buscopan intravenously for rectal palpation. Just off midline, running dorsoventrally, a thickened, turgid structure could be felt in the normal anatomical location of the cecal band. However, this mass was larger and firmer than the cecum. On palpation further cranially, a firm, turgid viscous of unknown origin could be felt. From Angel's diagnostic workup, it was determined that she likely had a right-sided large colon displacement, large colon impaction and small intestinal ileus. She was also hyperbilirubinemic (give value and normal range) and in a negative energy balance due to the prolonged duration off feed. Due to her ongoing discomfort and a willingness by her owners to pursue surgery, it was determined that Angel would undergo surgical correction of the suspected large colon displacement and impaction.

At approximately 5:15pm, Angel was prepared for surgery and pre-surgical antimicrobials, gentamicin 6.6mg/kg intravenously and potassium penicillin 22,000 iu/kg intravenously were administered as well as and flunixin meglumine at 1.1mg/kg also intravenously. Angel was then induced under general anesthesia. Immediately upon entering the peritoneal cavity, the gas distended cecum was visible in its normal position with healthy tissue characterized by pink serosa. The small intestine was diffusely dilated with gas and fluid ingesta. The colon was exteriorized and the pelvic flexure and remainder of the ascending colon were identified to the right of the cecum confirming the right dorsal colon displacement. At this time, a small area of petechial hemorrhage and an approximately 2.5cm rent could be seen in the mesentery near the right dorsal colon. A pelvic flexure enterotomy was performed to remove fecal content and resolve the impaction. The length of the small intestine was traced from the

ileocecal orifice to the duodenocolic band and found to be moderately distended with gas and ingesta, which was milked into the cecum and the intestines replaced in their normal anatomic position. The abdomen was lavaged with saline, gentamicin and penicillin infused saline and closed routinely. Recovery was slow and Angel appeared to be weak, making several unsuccessful attempts before eventually standing.

While catabolic states interfere with many important functions in the maintenance of homeostasis, one organ system prone to deadly consequences is the gastrointestinal tract. In the healthy horse, the mucosal epithelium provides a barrier against the translocation of bacteria through tight junctions, mucous lining the gut, epithelial cell enzymes, local immunity and circulating antibodies. Disease results when these protective mechanisms are overcome as is the case in intestinal lesions that cause hypoxia, mucosal damaging inflammation, mechanical trauma or intraluminal acidification. When specific components of gram negative bacteria, a normal inhabitant of the equine gastrointestinal tract, gain access and circulate in the bloodstream, a systemic, pro-inflammatory condition known as endotoxemia occurs. Horses are exquisitely sensitive to these components, known as lipopolysaccharides that are released from the cell wall structure of these bacteria.

The clinical signs associated with endotoxemia closely mirror those of sepsis and systemic inflammatory response syndrome (SIRS). They include a change in body temperature, tachycardia, tachypnea and white blood cell distribution changes, particularly leukopenia. While sepsis implies infection (either real or presumed) and endotoxemia refers to a circulating component of bacteria in the blood, their clinical manifestations are the same and can be thought of as the same condition. To add to the point of characterization, relatively little is known about sepsis in horses by causes other than endotoxemia (Smith, 2015). When endotoxin initially gains

access to the blood stream, considered the “hot phase”, an inflammatory cascade is initiated and results in clinical signs of mild tachypnea, depression, restlessness and rectal temperature begins to rise. Mucous membranes are pale in early phases but become congested and hyperemic with the classic toxic line around the gingiva after about 90 minutes (Smith, 2015). Intestinal sounds cease and heart rate increases as abdominal discomfort peaks leading to signs of colic. About 4-6 hours after endotoxin exposure, horses are febrile, tachycardic and tachypneic in attempts to compensate for systemic hypotension. As untreated inflammation progresses to SIRS, vascular damage from a massive influx of cytokines and local tissue damage leads to intravascular coagulation seen clinically as petechial and ecchymotic hemorrhages. Ventral edema appears around the pectorals as fluid leaks into the interstitial space. Severe sepsis occurs when SIRS progresses to organ dysfunction eventually leading to septic shock characterized by acute hypotension that is nonresponsive to efforts of volume resuscitation. As multiple organ systems begin to fail, known as MODS, signs of circulatory failure persist with weak pulses, cold extremities, muscle tremors, oliguria and often death.

