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ASSESSMENT OF THE SERUM AMYLOID A ASSAY FOR DIAGNOSING DISEASE IN NEONATAL FOALS

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ASSESSMENT OF THE SERUM AMYLOID A ASSAY FOR DIAGNOSING DISEASE IN NEONATAL FOALS

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
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By

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Lexington, Kentucky

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ABSTRACT OF THESIS

ASSESSMENT OF THE SERUM AMYLOID A ASSAY FOR DIAGNOSING DISEASE IN NEONATAL FOALS

Diagnosing disease in equine neonates poses a challenge for the equine industry because of the nonspecific manifestations of many diseases and the rapid deterioration that occurs. The differential diagnostic procedure requires many laboratory tests, whose results take days to receive. Serum amyloid A (SAA) is the only major acute phase protein identified in the horse; it exists in low levels in the healthy horse and increases over 100 fold in response to inflammatory stimulus 6-8 hours post stimulus. A point of care test allows veterinarians to obtain a SAA concentration within minutes that indicates the existence of infection. Being able to test and quantify this protein at the onset of illness may reduce the time before treatment is initiated and therefore increase the chance of survival for the equine neonate, which would greatly help a large problem in the industry.

KEYWORDS: equine neonate, neonatal disease, serum amyloid A, differential diagnosis, assay application

Samantha Whitney Strouss

September 13, 2018

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This thesis is dedicated to my grandfather, Dr. Albert ‘Bud’ Strouss. Although our time in this life together was brief, I am the scholar and avid horsewoman I am, because of the scholar and horseman you were. Grandpa, thank you for looking after me throughout this journey from above and thank you for giving me the inspiration to not only follow my passion for horses, but to turn it into a career and a way to give back to the animal that has given so much to me.

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Overview

The first chapter of this thesis is a literature review that serves to provide the reader with a foundation of information that will prepare them for the work presented in the following chapters. The literature review presents background information on the concepts presented in the chapters that follow and summarizes the literature published regarding the topics related to the research projects presented.

The second chapter provides an introduction to the original studies that are presented in the later chapters of the thesis. It explains the rationale behind the thesis. Finally, it presents the specific hypotheses and objectives of each study.

The third and fourth chapters of the thesis present original studies in the form of manuscripts submitted to peer-reviewed journals. They follow the style required by the specific journals to which they were, or will be, submitted. Each chapter presents an abstract, introduction, materials and methods, results, and discussion and clinical relevance section.

Chapter One: Literature Review

Assessing the application of measuring serum amyloid A (SAA) as a marker of disease in the equine veterinary practice

Mortality and morbidity rates of sick equine neonates are a cause of concern in the equine clinical setting. Equine neonatal diseases can progress quickly and despite aggressive treatment, neonates often succumb to the rapid progression of disease and are unable to recover. Diagnosing equine neonatal diseases based on clinical symptoms is difficult due to the nonspecific manifestations of many diseases; however, obtaining an accurate diagnosis within a critical time frame is essential to improving the prognosis of the patient. The following pages describe many common equine neonatal diseases that are diagnosed at equine veterinary hospitals as well as the diagnostic procedures and treatments commonly used to diagnose and treat these diseases.

Pneumonia

Lower respiratory tract infections are common in neonatal foals and are one of the leading causes of morbidity and mortality in equine neonates (Reuss and Cohen, 2015 and McKenzie, 2006). Pneumonia in neonates is most often associated with septicemia and can develop in utero or at the time of parturition (Reuss and Cohen, 2015 and McKenzie, 2006). In utero it is possible to develop pneumonia due to infected fetal membranes or via aspirating contaminated fetal fluids (Reuss and Cohen, 2015). During, or shortly after, parturition, pneumonia can develop due to foreign microbes invading the bloodstream via the respiratory tract, gastrointestinal tract, or umbilicus (Reuss and Cohen, 2015). This invasion can result in systemic inflammatory response syndrome, which can manifest as generalized or localized clinical signs of sepsis including acute

lung injury or respiratory distress (Reuss and Cohen, 2015). While it is possible for mixed infections and gram-positive infections to occur, neonatal pneumonia is most often caused by gram-negative bacteria (McKenzie 2006). The most common gram-negative bacteria that causes neonatal pneumonia is Escherichia coli, but other bacteria that cause neonatal pneumonia include *Klebsiella spp*, *Actinobacillus spp*, and *Salmonella spp* (Reuss and Cohen, 2015).

Pneumonia can develop secondary to meconium aspiration or milk aspiration in neonates (McKenzie, 2006). Meconium aspiration occurs in utero or during parturition (Reuss and Cohen, 2015). Meconium aspiration most commonly occurs due to mechanical airway obstruction, chemical pneumonitis, alveolar edema, or displacement of surfactant (Reuss and Cohen, 2015). Despite the meconium being sterile, bacterial pneumonia develops due to the compromised respiratory functions of the neonate (Reuss and Cohen, 2015). Milk aspiration most commonly occurs in neonates that have a weak suckle or dysphagia (Reuss and Cohen, 2015). Other causes of milk aspiration can include improper nasogastric feeding tube placement or improper bottle-feeding (Reuss and Cohen, 2015).

Diagnosing neonatal pneumonia includes a comprehensive patient diagnostic evaluation. A physical exam including thorough auscultation of the thorax and a rebreathing examination is conducted (Reuss an Cohen, 2015). Thoracic radiographs provide information regarding the distribution and severity of the pneumonia as well as giving insight to the cause of the bacterial infection (Reuss and Cohen, 2015). Ultrasonography may be used to determine pathologic abnormalities in the pleural space (Reuss and Cohen, 2015). Comprehensive bloodwork, including white blood cell

concentration with a differential cell count, plasma fibrinogen, and globulin determination can provide insight into the severity of the disease and a blood culture should be obtained to identify the causative bacterial organism (Reuss and Cohen, 2015).

Treatment of neonatal pneumonia involves a combination of antimicrobial, anti-inflammatory, and ancillary therapies (McKenzie, 2006). When determining which therapies to utilize, the veterinarian must consider the organisms involved, the severity or the illness, and the ability to administer the drug (McKenize, 2006). Because neonatal pneumonia is most commonly a result of septicemia, a broad-spectrum antimicrobial drug is used (McKenzie, 2006). Common antimicrobials used to treat neonatal pneumonia include β-lactam antibiotics such as ceftiofur or ampicillin in combination with an aminoglycoside antimicrobial such as amikacin (Reuss and Cohen, 2015). Once the results from the blood culture are obtained, it is possible to develop a more specifically targeted antimicrobial regimen. Nonsteroidal anti-inflammatory drugs are often included in a treatment plan to decrease fever and discomfort (McKenzie, 2006). Supplemental oxygen is often provided to foals with neonatal pneumonia via nasal insufflation (McKenize, 2006). Other ancillary therapies include rest, temperature control, the use of bronchidilators, and the use of gastric acid suppressive drugs (McKenzie, 2006). While neonatal pneumonia can be a severe and deadly disease, recovery with proper treatment and management is possible and does not have a negative impact on performance later in life (Reuss and Cohen, 2015).

Hypoxic Ischemic Encephalopathy

Hypoxic ischemic encephalopathy (HIE) is the most common neurological disorder in equine neonates (Lyle-Dugas et al., 2016). Hypoxic ischemic insults affect the central nervous system (CNS). Insufficient amounts of oxygen reach the brain and insufficient amounts of glucose reach the CNS system causing a cascade of changes to occur in order to allow neurons to react to the deficient supply of energy (Galvin and Collins, 2004). Maternal predisposing factors that contribute to HIE include respiratory disease, endotoxemia, hemorrhage, anemia, surgery, and cesarean delivery (Galvin and Collins, 2004). Placental predisposing factors that contribute to HIE include placentitis, chronic uteroplacental separation, and acute or premature uteroplacental separation (Galvin and Collins, 2004). Fetal predisposing factors that contribute to HIE include twinning, congenital abnormalities, dystocia, aspiration of meconium, sepsis, prematurity, and dysmaturity (Galvin and Collins, 2004).

Neonates with HIE may begin to display clinical signs immediately following birth, however, neonates with HIE may appear normal at birth and develop clinical signs between 12 and 72 hours of age (Tennet-Brown et al., 2015). The most common clinical signs of HIE are alterations of consciousness, inability to suckle, tongue protrusion, star-gazing, and local or generalized seizures (Lyle-Dugas et al., 2016). While HIE is a disease of the CNS, other systems are typically affected including gastrointestinal tract, kidneys, heart, lungs, liver, adrenal glands, and parathyroid glands (Tennet-Brown et al., 2015).

Diagnosing HIE is typically achieved by excluding infectious or congenital diseases including sepsis, hypoglycemia, prematurity, dysmaturity, bacterial meningitis,

hydrocephaly, epilepsy, liver disease, viral encephalitis, and toxicosis (Tennet-Brown et al., 2015; Lyle-Dugas et al., 2016). Laboratory results may assist in the diagnostic process. Often foal with HIE will have elevated creatinine levels, but does not indicate HIE outright (Tennet-Brown et al., 2015). A cerebral spinal fluid analysis will rule out meningitis. Electroencephalography and computed tomography may be useful diagnostic tools in equine neonates, but it is often difficult or impractical to perform these diagnostic tools (Tennet-Brown et al., 2015).

There is no definitive treatment for HIE, but most common treatment plans for HIE include supportive therapy, anticonvulsive therapy, nutritional support, fluid therapy, antimicrobial therapy, and respiratory support (Tennet-Brown et al., 2015; Gavin and Collins, 2004). Excellent nursing care and careful monitoring is crucial to the successful outcome of neonates with HIE (Tennet-Brown et al., 2015). Neonates should have stimuli reduced and should be kept clean and dry in sternal recumbency (Gavin and Collins, 2004). Seizure activity can be treated with diazepam, phenobarbital, or a midazolam constant rate infusion (Tennet-Brown et al., 2015; Gavin and Collins, 2004). Neonates that are unable to stand to nurse or nurse without aspirating require enteral feedings via an indwelling feeding tube (Tennet-Brown et al., 2015; Gavin and Collins, 2004). Foals that cannot tolerate enteral feedings require nutritional supplementation via parenteral nutrition (Tennet-Brown et al., 2015). Fluid therapy is typically used to optimize tissue perfusion and arterial oxygenation, however, it must be monitored carefully as administering too much fluid can results in an increase in cerebral edema (Tennet-Brown et al., 2015; Gavin and Collins, 2004). Broad-spectrum antibiotics are typically administered due to an increased risk of infection in neonates with HIE (Tennet-

Brown et al., 2015; Gavin and Collins, 2004). Supplemental oxygen may be administered and caffeine may be administered if apnea is noticed (Gavin and Collins, 2004). The prognosis for neonates with HIE is good with the survival rate being at least 80% (Tennet-Brown et al., 2015). Neonates that recover from HIE are not expected to have long-term health impacts and athletic performance is not thought to be impacted (Tennet-Brown et al., 2015).

Gastrointestinal Diseases

There are many different gastrointestinal diseases that can affect a neonate in the first days of life. Two categories of equine neonatal gastrointestinal diseases are nondairrheal disorders and diarrheal disorders. Within diarrheal disorders there are infectious and non-infectious disorders. Nondiarrheal disorders of the gastrointestinal system in equine neonates are relatively uncommon whereas diarrheal disorders are more common (Ryan and Sanchez, 2005).

Gastric ulceration is nondiarrheal gastrointestinal disease that can cause great harm to a neonate. Clinical signs include inappetence or sign of colic, but are often not displayed until the case is severe or until rupture has occurred (Ryan and Sanchez, 2005). Gastric ulceration is diagnosed via gastric endoscopy (Ryan and Sanchez, 2005). Treatment for gastric ulceration depends on the health status of the neonate and it is important to consider other conditions the may coexist when determining a treatment plan. Histamine type 2 antagonists inhibit gastric secretions and proton pump inhibitors block hydrogen secretion (Ryan and Sanchez, 2005). Sucralfate is commonly administered; it works by adhering to ulcerated mucosa, stimulating mucus secretion, and

enhancing prostaglandin E and epidermal growth factor synthesis (Ryan and Sanchez, 2005).

Ileus is a nondiarrheal gastrointestinal disease of equine neonates that has many causes, but it is commonly a secondary disease discovered in neonates with prematurity, sepsis, or HIE (Ryan and Sanchez, 2005). Foals with ileus typically present with gastric reflux or abdominal distention and typically display depressed mentation (Ryan and Sanchez, 2005). Reflux and abdominal circumference should be monitored closely and the abdomen should be examined via ultrasound (Ryan and Sanchez, 2005). The small intestine may appear fluid filled and amotile (Ryan and Sanchez, 2005). While reflux is present the neonate should not be fed and if large amounts of reflux are present it should be removed via a nasogastric tube (Ryan and Sanchez, 2005). Supportive care including fluid therapy and nutritional support is often necessary (Ryan and Sanchez, 2005).