In the horse, unlike other species, laminitis is a life-threatening component associated with MODS. Due to the unique blood supply of the hoof, it is intensely affected by ischemic events. Furthermore, when there are increased levels of inflammatory mediators, matrix metalloproteinases are upregulated, which play a role in the separation of the lamellar cells from their basement membranes, resulting in separation of dermal and epidermal laminae (Hyman, 2015). Clinically, horses with laminitis will first show increased digital pulses and shifting leg lameness, which progresses rapidly as the secondary epidermal laminae die and P3 rotates in the hoof capsule further compromising blood supply.

Endotoxemia as a clinical syndrome in equine patients was first recognized more than 45 years ago (Smith, 2015). The term endotoxin refers to the principal component of all gram-negative bacteria, lipopolysaccharide (LPS). It is estimated that LPS is detected in 25-35% of colics (Bowser, 2017). LPS is composed of three domains; the O-specific side chain, a core polysaccharide and finally the toxic principle, Lipid A. The pathogenesis of the body's response to endotoxin is a complex series of receptor interactions, beginning with the innate immune system. LPS is detected by the immune system through damage associated molecular patterns (DAMPs) and pathogen associated molecular patterns (PAMPs). Soluble and cell-associated receptors, known as pattern-recognition receptors (PRRs) circulate throughout the body to detect DAMPs and form the PRR-DAMP complex. The interaction between DAMPs and PRRs is the initial event in the innate immune response that results in the clinical presentation of endotoxemia (Smith, 2015). To form this interaction, LPS first complexes with the acute phase protein LPS binding protein (LBP) to facilitate recognition by the CD14 receptor on mononuclear phagocytes. This complex initiates a cytoplasmic signaling cascade of cytokines and inflammatory mediators via Toll-Like Receptors (TLR). Because TLRs have transmembrane and intracellular components, they initiate intracellular signaling pathways, assisted by the protein MD-2 (Moore, 2005). The result of this complex signaling stimulates the adaptive immune system as TLR receptors exist on many cells in many different tissues. Proinflammatory cytokines, chemokines, coagulants, enzymes and acute phase proteins create a viscous cycle of amplification as tissues subject to damage make more DAMPS and provide positive feedback to amplify the entire system.

While the stimulation of the innate and adaptive immune system produces a myriad of mediators of inflammation, there are a few key players in endotoxemia including eicosanoids,

tissue necrosis factor, interleukin-1 and tissue factor, which will be discussed here. When endotoxin binds with macrophages, it activates the arachidonic acid cascade responsible for the inflammation and hemodynamic dysfunction seen early in endotoxemia. The important biproducts of the arachidonic cascade include thromboxane A2, prostaglandin I2 and leukotrienes. Thromboxane A2 causes vasoconstriction and platelet aggregation while prostaglandin I2 counterbalances to cause vasodilation and inhibit platelet aggregation. Clinically this is seen as pulmonary hypertension and systemic arterial hypotension leading to inadequate tissue perfusion and coagulopathies (Morris, 1991). Leukotrienes also produced by the arachidonic cascade work on microcirculation and cause bronchoconstriction, vasoconstriction and increased capillary permeability for a combined effect of tissue ischemia. Simultaneously, Leukotriene B4 is a neutrophil chemoattractant that further leads to tissue ischemia and damage in the microcirculation (Morris, 1991).

Much of the acute phase response to endotoxemia is attributed to interleukin-1 (IL-1), which causes fever, chemotaxis, muscle degradation and a reduction in albumin synthesis. Clinically, IL-1 is associated with the congested mucous membranes and prolonged capillary refill time. Microvascular injury and hypotension continues as IL-1 promotes the accumulation of large numbers of neutrophils on the surface of endothelial cells. Clinicopathologically, this is seen as a profound leukopenia characterized by a neutropenia. As the bone marrow begins to respond to the dramatic increase in neutrophil use, colony stimulating factors churn out more neutrophils, leading to a left-shift.