Meconium impaction is a very common cause of colic in equine neonates (Ryan and Sanchez, 2005). Meconium is made of sloughed cells, swallowed amniotic fluid, and mucus and is formed during fetal development (Ryan and Sanchez, 2005). Clinical signs of meconium impaction typically develop between 12-24 hours of age (Ryan and Sanchez, 2005) and include depression, abdominal distention, rolling, straining, tail-raising, and restlessness (Ryan and Sanchez, 2005). A diagnosis of meconium impaction is based on history, clinical signs, and age (Ryan and Sanchez, 2005). Other diagnostic tools such as abdominal or rectal palpation or contrast radiography may assist in the diagnostic process (Ryan and Sanchez, 2005). Treating meconium impaction involves providing fluid therapy, nutritional support, and pain management (Ryan and Sanchez, 2005). Soapy water enemas or retention enemas with acetylcysteine are administered to

break up the impaction and to encourage the meconium to pass (Ryan and Sanchez, 2005).

Diarrheal gastrointestinal diseases are more common in equine neonates and can be infectious or noninfectious. Foals with HIE may present with diarrhea at a very young age due to organ dysfunction caused by tissue hypoxia (Dunkel 2008). Clinical signs include intolerance of feedings, meconium retention, abdominal distention, ileus, colic, nasogastric reflux, and watery or bloody feces (Dunkel, 2008). Diagnosing diarrhea caused by HIE occurs by ruling out infectious diseases and confirming the presence of other clinical sign associated with HIE (Dunkel, 2008). Treatment includes fluid therapy as well as supplemental nutrition and feedings should be withheld or limited until symptoms subside (Dunkel, 2008).

Necrotizing enterocolitis is a life threatening disease in premature human neonates as well as equine neonates (Dunkel, 2008). Due to a redistribution of cardiac output, ischemic injury or necrosis occurs in the intestine (Dunkel, 2008). Clinical signs include depression, changes in body temperature, feed intolerance, nasogastric reflux, ileus, hypotension, and glucose instability (Dunkel, 2008). Treatment includes parenteral nutrition and treating the metabolic instabilities and sepsis (Dunkel, 2008).

Infectious agents are known to cause diarrheal gastrointestinal diseases in neonates and can be detrimental to the health of a neonate. Common infectious agents that cause diarrhea in neonates are rotavirus, *Salmonella spp*., and *Cryptosporidium* (Dunkel, 2008). Diagnostic tools used to determine the causative agents of diarrhea in foals include commercially available assays, fecal cultures, PCR, and acid fast fecal staining (Dunkel, 2008). Treatment most often includes fluid therapy, correcting

electrolyte and acid-base abnormalities, antimicrobials, and nutritional support (Dunkel, 2008).

Sepsis

Sepsis is one of the leading causes of mortality in the equine neonate and is a large problem for equine practitioners because of the high mortality rate despite aggressive treatment (McKenzie and Furr, 2001). Sepsis is caused by a nonspecific inflammatory response that occurs in response to invasion of tissue by foreign microorganisms (Sanchez, 2005). There are both maternal and postnatal predisposing factors that can cause sepsis including dystocia, premature placental separation, maternal illness, placentitis, failure of passive transfer, improper umbilical care, and poor sanitary conditions (Sanchez, 2005). In utero the fetus may be exposed to microorganisms that invade the placenta and gain access to the neonate's bloodstream or they can infect the respiratory and gastrointestinal tract. After birth, infection usually occurs through the umbilicus, ingestion, inhalation, or via wounds (McKenzie and Furr, 2001). Gram-negative bacteria are the most common cause of equine neonatal sepsis, however, mixed infections is common and gram-positive bacteria are often present with mixed infections (McKenzie and Furr, 2001).

Clinical signs of sepsis vary but include depression, decreased suckling, lethargy, and recumbency (Sanchez, 2005). Sepsis also can result in fever, hypothermia, tachycardia, tachypnea, hypocapnia, leukocytosis, leukopenia, or increased number of immature forms of granulocytes and by definition two of these symptoms must be present (McKenize and Furr, 2001). Diarrhea is commonly present but other localized signs may

be noticed as well such as uveitis, seizures, joint effusion, lameness, respiratory distress, patent urachus, and omphalitis (Sanchez, 2005).

The blood culture is the gold standard for diagnosing sepsis (Sanchez, 2005). While the blood culture is useful because it identifies the causative agent, its limitations include that results are often delayed 48 hours and that it has poor sensitivity (Sanchez, 2005). A sepsis scoring system has been developed as an attempt to improve the diagnostic process of neonatal sepsis, but limitations still exist (McKenzie and Furr, 2001).

Treatment of neonatal sepsis is complex and aggressive treatment is typically required for a successful outcome, however, even with aggressive treatment survival is not always obtainable (Sanchez, 2005). Antimicrobial therapy must be used and typically a broad- spectrum antibiotic is used as soon as possible (Sanchez, 2005). The antimicrobial therapy may be altered when the results of the blood culture are received, but treatment should not be withheld while waiting for the results (Sanchez, 2005). A recommended approach is to combine amikacin with a penicillin or ampicillin (Sanchez, 2005). When this course of treatment is used, it is necessary to monitor the neonate's creatinine levels (Sanchez, 2005). Fluid therapy as well as nutritional support is necessary in most cases of sepsis (Sanchez, 2005). Current reported survival rates of neonates diagnosed with sepsis range from as low as 45% to as high as 72% (Sanchez, 2005). Performance and long-term survival of neonates with sepsis have been studied in limited scope (Sanchez, 2005).

Obtaining a differential diagnosis in the equine veterinary practice is a complex process that is essential to the outcome of the patient. Correctly and efficiently acquiring an accurate diagnosis allows the veterinarian to determine the best-suited treatment plan for the patient and to obtain a prognosis for the outcome. Most often the diagnostic process involves numerous blood tests run in a laboratory that provide awareness into what is occurring inside the horse in response to the disease process. Some of the laboratory blood work routinely performed includes measuring acute phase proteins, which are one of the results of the inflammatory response. Acute phase proteins give insight into how the body is reacting to the disease, which can show promise in diagnosing certain conditions.

The inflammatory response

Inflammation is the response of tissues to injury or the invasion of microorganisms. It plays a critical role by helping to move phagocytic cells and defensive molecules from the bloodstream to the affected site (McKenzie and Furr, 2001). The inflammatory response begins when tissue is either injured or invaded by microorganisms. This initiates macrophage activation and the injured cells release proinflammatory enzymes and proinflammatory cytokines including interleukin-1, tumor necrosis factor-α, and interleukin-6 (McKenzie and Furr, 2001). One of the purposes of the cytokines: interleukin-1, tumor necrosis factor-α, and interleukin-6 is to initiate the acute phase response (McKenzie and Furr, 2001).

The acute phase response

The acute phase response is a systematic chain of reactions that occur as part of the inflammatory response as a reaction to damage to the tissue caused by a wide variety of sources including bacteria, viruses, parasites, stress, and trauma (Jacobsen and Anderson, 2010; Satué et al., 2013). One of the outcomes of the acute phase response is an increase in the circulation and concentration of multiple plasma proteins referred to as acute phase proteins (Pepys et al., 1989). Acute phase response proteins are characterized as proteins which concentrations change by at least 25% as a reaction to the acute phase response (Jacobsen and Anderson, 2010). There are different classifications of acute phase response proteins including major, minor, positive and negative. Positive acute phase response proteins are proteins that increase in concentration during the acute phase response whereas negative acute phase response proteins decrease in concentration during the acute phase response. Minor acute phase proteins change in concentration by .5-1 times, moderate acute phase proteins change in concentration 5-10 times, and major acute phase proteins change in concentration 100-1000 times in response to the acute phase response (Jacobsen and Anderson, 2010).

Serum amyloid A

Serum amyloid A (SAA) is the only major acute phase protein identified in the horse (Jacobsen and Anderson, 2010). It qualifies as a major acute phase protein because it exists in low or undetectable levels in the healthy horse and increases 10-1000 times in response to inflammatory stimulus (Jacobsen and Anderson, 2010; Satué et al., 2013). Serum amyloid A has been studied as an acute phase protein and indicator of infection in

humans and other species, but there have been a limited number of studies conducted evaluating applications of SAA in equine medicine (Jacobsen and Anderson, 2010, Pepys et al., 1989).

Serum amyloid A is an apo-lipoprotein (Nunokawa et al., 1993) weighing 9-11 kilodalton (Jacobsen and Anderson, 2010; Satué et al., 2013). Sletton et al. (1989) published the full sequence of equine SAA and three isoforms of SAA have been identified (Jacobsen and Anderson, 2010; Satué et al., 2013). Serum amyloid A is primarily synthesized by hepatocytes. However, extrahepatic synthesis of the isoform SAA3 has also been identified in the equine endothelial cells in the gastrointestinal tract, mammary glands, and airways, which may give insight into the functions of SAA (Jacobsen and Anderson, 2010; Satué et al., 2013).

While SAA appears to be a particularly sensitive biomarker of infection, its exact functions remain unknown (Jacobsen and Anderson, 2010). Studies have shown that SAA performs a wide variety of functions including both influencing leucocyte function, impacting inflammatory mediator synthesis, assisting in lipid transportation, and encouraging extracellular matrix degradation by inducing enzymes (Jacobsen and Anderson, 2010).

The applications of SAA in the equine clinical setting have just started to be explored, but numerous characteristics of the protein suggest that it has many attractive diagnostic properties as well as potential to be an indicator of current health status (Satué et al., 2013). Serum amyloid A exists in low levels in the healthy horse regardless of age, sex, or breed. The normal range of SAA concentration in the horse is 0.5 mg/l to 20 mg/l with the majority of healthy horses having a SAA concentration under 7 mg/l (Satué et

al., 2013). In the presence of an inflammatory stimulus, SAA concentration begins to increase after 6-12 hours and peaks between 36 hours and 48 hours post stimulus (Jacobsen and Anderson, 2010). Serum amyloid A increases significantly and can reach concentrations of 3000 mg/L. Serum amyloid A begins to decrease in concentration very quickly once inflammatory stimuli are removed. Serum amyloid A is degraded in the liver 30 minutes to 2 hours after synthesis (Satué et al., 2013). Serum amyloid A concentrations reach baseline levels within 1-2 weeks with no presence of inflammatory stimulus (Satué et al., 2013).

Serum amyloid A compared to other common markers of inflammation

The first acute phase protein identified in humans was C-reactive protein (CRP) (Pepys et al., 1989). C-reactive protein exhibits many characteristics similar to SAA that indicates it is a good predictor of infection including a low normal concentration and rapid change in response to infection (Pepys et al., 1989). While CRP and SAA share similar characteristics, SAA increases quicker in response to inflammatory stimuli and increases much more drastically than CRP. C-reactive protein only increases up to 35 times its normal concentration and does not peak until 72-120 hours post inflammatory stimulus (Jacobsen and Anderson, 2010). These characteristics demonstrate why SAA may be a more sensitive, and, therefore, better biomarker of infection.

One of the most commonly used acute phase proteins in the clinical setting in horses is fibrinogen, a minor acute phase protein. There are many characteristics of fibrinogen, however, that imply SAA is a better indication of infection and disease in the horse. Serum amyloid A exists in low levels in the healthy horse whereas fibrinogen is

present in high concentrations and has a wide range of normal values ranging between 2000 and 4000 mg/l (Jacobsen and Anderson, 2010). Plasma concentrations of fibrinogen in inflammatory conditions range from 3000 mg/l to 11000 mg/l which is only a .5 to 5.5 times increase compared to hundreds or a thousand time increase observed in SAA in inflammatory conditions (Jacobsen and Anderson, 2010). Serum amyloid A also begins to increase 18-60 hours earlier than fibrinogen and peaks 24 to 96 hours sooner than fibrinogen in response to inflammatory stimulus (Jacobsen and Anderson, 2010). Finally, where SAA clears the body quickly after the inflammatory stimuli are removed and resolved, fibrinogen levels remain elevated for multiple days (Jacobsen and Anderson, 2010).

The specific characteristics of SAA that indicate it is a more sensitive biomarker of infection in horse has prompted studies that compare its diagnostic capabilities to other commonly used biomarkers. This research was conducted to identify SAA's possible role in the equine clinic. The idea that SAA is a more sensitive indicator of inflammation and infection in the horse is supported by the works of Chavette et al. (1992) and Hultén and Demmers (2010). In those studies there was poor correlation between SAA and fibrinogen and white blood cell (WBC) count in foals with infectious and noninfectious diseases. Belgrave et al. (2013) compared SAA's diagnostic accuracy to the diagnostic accuracy of total WBC count, albumin globulin ratio, and plasma fibrinogen. Serum amyloid A accurately diagnosed a clinically abnormal horse 75% of the time, which was higher than the diagnostic accuracies of total WBC count, albumin globulin ratio and plasma fibrinogen, which ranged between 59% and 62%, respectively (Belgrave et al.

2013). In newborn foals SAA concentration was three times better at diagnosing non-healthy foals than WBC count (Stablelab Studies).

Measuring SAA

Many different methods of measuring SAA in the horse have been evaluated including ELISA, single radial immunodiffusion, electroimmunoassay, latex agglutination immunoturbidemetric assay, and lateral flow immunoassay (Jacobsen et al. 2006). A turbidometric immunoassay (TIA) developed for use with human serum showed slight, but negligible signs of inaccuracy when used to test equine serum. Jacobsen et al. (2006) concluded that equine SAA concentrations can be measured reliably using the TIA designed for human SAA. This type of test is easily automated and fast which makes it particularly well suited for routine diagnostic procedures and an appropriate way to measure SAA concentration in a veterinary laboratory (Jacobsen and Anderson, 2010).

Commercially available point of care lateral flow immunoassay tests have been developed to measure SAA concentration. For ne point of care lateral flow test (StableLab Equine Blood Analysis Kit), the precision and accuracy have been determined as 98.6% and 95.6% respectively (Viner et al 2017). This lateral flow test has good agreement with a TIA as well (Viner et al., 2017). Schwartz et al. (2018) found the correlation coefficeint for results obtained by the point of care test and the TIA to be 0.836. The point of care test showed acceptable linearity and precision in equine serum and plasma for SAA concentrations (Schwartz et al. 2018). These types of tests are particularly useful where access to a laboratory is not available or where rapid results are desired.

Neonatal Disease and SAA

As previously discussed diagnosing neonatal disease is a difficult process due to the nonspecific nature of the clinical signs of many neonatal diseases, and it is crucial to the survival of the neonate to obtain an accurate diagnosis as quickly as possible. Currently, diagnosing disease in equine neonates requires multiple laboratory tests, including a blood culture, in conjunction with a patient history and physical exam. The results from the laboratory results are often delayed, frequently taking 48-72 hours to obtain. Serum amyloid A assays have been evaluated as a tool to diagnose disease in equine neonates in a limited scope.

Chavatte et al. (1992) compared SAA concentrations by electroimmunoassay in normal foals and foals with different clinical conditions including foals suffering from infection, foals showing signs of weakness and prematurity, and foals suffering from neonatal maladjustment syndrome, traumatic birth, or meconium colic. The normal foals had SAA concentrations that agreed with the previously established normal reference range of SAA concentration of 0-20 mg/l. All foals with clinical conditions had raised SAA concentrations, however, the foals in the infection group had significantly higher SAA concentrations than the normal group and the other clinical groups. Chavatte et al. (1992) proposed that SAA concentrations above 200 mg/l were indicative of infection, SAA concentrations between 20 and 100 mg/l were indicative of a non-infective process associated with trauma or prematurity, and SAA concentrations below 20 mg/l were indicative of a normal health status. The results of this study suggest that measuring SAA concentration is useful in the differential diagnosis process in regards to infection the neonate and foal, but SAA concentrations also rise in other conditions.

In a similar study to Chavette et al. (1992), Stoneham et al. (2001) evaluated using a latex agglutination assay to measure SAA in the normal neonate in the first 3 days of life and in response to clinical diseases in the equine neonate. This study evaluated SAA concentration in the normal foal and in foals with septicemia, focal infection, failure of passive transfer of immunity, and noninfectious diseases. The results of the study showed that SAA concentration was increased on day 2 of life in normal foals, but the median SAA concentration values on days 1, 2 and 3 of life fell within previously established normal ranges. The median SAA concentration for the septicemia, focal infection, failure of passive transfer, and noninfectious disease group were 279.9 mg/l, 195 mg/l, 5.1 mg/l and 3.1 mg/l, respectively. There was a significant difference in the SAA concentrations between the normal group and the septicemia group and focal infection group. There was also a significant difference between the septicemia group and the groups with failure of passive transfer and noninfectious diseases. Stoneham et al. (2001) concluded that measuring SAA concentration is useful in diagnosing infectious disease in neonates, but they suggested that a value over 100 mg/l was indicative of infection where as a value of 200 mg/l was suggested by Chavatte et al (1992).

Further research regarding using measuring SAA concentrations for diagnostic purposes in equine neonates supports Stoneham et al. (2001) and Chavatte et al. (1992) concluded that measuring SAA concentrations is useful in diagnosing infection and septicemia in equine neonates (Paltrinieri et al. 2008; Hultén and Demmers, 2002). Similar to studies evaluating SAA concentration in adults with infection, compared with those of other commonly used biomarkers of infection, including WBC count and

fibrinogen, it is suggested that SAA concentration is a more accurate indicator of infection in equine neonates (Hultén and Demmers, 2002; Stablelab Studies).

Respiratory Diseases and SAA

While it is established that SAA concentration is a reliable marker of infection, its response to different respiratory diseases has been an area of interest. Viner et al. (2017) studied SAA concentration in horses with different infectious and noninfectious respiratory diseases, including equine influenza virus (EIV), equine herpesvirus-4 (EHV-4), *Streptococcus equi* (*S. equi*), inflammatory airway disease (IAD), and healthy horses. The median SAA concentrations of the healthy, IAD, EIV, EHV-4, and *S. equi* groups were 0 mg/l, 0 mg/l, 731 mg/l, 1173 mg/l, and 1953 mg/l, respectively. There were significant differences in SAA concentration between horses with infectious diseases (EIV, EHV-4, *S. equi*) and the horses in the IAD group, although all horses with respiratory diseases were significantly different than the control group of healthy horses. This study concluded that SAA is more elevated in horses with infections in the respiratory tract when compared to those with noninfectious diseases of the respiratory tract. The authors of this study suggest that monitoring SAA in horses with respiratory diseases can assist in early detection, track disease progression, and help make decisions regarding biosecurity and isolation issues regarding contagious horses.

Further studies regarding SAA concentrations in horses with respiratory diseases have produced similar results as Viner et al. (2017). Acute equine influenza A2 (H3N8) virus infection is a highly contagious virus that not only causes clinical abnormalities, but also predisposes them to secondary bacterial infections and causes performance to be

impaired. Serum amyloid A concentrations in horses with equine influenza increase during the first 48 hours of onset clinical signs and return to baseline values within 11-22 days (Hultén et al., 1999). In horses with bacterial pneumonia both SAA concentrations and surfactant protein D (SP-D), a protein commonly used to indicate pulmonary injury, were elevated (Hobo et al., 2007). The increase in SAA preceded the increase in SP-D, which increased with the presence of clinical signs suggesting that measuring SAA concentrations could help identify bacterial pneumonia before clinical signs are present (Hobo et al., 2007). Elevated SAA concentration was also detected in horses with heaves compared to healthy horses (Lavoie-Lamoureux et al., 2012).

One area of particular interest regarding SAA concentration and respiratory diseases has been regarding using SAA concentration to detect and monitor *Rhodococcus equi* pneumonia in foals. Passamonti et al. (2015) and Cohen et al. (2010) both evaluated the usefulness of monitoring SAA concentration as a means of early detection for *R. equi* pneumonia. This is of particular interest due to the subtle clinical signs presented in the early stages of *R. equi* pneumonia. The results of both studies showed that SAA concentrations in foals with *R. equi* pneumonia were varied and therefore it is suggested that SAA concentration is not a helpful tool in the early diagnosis process of *R. equi* pneumonia in foals. While measuring SAA concentration is not useful in early detection, it may be useful in determining real time health status and effectiveness of therapy.

Surgery and SAA

The effects of surgery on SAA concentration have been examined in several different contexts. Surgery induces the acute phase response and therefore prompts a rise in SAA concentration (Jacobsen et al., 2010). It is suggested, however, that general anesthesia alone does not cause an increase in SAA concentration (Pepys et al., 1989). In most cases SAA concentration is elevated 24 hours post surgery and remains elevated for a short period of time before returning to normal levels. There is variation in the degree of the increase as well as the longevity of the increased concentration, but a SAA concentration that remains elevated may suggest post-surgical complications such as infection (Jacobsen et al., 2010).

Pollock et al. 2005 evaluated SAA concentration in horses after elective surgery and non-elective surgery compared to normal horses and found that both groups of horses who had surgery had significantly higher mean SAA concentrations compared to the control group of healthy horses 24 hours after the surgery.

Jacobsen et al. 2010 found that horses had elevated SAA concentrations 3 days post castration. In cases that had mild inflammation and uncomplicated surgery, SAA concentrations returned to normal levels 8 days post surgery. In cases that had moderate to severe inflammation post surgery, SAA concentration remained elevated throughout the course of the study. The results of this study suggested that fever and leucocyte count were not useful tools when monitoring post castration surgery and SAA may improve post operative monitoring because its concentration revealed the level of inflammation present and correlated with the level of clinical severity of the inflammation.

Pepys et al. 1989 saw increased SAA concentrations post surgery for horses that underwent exteriorization of the carotid artery and division of flexor tendons that peaked 2-3 days and returned to normal values within 7-14 days post surgery.

Monitoring SAA concentration may be useful in post surgery management. Serum amyloid A concentrations that remain elevated for prolonged periods of time post surgery may indicate infection or a complication from the surgery process or a delay in the healing process. Measuring SAA concentration as a management tool may provide insight into the health status of the individual post surgery and indicate when the healing process is complete.

Conclusion

Serum amyloid A is an acute phase protein that has potential to be a useful indicator of infection and disease in the equine clinic. Because it increases in response to the initiation of the acute phase response, its use as a differential diagnostic tool may be somewhat limited as it increases in response to infection and not to a specific disease process. The ability of SAA to rise significantly and quickly in response to inflammatory stimuli makes it a particularly valuable diagnostic tool that can aid in early detection of infection as well as current health status and response to treatment. While research regarding the applications of SAA in the equine clinical setting is limited to date, it is apparent that SAA has usefulness as a diagnostic tool. The development of a point of care test able to measure SAA concentrations without access to a laboratory may be particularly useful to some veterinarians in remote areas.

Chapter Two: Introduction

Rationale

The current diagnostic process for diagnosing disease in the equine neonate is a complex process that combines knowledge of patient history and physical examination and diagnostic tests including extensive laboratory blood work. While this information provides the veterinarian with a comprehensive overview of the patient, the diagnostic process is often slowed by delayed results of the laboratory blood work. Also, veterinarians in remote areas may experience significant delays in laboratory results due to limited access to a lab. Obtaining an accurate diagnosis in a rapid time frame greatly improves the neonate's chance of survival because many neonatal diseases progress quickly and foal succumb to rapid deterioration. Identifying a biomarker of disease that can be quantified early in the disease process and easily obtained by a veterinarian, would improve diagnostic procedures by allowing the veterinarian to receive valuable information within a critical time frame. Because it is a sensitive acute phase protein and there is a point of care test kit available, serum amyloid A has been suggested as a possible biomarker of disease in equine neonates. The studies presented in this thesis whether serum amyloid A can serve as a biomarker of disease in equine neonates in order to remedy a large problem that exists in the equine veterinary practice.

Hypotheses and Objectives

Hypothesis 3.1: A SAA concentration above 100 mg/l is associated with equine neonatal sepsis

Objective 3.1: To assess measuring serum amyloid A (SAA) using a commercially available point of care lateral flow kit to diagnose sepsis in neonates less than one week of age.

Hypothesis 4.1: Median SAA concentrations will differ between diagnostic groups

Hypothesis 4.2: A SAA value greater than 100 mg/l is indicative of disease

Hypothesis 4.3: The optimum age to test SAA concentration is over 12 hours of age in order to allow for the SAA concentration to accumulate in response to a disease.

Objective 4.1: To assess measuring SAA using a point of care test to diagnose disease in the equine neonate

Objective 4.2: To identify differences in SAA concentration between diagnoses

Objective 4.3: To identify changes in SAA concentration over the course of 72 hours post admission

Chapter Three

Assessing the use of a commercially available point of care serum amyloid A test for diagnosing sepsis in equine neonates

Abstract

Objective - To assess measuring serum amyloid A (SAA) using a commercially available point of care lateral flow kit to diagnose sepsis in equine neonates less than one week of age.

Design – Retrospective case series of equine neonates from one equine hospital between the years 2014-2016.

Animals – 126 neonates under one week of age grouped based on primary diagnosis (colitis, contracted limbs, enteritis, hypoxic-ischemic encephalopathy (HIE), pneumonia, premature birth, sepsis, weak foal and normal).

Procedures – Peripheral blood was obtained from each neonate by jugular catheter upon admission or immediately after birth at the hospital. The blood serum was tested using the StableLab Equine Blood Analysis Kit. The data were analyzed using the Wilcoxon rank sum test in SAS.

Results - The median SAA concentration varied by diagnostic group. There was a significant difference between the SAA concentrations for the colitis, premature birth, contracted limbs, weak foal, HIE and normal foal groups when compared to the sepsis group. There was no significant difference between the SAA concentrations for the enteritis or pneumonia groups when compared to the sepsis group.

Discussion and Clinical Relevance - The results of this study suggest that a commercially available point of care lateral flow kit is a helpful tool in diagnosing sepsis

in neonates less than one week of age because it allows for rapid results and does not require access to a laboratory. While additional testing is needed to differentiate between infectious diseases, the test can provide veterinarians with valuable diagnostic information within a critical time frame.

Introduction

Equine neonatal sepsis has been identified as one of the major causes of mortality in the young foal (Sanchez, 2005). Sepsis is a nonspecific inflammatory response initiated by the presence of foreign microorganisms in sterile tissue (Sanchez, 2005). The disease manifests itself in a variety of ways, including fever, tachycardia, tachypnea, and leukocytosis/leukopenia (McKenzie and Furr, 2001). The systemic nature of the disease allows for rapid progression and quick deterioration of the body, which must be combated by early diagnosis and aggressive treatment for a successful outcome McKenzie and Furr, 2001).