The central mediator of endotoxemia is tumor necrosis factor (TNF), or cachectin. In a study performed on chimpanzees, anti-TNF monoclonal antibody was administered immediately following administration of endotoxin. In these animals, the anti-TNF completely prevented

serum activity and reduced the production of other important inflammatory mediators, supporting its central role in endotoxemia (van der Poll, 1994). TNF acts quickly, reaching peak serum concentrations in 90-120 minutes and initiates a cascade of responses that include hypotension, hemoconcentration, metabolic acidosis, and disseminated intravascular coagulation (Moore, 2001). As systemic hypotension leads to poor perfusion, tissues become hypoxic and anaerobic metabolism takes over producing lactic acid. Clinicopathologically, hyperlactatemia occurs when serum concentrations exceed 2mmol/L.

Coagulopathies associated with endotoxemia can have deadly outcomes when pro-coagulation exceeds anticoagulation. Tissue factor, or thromboplastin is synthesized by monocytes, macrophages and endothelial cells in response to endotoxin and is exposed to coagulation factor VII, which stimulates the extrinsic arm of the coagulation cascade (Moore, 2001). Simultaneously, endotoxin binds to complement proteins to initiate the lectin-dependent and alternative pathways of complement activation through coagulation factor XII (Hageman factor) (Smith, 2015). The result is intravascular coagulation exacerbated by cellular damage products that activate complement. Coagulation abnormalities show up in blood work as prolonged PT, PTT, APTT, FDP, ATIII and decreased platelet counts.

Dr. James Moore describes four main goals for the treatment of endotoxemia: 1) prevention of movement of endotoxin into the circulation, 2) neutralization of endotoxin before it interacts with inflammatory cells, 3) prevention of the synthesis, release, or action of inflammatory mediators, and 4) prevention of endotoxin-induced cellular activation (Moore, 2001). However, clinically the first goal of treatment of endotoxemia is to stabilize, support and monitor the patient. Early interventional strategies in treating the source of colic address the continued movement of endotoxin into the circulation. The mainstay of treatment for

neutralizing endotoxin has been polymyxin B, an antibiotic that prevents lipid A from binding to inflammatory receptors. However, polymyxin B is associated with dose dependent acute tubular nephrosis and must be used with caution. Biosponge is a smectite product used to bind *Clostridium dificile* enterotoxins in the GI tract that is commonly used in cases of colitis. Less commonly used products include hyperimmune serum containing anti-endotoxin antibodies from animals vaccinated against pathogenic gram-negative bacteria. However clinical and experimental trials have reported mixed results on successful treatment.

Controlling the immune system's response to endotoxin is arguably the most important aspect of treatment. Nonsteroidal anti-inflammatories, specifically flunixin meglumine is the most effective in preventing endotoxin-induced synthesis of inflammatory mediators (Moore, 2001). However, non-steroidals are not without their drawbacks including gastrointestinal ulceration and renal papillary necrosis. Other immunomodulating therapies have been described such as steroids, omega-3 fatty acids, monoclonal antibodies, platelet activating factor receptor antagonists and pentoxifylline. Controlling intravascular coagulation is centered on subcutaneous heparin administration, oral aspirin and less commonly plasma and whole blood transfusion. The free radical scavengers Lidocaine and DMSO are also frequently employed in endotoxin therapy. Last, and probably most important, is to institute immediate laminar cryotherapy to protect the hooves from laminitis. Many would argue that, "we can't save their lives if we don't save their feet."

Over the course of the first night and the duration of Angel's stay, she became intermittently febrile, which was controlled with flunixin meglumine. She was maintained on intravenous fluid therapy consisting of a balanced polyionic solution (Hartman's Solution) at a rate of 3L/hr . To treat her pain, small intestinal ileus, and provide anti-endotoxic effects, she was