Due to the non-specific manifestations of the disease, it can be difficult for practitioners to diagnose equine neonatal sepsis (Palmer, 2014). Currently the gold standard for the definitive diagnosis of sepsis in equine neonates is the blood culture test, which allows the causative agent to be identified (Sanchez, 2005). The disadvantages of the blood culture are that results are delayed, and that it has poor sensitivity and will only detect bacterial infection (Sanchez, 2005; Palmer, 2014). In 1988 Brewer and Koterba developed a sepsis scoring system to be used to identify sepsis in hospitalized equine neonates by weighting laboratory results, patient history information, and a physical examination parameters (Palmer, 2014). While the scoring system is a useful tool, it still

possesses limitations that leave a need for the identification of a practical biomarker of disease (Palmer, 2014). Limitations of the sepsis scoring system include requiring multiple blood tests as well as access to a laboratory, which can often cause a delay in the initiation of treatment.

Finding a marker of infection that is sensitive enough to detect infection, specific enough to discriminate between infections and other stimuli, and easily measured early in the course of disease would be a significant advancement in diagnosing equine neonatal sepsis (Palmer, 2014). Serum amyloid A is an apolipoprotein that plays a role in host defense mechanisms and provides local protection against invading microorganisms (Jacobsen and Anderson, 2010). SAA is the only major acute phase protein identified in the horse (Belgrave et al., 2013). The low or nonexistent normal levels, significant and quick increase in response to stimuli, and short half-life of SAA in equines makes it an attractive biomarker for equine neonatal sepsis (Jacobsen and Anderson, 2010; Belgrave et al., 2013; Satué et al., 2013).

The objective of this study was to evaluate the ability of SAA concentration to serve as a biomarker for bacterial sepsis using a commercially available stall-side lateral flow kit in neonates less than one week of age. The working hypothesis was that a SAA concentration above 100 mg/l is associated with equine neonatal sepsis.

Materials and methods

The clinical records of neonates admitted to, or born at, the Rood and Riddle Equine Hospital in Lexington, Kentucky between the years of 2014-2016 were examined retrospectively. A neonate was defined as a foal less than 7 days old. Records from

neonates that were tested for SAA were used in the study. Of the 278 foals admitted to the neonatal intensive care unit during the study period, 167 were tested for SAA based on clinician discretion. Thirty-one of these foals were eliminated from the study due to being over seven days of age. Of the 136 neonates 7 days of age and under who were tested for SAA, 10 foals were excluded from the study due to incomplete medical records. The clinical records of 126 neonates were included in the analysis and grouped into 9 categories based on primary diagnosis: colitis, contracted limbs, enteritis, hypoxic-ischemic encephalopathy (HIE), pneumonia, premature birth, sepsis, weak foal and normal. One of the three attending veterinarians determined primary diagnosis after obtaining the patient's history, completing a physical examination, interpreting blood test results and performing other diagnostics such as ultrasound or radiographs. The characteristics of each diagnostic group that must be present in order for the attending veterinarian to determine a primary diagnosis are presented in Table 3.1.

Blood was obtained from each neonate by jugular venipuncture or a jugular catheter upon admission, or immediately after, birth at the hospital. Each sample was immediately placed into vacutainer blood collection tube with no additive and allowed to clot. The serum from the samples was separated via centrifugation at 2500 RPM for 5 minutes. All serum samples were analyzed for SAA concentration the same day they were collected using a commercially available point of care lateral flow immunoassay test that determines SAA concentration (Stablelab SAA test, Sligo, Ireland). According to the manufacturer of the kit the precision and accuracy of the test are 98.6% and 95.6% respectively (Viner et al., 2017). The test has shown good linearity and agreement with a TIA (Viner et al., 2017).

The distribution of data for SAA concentration was tested for normality using the Shapiro-Wilks test. The data had a nonnormal distribution so median values were used and the Wilcoxon rank sum test was used to determine significant differences of SAA concentrations between the sepsis and other diagnostic groups and the normal and other diagnostic groups. The statistical analysis was performed using a statistical software (PROC NPAR1WAY, SAS, version 9.3, SAS Institute Inc., Cary, NC). P-values less than < 0.05 were deemed significant.

Results

The median SAA concentrations for the normal, contracted, premature, weak foal, and HIE were within the normal range for neonates of 0-20mg/l, which was determined by the manufacturer (0, 7, 5, 0, and 6 mg/l, respectively) (Table 3.2) (Di-Sien Chan, Stablelab, Sligo, Ireland). The median SAA concentration for the colitis group was 65 mg/l, which indicated possible infection (Table 2) (Di-Sien Chan, Stablelab, Sligo, Ireland). The median SAA concentrations for the enteritis, pneumonia, and sepsis were above 100 mg/l (390, 1834, and 510 mg/l respectively), which was indicative of infection (Table 2) (Di-Sien Chan, Stablelab, Sligo, Ireland). The ranges of SAA concentrations by diagnostic group are presented in Table 3.2.

There were significant differences in SAA concentration between the sepsis compared to the colitis ($p = 0.0151$), contracted ($p=0.0052$), HIE ($p=0.0007$), normal ($p=0.0001$), premature ($p=0.0009$), and weak foal ($p<0.0001$) groups (Table 3.2). There was no significant difference between the sepsis group and enteritis and pneumonia groups (Table 3.2). There was a significant difference in SAA concentration between the normal group compared to the sepsis, pneumonia, and enteritis groups (Table 3.2). There

was no significant difference between the normal group and the colitis, contracted, HIE, normal, premature, and weak foal groups (Table 3.2).

Discussion

It is difficult to obtain a diagnosis for equine neonates because clinical signs vary from case to case and are often not specific to one differential diagnosis. While monitoring SAA levels is shown to be a helpful tool in diagnosing sepsis in equine neonates, it should be used in conjunction with other diagnostic tools and should not be used as the sole criteria for diagnosis (Sanchez, 2005; Palmer, 2014). In this study SAA values were significantly different than the normal group for multiple diagnostic groups including the sepsis, pneumonia and enteritis groups. To differentiate between each of these diagnosis, further testing and clinical observation is required. Decreasing the time between onset of disease and initiation of treatment is essential to achieving a successful outcome of disease. Even though further testing is necessary to obtain a diffinitive differential diagnosis, observing an SAA value above 100 mg/l provides a veterinarian with enough information to initiate treatment, which most often includes antibiotics and supportive care.

Because SAA is the only identified major acute phase protein in the horse it possesses qualities that make it a more useful diagnositc tool than commonly used proteins such as fibrinogen, which is a minor acute phase protein. Serum amyloid A exists in the healthy horse in a low and narrow range of concentrations (0-20 mg/l) in comparison to fibrinogen, which has a normal value between 2000-4000 mg/l (Jacobsen and Anderson, 2010). Serum amyloid A increases up to 1000 times in response to inflammatory stimuli whereas fibrinogen only increases up to 10 times its normal value (Jacobsen and

Anderson, 2010). Serum amyloid A begins to increase just 6 hours post inflammatory stimuli and peaks just 48 hours post stimuli (Jacobsen and Anderson, 2010). Fibrinogen levels do not begin to increase until 24 hours post inflammatory stimuli and does not reach peak values until up to 144 hours post stimuli (Jacobsen and Anderson, 2010).

A limitation of this study was that single samples were used, rather than serial samples. Because SAA begins to increase 6 hours post inflammatory stimuli, the SAA values obtained in this study may reflect rising values as opposed to peak values. The range for each diagnostic group had a minimum of 0 mg/l with the exception of the pneumonia group which had a minimum value of 3 mg/l. This was a result of testing foals that were 0 hours of age. At this time point, SAA had not had a chance to increase in circulation. Collecting serial samples would decrease the risk of obtaining false negatives and, therefore, incorrectly declaring a neonate free of infection because the value has not reached its peak. Serial testing of SAA values over time may also provide insight into efficacy of treatment and convalescence of disease. Additionally, obtaining serial samples may help determine the optimum time to test SAA concentration in order to detect sepsis. Further studies in this area are necessary to evaluate the importance of serial SAA samples in diagnosing sepsis and disease in the equine neonate.

Serum amyloid A values in this study were determined using a commercially available stall side test. This type of test eliminates the time period between the initial examination by the veterinarian and initiation of treatment that exists when laboratory tests are used. A stall side SAA kit allows an ambulatory veterinarian to perform an initial examination and initiate treatment within minutes of each other, which may

eliminate an additional visit to the patient. This type of test would be especially useful in remote areas or in locations that do not have access to a laboratory.

The specific age range (foals less than one week of age) and large number of cases in this study make the study population unique compared to other studies evaluating using SAA to diagnose disease in foals. One of the first studies in this area suggested that SAA concentrations over 200 mg/l is associated with infection and that SAA concentrations between 20-100 indicate a non-infective process (Chavatte et al., 1992), however, a more recent study suggested that a value over 100 mg/l is indicative of infection (Stoneham et al, 2001). Stoneham et al. (2001) concluded that the measurement of SAA concentrations is a useful tool in diagnosing infection in young foals; the results of the present study also support measuring SAA concentrations for diagnosing infection in equine neonates less than one week of age.

In conclusion, the results of this study indicate that measuring SAA concentrations using a commercially available stall side test is helpful in diagnosing equine neonatal sepsis, enteritis, and pneumonia. The results support that a SAA concentration greater than 100 mg/l is associated with equine neonatal sepsis; however, a SAA concentration greater than 100 mg/l does not specifically indicate sepsis. Further diagnostic evaluation is required to differentiate between infectious diseases, including pneumonia, sepsis, and enteritis.

Table 3.1: Characteristics required to be present to obtain primary diagnosis

Primary Diagnosis	n	Required characteristics used for veterinarian to determine primary diagnosis
Colitis	**9**	Diarrhea, abdominal discomfort/distention
Contracted Limbs	**6**	Difficulty standing, abnormal position of limb/joint
Enteritis	**11**	Abdominal discomfort, nasogastric reflux, immobile/dilated small intestine on ultrasound
HIE	**16**	Neurologic signs not caused by infection
Pneumonia	**10**	Abnormal lung auscultation, consolidation/abscesses on ultrasound
Premature Birth	**9**	Gestational length, low birth weight, domed forehead, short hair coat, periarticular laxity, floppy ears, weak suckle, small frame, generalized weakness
Sepsis	**18**	Positive blood culture
Weak Foal	**33**	Slow to stand/nurse, normal thorax/abdomen ultrasound
Normal	**13**	Normal blood work and physical exam

Table 3.2: SAA concentration data by primary diagnosis

Primary Diagnosis	No. of foals	Median mg/l	Range mg/l	P-value
Normal*	13	0	0-215	0.0001
Colitis*	9	65	0-1470	0.0151
Contracted*	7	7	0-538	0.0052
Enteritis^	11	390	0-2640	0.1375
HIE*	16	6	0-953	0.0007
Pneumonia^	10	1834	3-4724	0.2776
Premature*	9	0	0-46	0.0009
Sepsis^	18	510	0-4764	
Weak Foal*	33	5	0-1106	<0.0001

* Significantly different from sepsis group (P<0.05)
^ Significantly different from normal group (P<0.05)

Figure 3.1: SAA concentration min, Q1, median, Q3, max by primary diagnosis

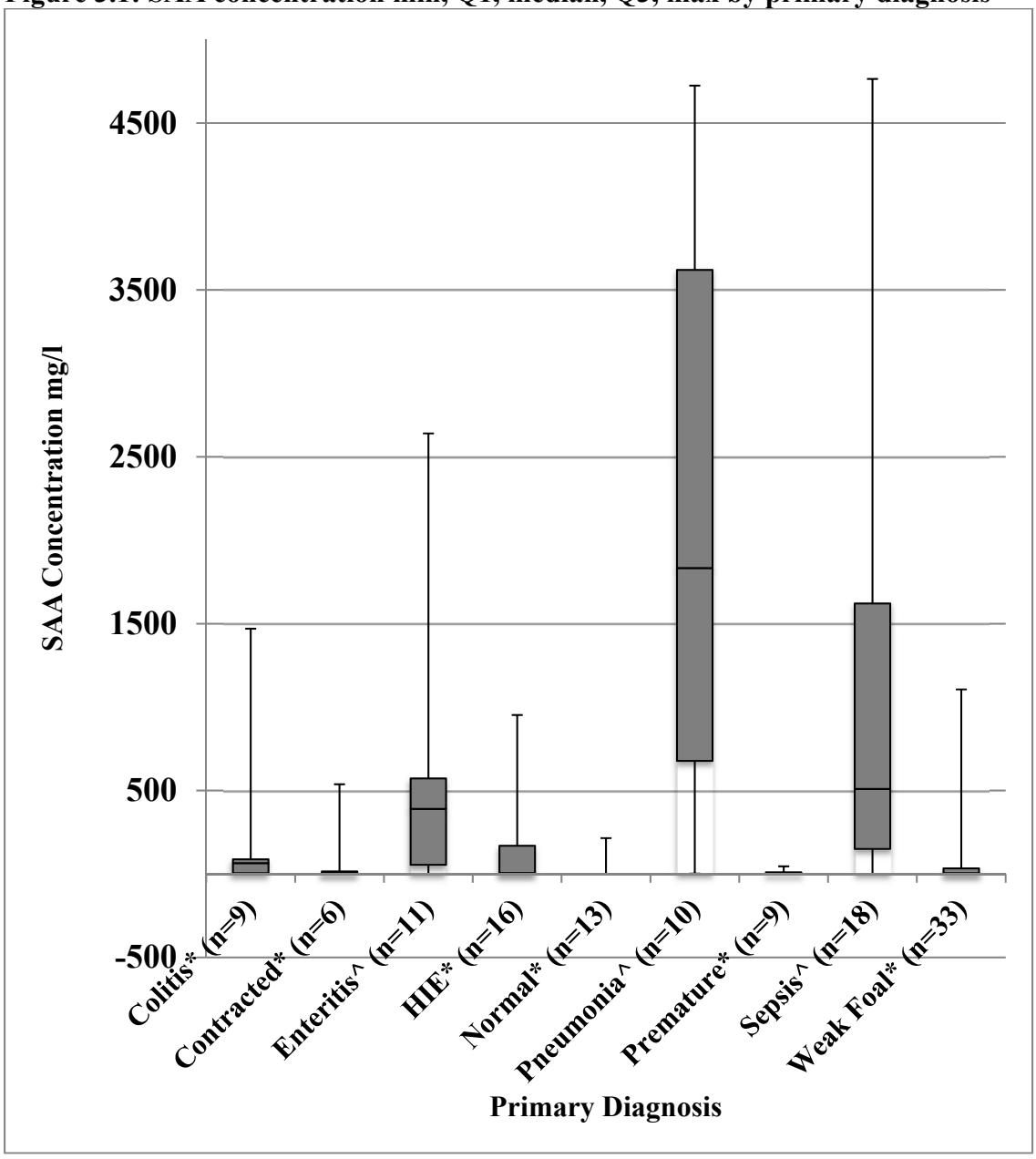


Figure 3.1: Min, Q1, median, Q3, and max SAA concentration for colitis, contracted, enteritis, HIE, normal, pneumonia, premature, sepsis and weak foal diagnostic groups.
*** Significantly different from sepsis group (P<0.05)**
^ Significantly different from normal group (P<0.05)

Chapter Four

Time series analysis of serum amyloid A concentration in hospitalized equine neonates

Abstract

Objective - To assess measuring SAA using a stall side test to diagnose disease in the equine neonate, to identify differences in SAA concentration between diagnoses, and to identify differences in SAA concentration at different time points

Design – Time series analysis of equine neonates from one equine hospital between the January 2017 and July 2017.

Animals – 24 neonates under 48 hours of age at admission with primary diagnoses of pneumonia, gastrointestinal disease and hypoxic-ischemic encephalopathy (HIE).

Procedures – Peripheral blood was obtained from each neonate by jugular catheter 0, 12, 24, 48, and 72 hours post admission. Serum was tested using the StableLab Equine Blood SAA Analysis Kit. Statistical analysis was performed using SAS PROC GLIMMIX. P-values less than 0.05 were deemed significant.

Results - There were significant differences in median SAA concentration between the encephalopathy group and the pneumonia group ($p=0.0025$) and the encephalopathy group and the gastrointestinal group ($p=0.0002$) over the course of the study. There was no significant difference in SAA concentration between the pneumonia and gastrointestinal groups.

Discussion and Clinical Relevance - The results of this study suggest that a commercially available point of care lateral flow kit is a helpful tool in diagnosing disease in neonates when tested after 12 hours of age because it allows for rapid results

and does not require access to a laboratory. While additional testing is needed to differentiate between diseases, the test can provide veterinarians with valuable diagnostic information within a critical time frame.

Introduction

Diagnosing equine neonatal diseases is a difficult process due to the nonspecific or subclinical manifestations of many common neonatal diseases (Chavatte et al. 1992; Hultén and Demmers, 2002; Jacobsen and Anderson, 2010; Stoneham et al., 2001). Diagnostic testing is often extensive and includes multiple laboratory tests. This is not an ideal practice because it is important to minimize the amount of blood obtained from a neonate and because laboratory test results may take hours to days to obtain, thus delaying the initiation of treatment (Paltrinieri et al.; 2008). Compromised neonates have a high risk of rapid deterioration and therefore a quick and accurate diagnosis is essential in order to obtain a successful outcome for the individual (Hultén and Demmers, 2002; Stoneham et al., 2001). Utilizing a biomarker of disease that is sensitive enough to detect infection at an early stage of disease would greatly improve the diagnostic process and would result in an improved prognosis (Stoneham et al., 2001).

Serum amyloid A (SAA) is the only major acute phase response protein identified in the equine. Serum amyloid A has been studied extensively in humans, but its research in the horses is limited (Jacobsen and Anderson, 2010). While the exact functions of SAA in the horse are unknown, it has several purposes in the inflammatory response. These include enhancing and inhibiting leukocyte functions, inducing enzymes involved in the degradation process, affecting lipid transportation, and influencing inflammatory mediator synthesis (Jacobsen and Anderson, 2010). Serum amyloid A is a more sensitive

acute phase protein than other commonly used biomarkers (Satué et al., 2013). It exists in low or nonexistent levels in the healthy horse and responds significantly and quickly in response to inflammatory stimulus. Serum amyloid A concentrations begin to rise 6-8 hours post inflammatory stimulus and peak at 36-48 hours post stimulus (Satué et al., 2013). In comparison, fibrinogen begins to increase 24-72 hours post inflammatory stimulus and peaks between 72-144 hours post inflammatory stimulus (Jacobsen and Anderson, 2010). Being able to detect a noticeable difference in SAA concentration within the first 24 hours of life, compared to the first few days of life, allows treatment to be started sooner and increases the chance of survival for the neonate.

The objectives of this study were to assess measuring SAA using a point of care test to diagnose disease in the equine neonate, to identify differences in SAA concentration between diagnoses, and to identify changes in SAA concentration over the course of 72 hours post admission. The working hypotheses of the study were that median SAA concentrations will differ between diagnostic groups, a SAA value greater than 100 mg/l is indicative of disease, and that the optimum age to test SAA concentration is over 12 hours of age in order to allow for the SAA concentration to accumulate in response to a disease.

Materials and Methods

This study was a time series analysis that was comprised of 24 neonates between 0 and 48 hours of age at admission. The neonates included in this study were admitted to Rood and Riddle Equine Hospital between February 2017 and June 2017. Inclusion criteria for the study consisted of being between 0 and 48 hours of age at admission and having blood samples collected at each time point of the study. The foals were grouped

into three primary diagnoses: pneumonia (n=8), HIE (n=8), and gastrointestinal disease (n=8). One of the three attending veterinarians determined primary diagnosis after obtaining the patient's history, completing a physical examination, interpreting blood test results and performing other diagnostics such as ultrasound or radiographs. The specific characteristics required to diagnose pneumonia, HIE, and gastrointestinal disease are presented in Table 4.1.

Blood was obtained from each foal via jugular catheter at 0, 12, 24, 48 and 72 hours post admission. The blood was stored in a 5ml vacutainer with no additive and allowed to clot. The serum was separated via centrifugation at 2500 RPM for 5 minutes and stored in a freezer at -30° C. After thawing the serum and allowing it to reach room temperature, the serum was tested for SAA concentration using a commercially available point of care lateral flow kit (Stablelab SAA test, Sligo, Ireland). For the purpose of this study a SAA concentration greater than 100 mg/l was considered indicative of infectious disease.

Because the data had a non-Gaussian distribution median values were reported. The statistical analysis was performed using statistical software (SAS, version 9.3, SAS Institute Inc., Cary, NC). Differences in SAA concentration between primary diagnoses were determined by using Tukey grouping of the least square means to adjust for multiple comparisons. PROC GLIMMIX was used to obtain differences in SAA concentration between primary diagnoses. PROC GLIMMIX estimates trends in disease rates, modeling proportions over time in a clinical trial, and predicts the probability of occurrence in times series. P-values less than 0.05 were deemed significant.

Results

The median SAA concentrations for all neonates in this study at 0, 12, 24, 48, and 72 hours post admission were 32.5 mg/l, 566 mg/l, 712 mg/l, 466 mg/l, and 161.5 mg/l respectively (Fig 4.1). The range of SAA concentrations for all neonates in this study at 0, 12, 24, 48, and 72 hours post admission were 0-3000 mg/l, 26-2784 mg/l, 33-2777 mg/l, 11-2679 mg/l, and 0-2234 mg/l respectively (Fig 4.1). The median SAA concentrations for the pneumonia and encephalopathy groups at admission were under 100 mg/l (41 mg/l and 2 mg/l respectively) whereas the median SAA concentration at admission for the gastrointestinal foals was greater than 100 mg/l (463 mg/l) (Fig 4.2). The median SAA concentration was above 100 mg/l for all three diagnostic groups at the 12, 24, and 48-hour post admission. All three diagnostic groups had increasing median SAA concentrations during the 0, 12 and 24-hour post admission time samples with the greatest increase occurring between the 0 and 12 hour time samples. The median SAA concentration for the encephalopathy group at the 72-hour post admission time sample was below 100 mg/l (55 mg/l), while the median SAA concentration at 72-hour post admission for the pneumonia and gastrointestinal groups remained elevated at 581 mg/l and 266 mg/l respectively. The ranges of SAA concentration for the pneumonia group at 0,12, 24, 48, and 72 hours post admission were 6-84 mg/l, 162-2673 mg/l, 136-2432 mg/l. 41-2679 mg/l and 0-2234 mg/l respectively. The ranges of SAA concentration for the gastrointestinal group at 0, 12, 24, 48, and 72 hours post admission were 0-3000 mg/l, 450-2784 mg/l, 448-2528 mg/l, 159-2345 mg/l, and 12-2206 mg/l respectively. The ranges of SAA concentration for the HIE group at 0, 12, 24, 48, and 72 hours post admission were 0-53 mg/l, 26-420 mg/l, 33-735 mg/l, 11-476 mg/l, ad 0-317 mg/l

respectively. The median SAA concentrations and ranges of SAA concentration for each diagnostic at each time point are presented in Figures 4.2-4.6.

There were significant differences in median SAA concentration between the encephalopathy group and the pneumonia group ($p=0.0025$) and the encephalopathy group and the gastrointestinal group ($p=0.0002$) over the course of the study (Table 4.2). There was no significant difference in SAA concentration between the pneumonia and gastrointestinal groups.

At 0 hours post admission 100% of the neonates in the pneumonia group, 25% neonates in the gastrointestinal group, and 100% of the neonates in the encephalopathy group had SAA values below 100 mg/l. At 12 hours post admission 0% of the neonates in the pneumonia group, 0% of the neonates in the gastrointestinal group, and 25% of the neonates in the encephalopathy group had SAA values below 100 mg/l. At 24 hours post admission 0% of the neonates in the pneumonia group, 0% of the neonates in the gastrointestinal group, and 37.5% of the neonates in the encephalopathy group had SAA values below 100 mg/l. At 48 hours post admission 12.5% of the neonates in the pneumonia group, 0% of the neonates in the gastrointestinal group, and 37.5% of the neonates in the encephalopathy group had SAA values below 100 mg/l. At 48 hours post admission 12.5% of the neonates in the pneumonia group, 37.5% of the neonates in the gastrointestinal group, and 62.5% of the neonates in the encephalopathy group had SAA values below 100 mg/l. These values are displayed in Figure 4.7.

Discussion

The differential diagnostic procedure in neonates is a complicated process that poses many challenges for the equine industry. It is important to note that no single

diagnostic test should be used to differentially diagnose a neonate in the clinical setting (Chavatte et al., 1992). For the most accurate diagnosis, a combination of diagnostic procedures should be considered.

The median SAA concentrations, regardless of diagnostic groups, were above 100 mg/l at all time points except for at 0 hours post admission. This corresponded to 0 hours of age for all but 6 foals in the study. At 12 hours post admission, however, all foals in the pneumonia and gastrointestinal groups had SAA concentrations above 100 mg/l. These results suggest that testing for SAA concentration at 0 hours of age would not be beneficial to the diagnostic process. SAA concentrations do not begin to increase until 6-8 hours post inflammatory stimulus, thus it is more worthwhile to test SAA concentration after it has had sufficient time to accumulate. The authors of this study recommend assaying SAA concentration no earlier than 12 hours of age to detect elevated levels associated with disease.

While the encephalopathy group had a significantly lower SAA concentration than the pneumonia and gastrointestinal groups, it did have median SAA concentrations over 100 mg/l. This is likely because SAA increases in response to other conditions besides infection, including trauma and stress (Belgrave et al., 2013). Continued testing for SAA should be used for these cases to monitor for decreasing or slightly increasing SAA concentrations. Neonates with encephalopathy are compromised and have a larger susceptibility to infection compared to a healthy neonate (McKenzie and Furr, 2001).

The results of this study did show a significant difference in the SAA concentrations of the pneumonia group and the encephalopathy group and the gastrointestinal group and the encephalopathy group. These results support that the SAA

test can be a useful tool in diagnosing disease in neonates. This finding agrees with previous authors' conclusions as well (Chavatte et al., 1992; Stoneham et al., 2001). A SAA concentration above 100 mg/l should be recognized as a possible indication of disease or infection, and further diagnostic testing should be pursued. Follow-up SAA testing should be performed in order to follow the current health status of the patient.

A point of care test that is able to quantify SAA concentration within minutes is especially useful for veterinarians in remote locations without access to a laboratory. A low SAA concentration obtained from a foal less than 12 hours of age, indicates a healthy foal that may not need further testing and does not require treatment such as antibiotics. A high SAA concentration indicates need for further testing and alerts veterinarians that the foal will require further testing and treatment to some extent.