given a Lidocaine constant rate infusion at 90mg/kg/hour, which was adjusted according to her level of pain control and side effects of ataxia. Antimicrobial therapy was administered in the form of potassium penicillin (22,000iu/kg intravenously every 6 hours) and gentamicin (6.6mg/kg intravenously every 24 hours) for the first two days and enrofloxacin (5mg/kg intravenously every 24 hours) thereafter as she continued to show signs of a fever of unknown origin. On the second day post-operatively, she began refluxing large volumes of gastrointestinal fluids and ultrasound imaging showed she was having small intestinal ileus. Metoclopramide (0.3mg/kg/hr intravenously) and acepromazine (0.01mg/kg intramuscularly)were administered as promotility treatments. As she continued to receive flunixin meglumine for pain control, ranitidine (7mg/kg orally every8 hours), sucralfate (25mg/kg orally every 8 hours) and Gastrogard (1per pound dose orally every 24 horus) were given orally for gastroprotection. She received DMSO for inflammation at 480ml every 24 hours and polymixin B at 6,000U/kg every 8 hours to help control the inflammatory effects of endotoxemia. Angel's feet were continuously iced from the fetlock down and changed every hour as needed to help prevent laminitis.

Clinicopathologically, the white blood cell count dramatically dropped to 1.8 (reference 5-11.9) but then rose to within reference values on the 5th day. Postoperatively her total bilirubin remained high at 10.7 mg/dL (normal range: 0.2-3.5) and hyperlipidemia was suspected, given her high body condition score. Triglycerides were measured on each day thereafter (115mg/dl, 488mg/dl, 257mg/dl 120mg/dl respectively, normal reference range: 11-59) indicating that Angel was mobilizing abnormally high volumes of fat to replace her nutritional deficit. Her ALP liver values were moderately elevated (167 U/L, 332 U/L, 388 U/L, 510 U/L on days 2-5 respectively. Normal range: 61-153) but other liver parameters were within normal limits save for a low total protein (5.4 g/dl, 5.6 g/dl, 5.9 g/dl, 5.8 g/dl on days 2-5 respectively. Normal range: 6.1-8.4).

Heparin at 0.3mg/kg was started subcutaneously in the pectoral muscles to aid in reducing activity of Hormone Sensitive Lipase, reduce adhesion formation at the enterotomy site and help to treat intravascular coagulation. She began receiving IV parenteral nutrition in the form of a 2% dextrose solution. Her fluids were also supplemented with calcium, magnesium and potassium chloride.

Throughout her stay post-operatively, Angel showed the classic clinical signs of endotoxemia including fever, tachypnea, tachycardia, injected mucous membranes and abdominal discomfort. She developed ventral edema around her mandible and pectorals and was rested on a head stand. On the 5th day of hospitalization she also began to have foul smelling, watery diarrhea, which she was very uncomfortable passing. Around 6:30pm on January 9, 2018, Angel's heart rate rose to 80 beats per minute and she became increasingly uncomfortable. Her mucous membranes were bright red, she was extremely agitated and was seen kicking at her flank. She began to have widespread muscle fasciculations and became laterally recumbent. She was sedated with xylazine (0.8mg/kg intravenously) and butorphanol (0.01mg/kg intravenously) with dexmedetomidine (0.01mg/kg intravenously) to control her pain, which was unsuccessful, and a solution of morphine (160mg) , lidocaine (2,000mg) and ketamine (3,840mg) in lactated ringers was titrated and administered according to pain control. Unfortunately, Angel died at approximately 3:30am.

Postmortem exam revealed fibrinonecrotic colitis and with widespread petechiation and purpuric hemorrhage in multiple organs. Histopathology showed the superficial 2/3 of the colonic mucosa was diffusely necrotic and covered by a layer of fibrin and cellular debris. The underlying lamina propria was infiltrated by moderate numbers of lymphocytes and plasma cells

and the tunica submucosa was edematous and contain dilated, blood filled vessels, some of which contain fibrin thrombi.

Horses that have been off feed for a prolonged period can present in a negative energy balance, which results in the mobilization of fat to the liver to used for energy. Supplementing nutritional needs in horses with large colon impaction and displacement can be challenging as small intestinal ileus can prevent enteral feeding, which is critical for health and maintenance of the gastrointestinal tract and mucosal barrier. As the gastrointestinal tract is further starved for nutrients, a proliferation of gram negative anaerobic bacteria occurs including *Salmonella* and *Clostridium*. This proliferation combined with a breakdown in the mucosal barrier can lead to bacterial translocation and endotoxemia, a life-threatening condition of uncontrolled inflammatory response. Therefore, while right dorsal displacements are a relatively benign form of colic and associated with good surgical success rates, complicating factors endotoxemia can drastically increase morbidity and mortality.

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