One limitation of this study is that the average age of the gastrointestinal group was higher than the other two diagnostic groups. This could explain the higher median SAA concentration at 0 hours post admission for this diagnostic group. Ideally, all foals included should have been the same age at admission. A second limitation of this study is that there was no normal neonate group to compare to the clinically abnormal cases. Accumulating this data would give insight to patterns of SAA concentration over time in clinically normal foals and allow us to compare this data to clinically abnormal foals; however, data regarding SAA concentrations in healthy foals has been obtained and analyzed in previous works including the study in chapter 3 of this thesis. Finally, the neonates in this study were grouped based on primary diagnosis, but other diseases are often present. This could explain elevated SAA in the encephalopathy group because, even though pneumonia or a gastrointestinal disease was not the neonate's primary

diagnosis, it is possible that the neonate subsequently presented with symptoms of these diseases as well.

Given the limited number of studies regarding SAA concentration in the equine, and especially in the equine neonate, equine clinicians are determining where the SAA test best fits into their wide variety of tools. This study does support that a point of care test for SAA is useful in the diagnostic process for equine neonates and is especially helpful for veterinarians without access to a laboratory. Obtaining a SAA concentration provides a veterinarian with a good starting point and can help the veterinarian determine what the next step should be. It would be beneficial for future studies in this area to evaluate using SAA as a tool to determine efficacy of treatment and convalescence of disease.

Table 4.1: Characteristics used to obtain primary diagnosis

N	Primary Diagnosis	Required characteristics used for veterinarian to determine primary diagnosis
8	Gastrointestinal Disease*	Abdominal discomfort/distention, nasogastric reflux, immobile/dilated small intestine on ultrasound, diarrhea
8	HIE	Neurologic signs not caused by infection
8	Pneumonia	Abnormal lung auscultation, consolidation/abscesses on ultrasound

* Not all characteristics must be present to obtain this diagnosis

Table 4.2: Median SAA concentrations for each primary diagnosis at different time points post admission

			Median SAA Concentration, mg/l				
Diagnostic Group	**n**	**Median Age (hrs)**	**0 Hrs**	**12 Hrs**	**24 Hrs**	**48 Hrs**	**72 Hrs**
Pneumonia[A]	8	0	41	677	843	1076	581
Gastrointestinal[A]	8	24	463	951	1201	455	266
Encephalopathy[B]	8	0	2	273	489	144	56

Diagnostic groups denoted with the same letter were not significantly different from each other
Diagnostic groups denoted with different letters were significantly different from each other

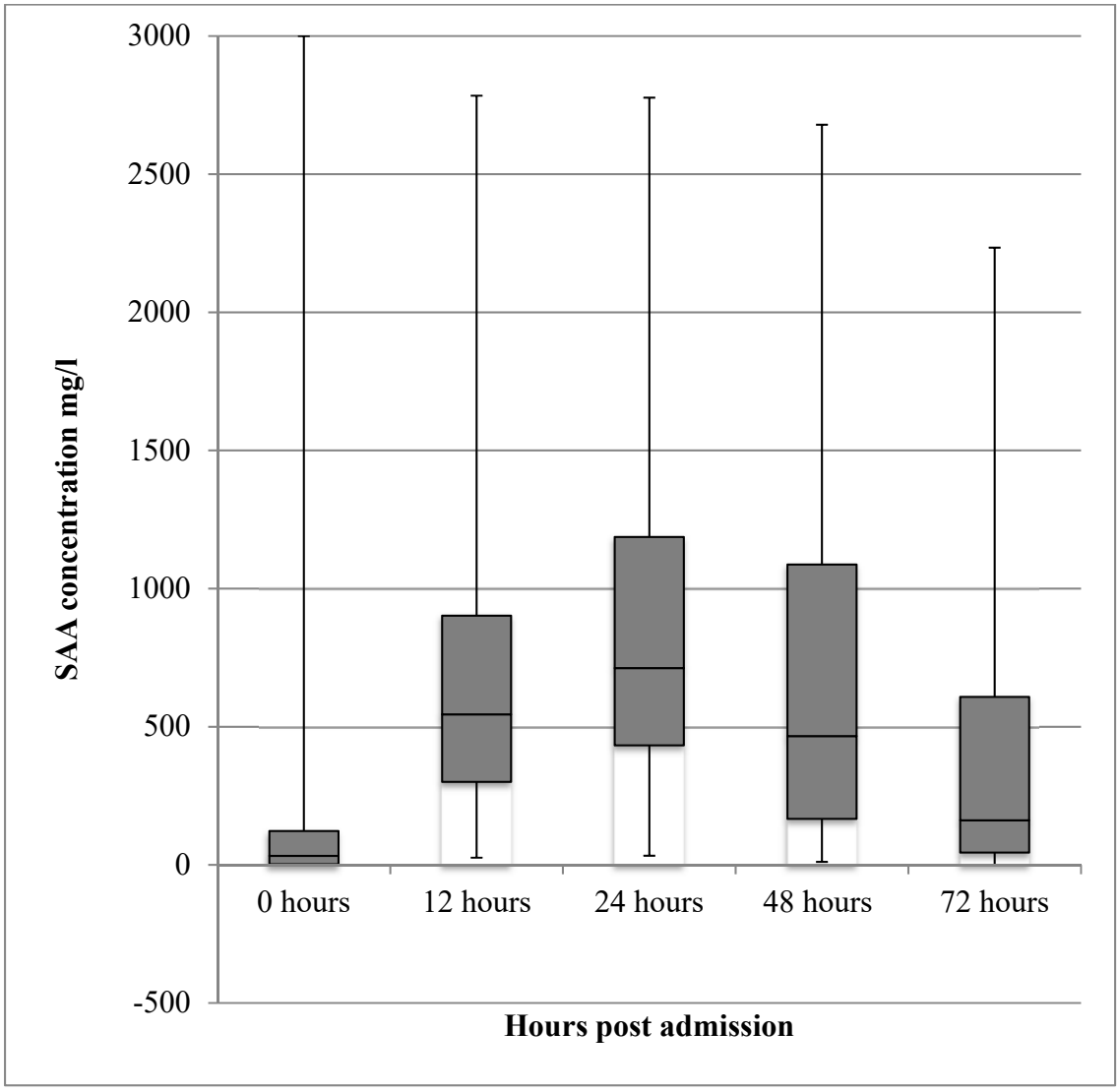


Figure 4.1: Min, Q1, medians, Q3, and max SAA concentrations of all neonates (n=24) at 0, 12, 24, 48, and 72 hours post admission.

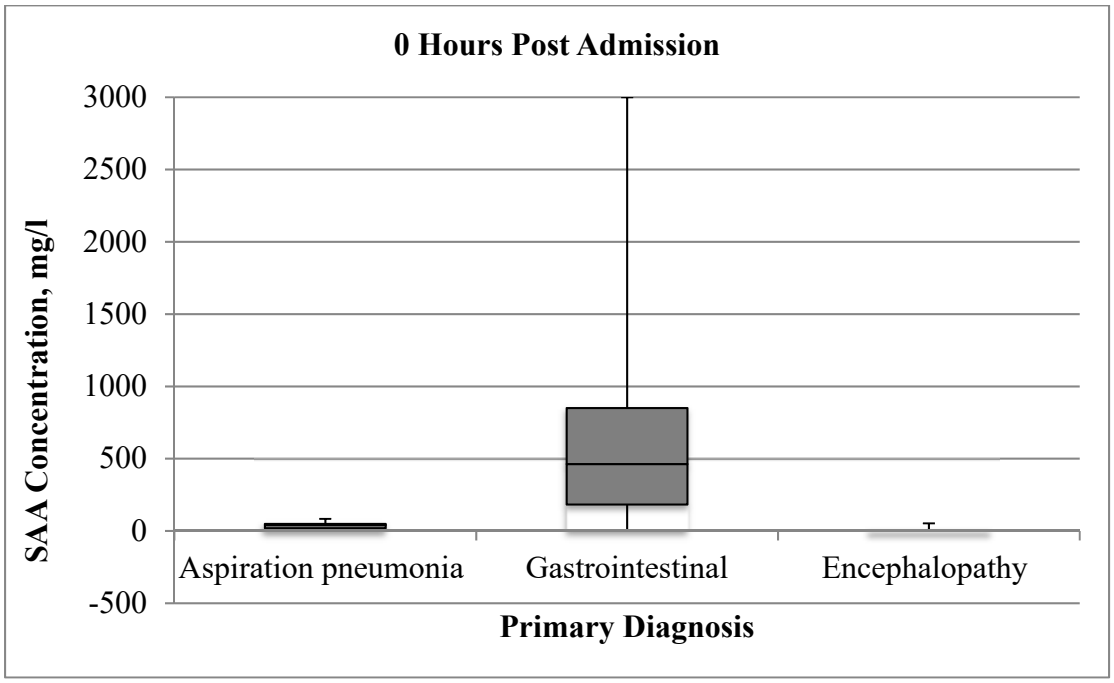


Figure 4.2: Min, Q1, median, Q3, and max SAA Concentration for pneumonia (n=8), gastrointestinal (n=8) and encephalopathy (n=8) diagnostic groups at 0 hours post admission

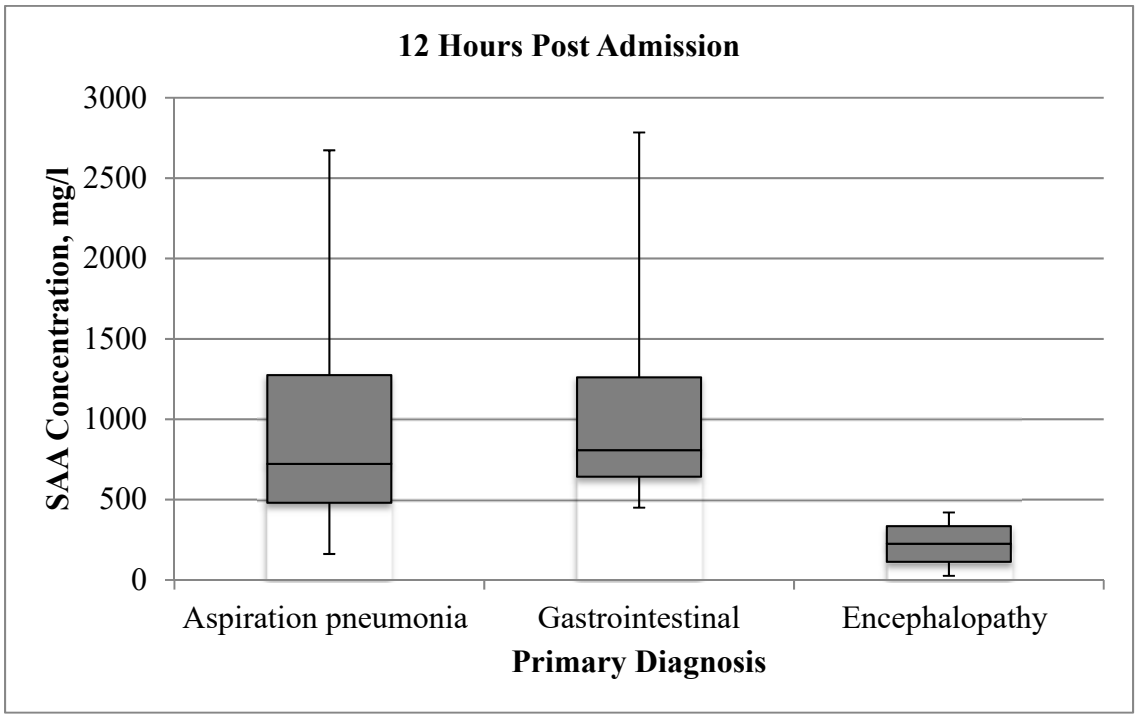


Figure 4.3: Min, Q1, median, Q3, and max SAA Concentration for pneumonia (n=8), gastrointestinal (n=8) and encephalopathy (n=8) diagnostic groups at 12 hours post admission

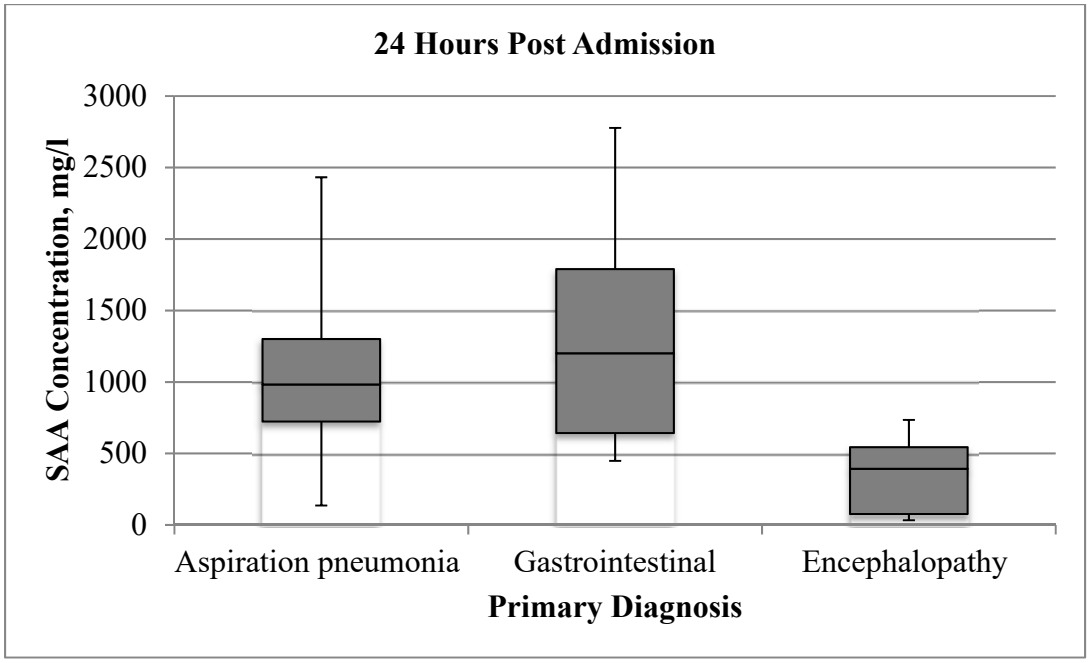


Figure 4.4: Min, Q1, median, Q3, and max SAA Concentration for pneumonia (n=8), gastrointestinal (n=8) and encephalopathy (n=8) diagnostic groups at 24 hours post admission

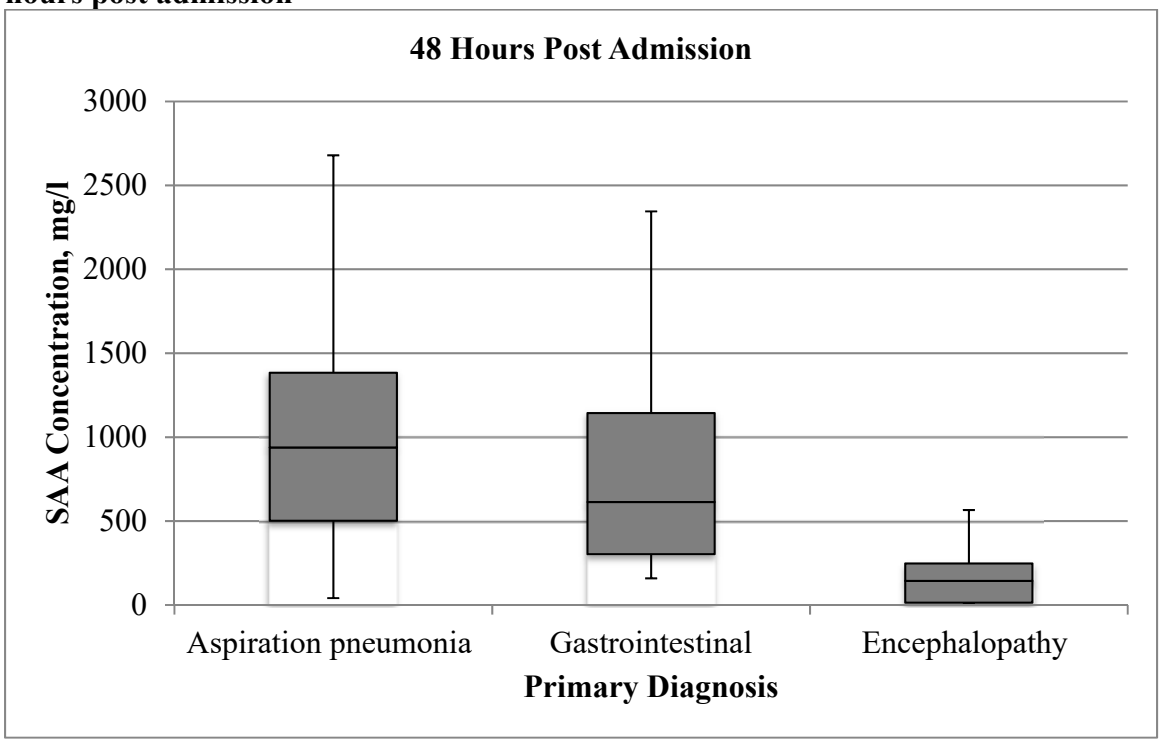


Figure 4.5: Min, Q1, median, Q3, and max SAA Concentration for pneumonia (n=8), gastrointestinal (n=8) and encephalopathy (n=8) diagnostic groups at 48 hours post admission

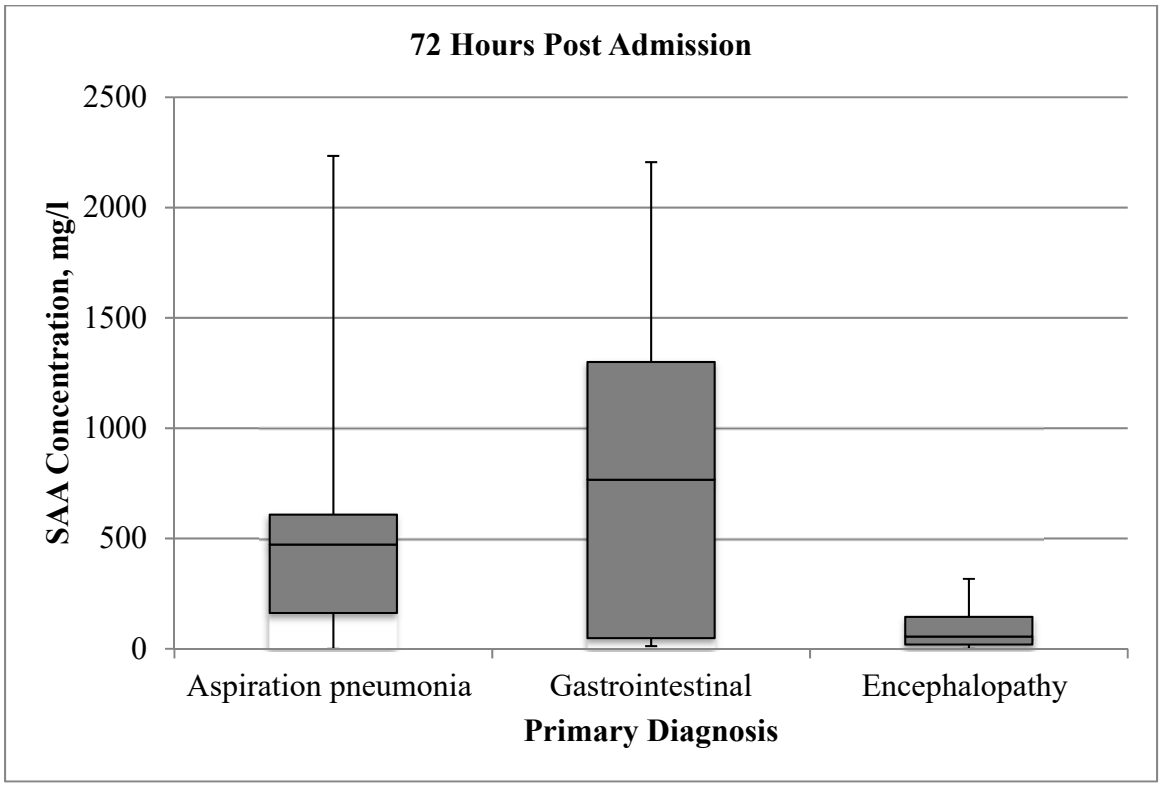


Figure 4.6: Min, Q1, median, Q3, and max SAA Concentration for pneumonia (n=8), gastrointestinal (n=8) and encephalopathy (n=8) diagnostic groups at 72 hours post admission

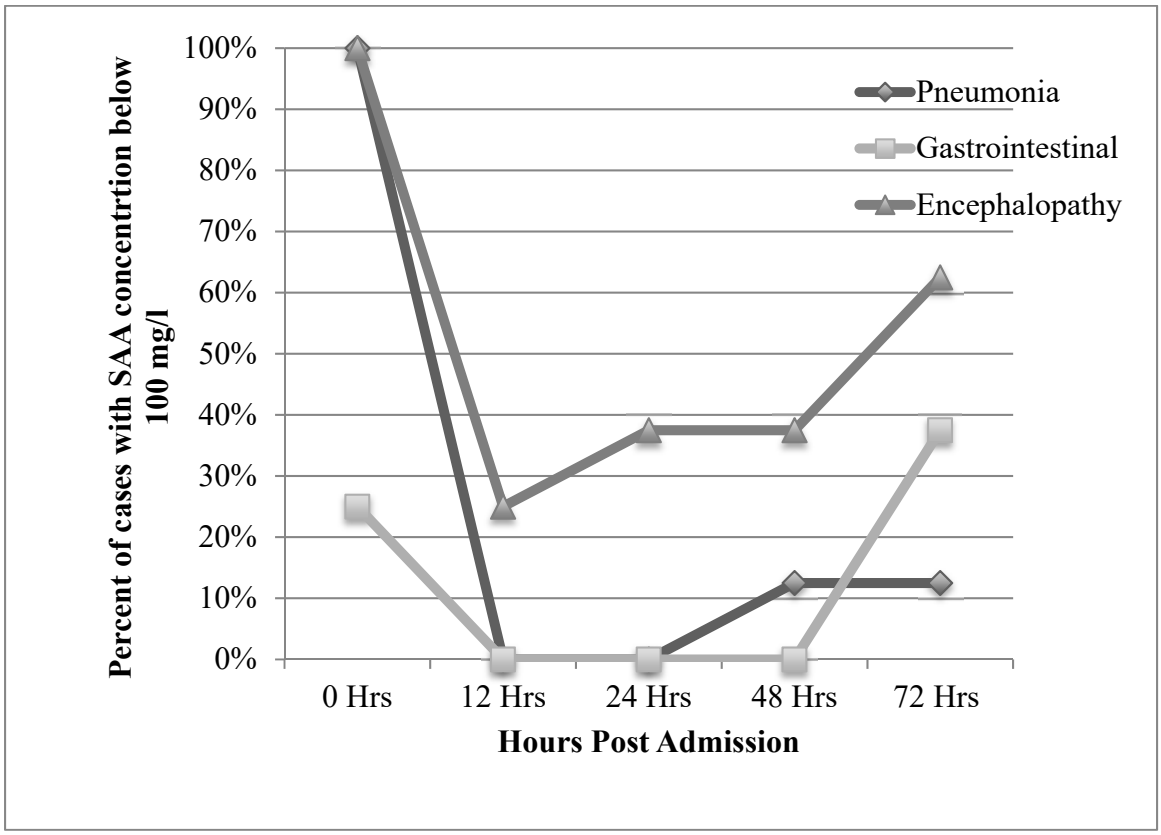


Figure 4.7: Percent of cases in pneumonia (n=8), gastrointestinal (n=8), and encephalopathy (n=8) diagnostic groups at 0, 12, 24, 48, and 72 hours post admission with an SAA concentration below 100 mg/l

Chapter Five: Conclusions and Future directions

Being able to quantify serum amyloid A concentration in neonates using a point of care test allows veterinarians to obtain valuable information with regard to the health or disease status of a neonate in a rapid time frame, without requiring access to a laboratory. The studies described in this thesis expand our knowledge of serum amyloid A concentrations in both the healthy and sick neonate. The work of this thesis supports previous studies in this area and demonstrates that a point of care SAA test kit provides veterinarians with a useful tool when diagnosing disease in equine neonates. The studies in this thesis demonstrate that limitations exist regarding using a point of care SAA test kit to diagnose disease in equine neonates. In order for this test to give the most insight into the occurring disease processes, the test needs to be executed once SAA concentrations have had ample time accumulate. Testing for SAA concentration too early (before 12 hours of age) may result in false negative results and misdirect a veterinarian's diagnosis. An alternative way to avoid this limitation is to continue to test SAA concentration as time progresses.

In follow up to the studies executed in this thesis, it would be beneficial for future studies in this area to focus on how to utilize a point of care serum amyloid A kit to monitor efficacy of treatment and convalescence of disease. Also, knowing which cases to continue to monitor and how often to test serum amyloid A concentration in particular cases would offer valuable information to veterinarians. Future studies in these areas would not only continue to improve diagnostic procedures, but would also advance equine neonatal disease management.

APPENDIX I

LIST OF ABBREVIATIONS

SAA……...……Serum amyloid A

CRP……C Reactive Protein

WBC……..……White Blood Cell

TIA.……..……Turbidometric Immunoassay

EIV……..……..Equine Influenza Virus

EHV-4……. Equine Herpesvirus-4

IAD…….…….. Inflammatory Airway Disease

SP-D……..Surfactant Protein D

HIE…….……Hypoxic Ischemic Encephalopathy

Appendix II: Foal Admission Form

ROOD & RIDDLE
EQUINE HOSPITAL

FOAL ADMISSION FORM

PATIENT NAME: **DATE:** **TIME:** **WEIGHT:**
CLINICIAN: **DATE / TIME OF BIRTH:**
TECH / SUPERVISOR: **SEX:** **COLT** ☐ **FILLY** ☐
HISTORY:

PHYSICAL EXAMINATION: **T:** **P:** **R:** **MM:** **CRT:**
CV:
RESP:
GI:
MS: Cold Extremities _____
OU:
UMB:
RESUSCITATION:
EPINEPHRINE: DOPRAM: DOBUTAMINE:.
NON-INVASIVE RES: ☐ INTUBATED: ☐ AMBU BAG: ☐ CHEST COMPRESSIONS: ☐
NOTES:

ULTRASOUND NOTES:

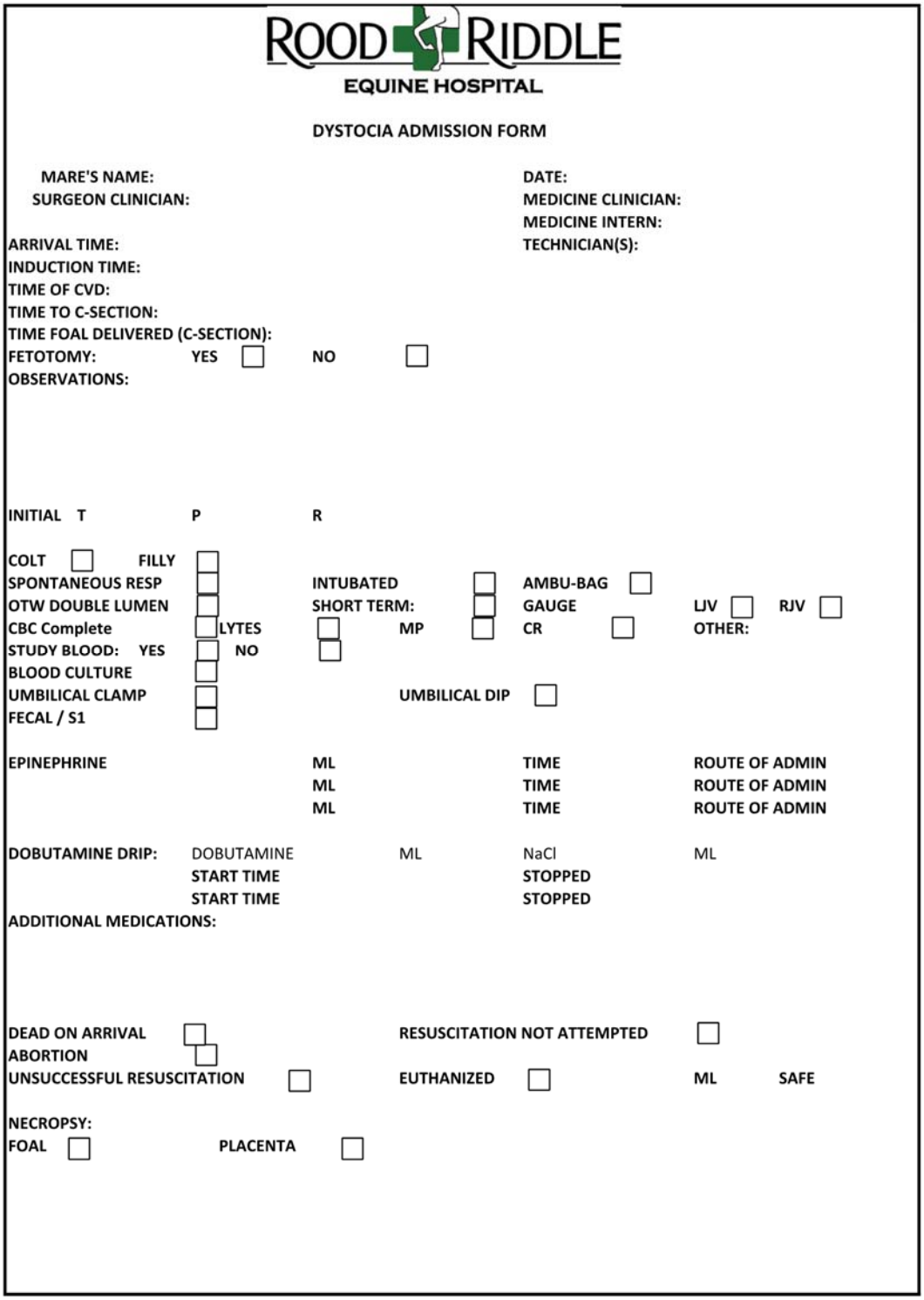
SEDATION:
VALIUM: **ML** **SAFE** **OTHER:**
BUTORPHANOL: **ML** **SAFE**

CATHETER: ☐ **TYPE / VEIN:** **RDVM CATHETER IN PLACE:** ☐
OXYGEN CANULA ☐ **L/MIN:**
INDWELLING NGT: ☐
UMBILICAL DIP: ☐
UMBILICAL CLAMP ☐
FECAL / S1 ☐
BLOODWORK / TESTING: **CBC/C** ☐ **MP** ☐ **CR** ☐ **LYTES** ☐ **SAA** ☐
ACCU CHECK **LACTATE** **BLOOD Cx** ☐ **ARD** ☐
ISTAT: TYPE:. ISTAT RESULTS:
STUDY BLOOD:
SURGERY: **SURGEON / NOTES:**

RADIOGRAPHS:
ENEMA: **SOAPY** ☐ **RETENTION:** ☐
NOTES:
REFLUX TUBE ☐ **AMOUNT:** **TUBE REMOVED** ☐
FLUIDS **AMOUNT:** **TYPE:**
ADDITIVES:
ADDITIONAL ADMISSION NOTES AND DRUGS:

MARE TRANQ ☐ **T:** **P:** **R:**
COLOSTRUM **VALUE:** **NI** ☐
MARE NOTES:

Appendix III: Dystocia Admission Form



ROOD & RIDDLE
EQUINE HOSPITAL

DYSTOCIA ADMISSION FORM

MARE'S NAME: DATE:
SURGEON CLINICIAN: MEDICINE CLINICIAN:
MEDICINE INTERN:
ARRIVAL TIME: TECHNICIAN(S):
INDUCTION TIME:
TIME OF CVD:
TIME TO C-SECTION:
TIME FOAL DELIVERED (C-SECTION):
FETOTOMY: YES ☐ NO ☐
OBSERVATIONS:

INITIAL T P R

COLT ☐ FILLY ☐
SPONTANEOUS RESP ☐ INTUBATED ☐ AMBU-BAG ☐
OTW DOUBLE LUMEN ☐ SHORT TERM: ☐ GAUGE LJV ☐ RJV ☐
CBC Complete ☐ LYTES ☐ MP ☐ CR ☐ OTHER:
STUDY BLOOD: YES ☐ NO ☐
BLOOD CULTURE ☐
UMBILICAL CLAMP ☐ UMBILICAL DIP ☐
FECAL / S1 ☐

EPINEPHRINE ML TIME ROUTE OF ADMIN
ML TIME ROUTE OF ADMIN
ML TIME ROUTE OF ADMIN

DOBUTAMINE DRIP: DOBUTAMINE ML NaCl ML
START TIME STOPPED
START TIME STOPPED
ADDITIONAL MEDICATIONS:

DEAD ON ARRIVAL ☐ RESUSCITATION NOT ATTEMPTED ☐
ABORTION ☐
UNSUCCESSFUL RESUSCITATION ☐ EUTHANIZED ☐ ML SAFE

NECROPSY:
FOAL ☐ PLACENTA ☐

Appendix IV: Data used in original study in Chapter 3

Patient #	Primary Diagnosis	SAA Concentration, mg/l
1	Colitis	0
2	Colitis	0
3	Colitis	5
4	Colitis	32
5	Colitis	65
1	Colitis	68
6	Colitis	88
7	Colitis	677
8	Colitis	1470
9	Contracted	0
10	Contracted	0
11	Contracted	0
12	Contracted	13
13	Contracted	16
14	Contracted	538
15	Contracted	
16	Enteritis	0
17	Enteritis	4
18	Enteritis	46
19	Enteritis	64
20	Enteritis	108
21	Enteritis	390
22	Enteritis	517
23	Enteritis	526
24	Enteritis	619
25	Enteritis	718
26	Enteritis	2640
28	HIE	0
29	HIE	0
30	HIE	0
31	HIE	0
32	HIE	0
33	HIE	0
34	HIE	0
35	HIE	1
36	HIE	11

37	HIE	13
38	HIE	107
39	HIE	167
40	HIE	176
41	HIE	225
42	HIE	782
43	HIE	953
44	Normal	0
45	Normal	0
46	Normal	0
47	Normal	0
48	Normal	0
49	Normal	0
50	Normal	0
51	Normal	0
52	Normal	0
53	Normal	0
54	Normal	1
55	Normal	34
56	Normal	215
57	Pneumonia	3
58	Pneumonia	61
59	Pneumonia	563
60	Pneumonia	1023
61	Pneumonia	1280
62	Pneumonia	2387
63	Pneumonia	2783
64	Pneumonia	3899
65	Pneumonia	4081
66	Pneumonia	4724
67	Premature	0
68	Premature	0
69	Premature	0
70	Premature	0
71	Premature	0
72	Premature	0
73	Premature	10
74	Premature	45

75	Premature	46
76	Sepsis	0
77	Sepsis	6
78	Sepsis	15
79	Sepsis	43
80	Sepsis	121
81	Sepsis	238
82	Sepsis	308
3	Sepsis	324
84	Sepsis	455
85	Sepsis	565
86	Sepsis	701
87	sepsis	878
88	Sepsis	1212
89	Sepsis	1757
90	Sepsis	1917
91	Sepsis	2348
92	Sepsis	3107
93	Sepsis	4764
94	Weak Foal	0
95	Weak Foal	0
96	Weak Foal	0
97	Weak Foal	0
98	Weak Foal	0
99	Weak Foal	0
100	Weak Foal	0
101	Weak Foal	0
102	Weak Foal	0
103	Weak Foal	0
104	Weak Foal	0
105	Weak Foal	2
106	Weak Foal	2
107	Weak Foal	2
108	Weak Foal	3
109	Weak Foal	3
110	Weak Foal	5
111	Weak Foal	7
112	Weak Foal	8

113	Weak Foal	9
114	Weak Foal	14
115	Weak Foal	23
116	Weak Foal	26
117	Weak Foal	32
118	Weak Foal	34
119	Weak Foal	40
120	Weak Foal	59
121	Weak Foal	70
122	Weak Foal	83
123	Weak Foal	198
124	Weak Foal	849
125	Weak Foal	1046
126	Weak Foal	1106

Appendix V: Data used in original study in Chapter 4

		SAA Concentration mg/l				
Age at admit (hrs)	**Primary Diagnosis**	**0 Hrs post admission**	**12 Hrs post admission**	**24 Hrs post admission**	**48 Hrs post admission**	**72 Hrs post admission**
0	aspiration pneumonia	6	523	836	1264	581
0	aspiration pneumonia	5	354	385	802	143
0	aspiration pneumonia	24	767	1123	1076	592
0	aspiration pneumonia	44	677	2432	2679	2234
0	aspiration pneumonia	84	1368	1135	1743	657
0	aspiration pneumonia	42	2673	843	517	365
0	aspiration pneumonia	41	162	136	41	0
0	aspiration pneumonia	63	1243	1798	456	169
48	gastrointestinal	3000	2784	2528	453	1186
24	gastrointestinal	646	763	448	159	59
48	gastrointestinal	760	1049	1228	1213	1169
48	gastrointestinal	279	668	627	340	12
12	gastrointestinal	1123	1895	2777	775	1642
12	gastrointestinal	238	853	1173	1121	363
0	gastrointestinal	0	566	677	190	17
0	gastrointestinal	17	450	1544	2345	2206
0	HIE	1	26	81	12	0
0	HIE	0	324	689	566	317
0	HIE	12	222	735	476	154
0	HIE	53	420	482	169	51
0	HIE	0	370	496	14	60
0	HIE	3	141	304	118	26
0	HIE	0	28	33	11	0
0	HIE	0	229	61	171	142

Appendix VI: Diagnostic values for differentiating between infectious and non-infectious disease as determined by the test manufacturer

SAA cut-off value	15 ug/ml	30 ug/ml	50 ug/ml	100 ug/ml	200 ug/ml
Sensitivity	93	91.5	90.7	89.1	83.7
Specificity	100	100	100	100	100
Pos. Predictive Value	100	100	100	100	100
Neg. Predictive Value	81.6	78.4	76.9	74.1	65.6
Accuracy	94.7	93.5	92.9	91.7	87.6

The diagnostic values for differentiating between infectious and non-infectious disease as determined by the test manufacturer were used to determine the threshold (100mg/l) that would indicate disease in the original studies in this thesis.

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Night Shift Intensive Care Unit Technician Supervisor, Nov 2015 – Current
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PEER-REVIEWED PUBLICATIONS

- **Strouss, SW**., Barr, BS., Rossano, MG. Assessing the use of a stall-side serum amyloid A test to diagnose sepsis in equine neonates. *J Am Vet Med Assoc* (2018). Manuscript submitted for publication.
- **Strouss, SW**., Barr, BS., Rossano, MG. Time series analysis of serum amyloid A concentration in equine neonates. *J Am Vet Med Assoc* (2018). Manuscript in preparation.

CONFERENCE PRESENTATIONS

- Oral Presentations
  - **Strouss, SW**., Barr, BS., Rossano, MG. (December, 2017) *Assessing the use of a stall-side serum amyloid A test to diagnose sepsis in equine neonates.* Conference of Research Workers in Animal Diseases, Chicago, IL. (Peer-reviewed)
- Poster Presentations
  - **Strouss, SW**., Barr, BS., Rossano, MG. (May, 2018) *Assessing using serum amyloid A to diagnose disease in neonates.* Animal & Food Sciences Graduate Association Poster Symposium, Lexington, KY.