

**Efficacy of hyperimmunized plasma in the treatment of
horses with acute diarrhea**

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(ABSTRACT)

The aim of this study was to evaluate the use of a hyperimmunized plasma containing high concentration of antibodies against *Clostridium difficile*, *Clostridium perfringens* and *Salmonella sp* in a referral population of equine colitis cases.

A prospective, blinded clinical trial was undertaken. Horses were enrolled if they were over 1 year old, duration of diarrhea at presentation was less than 72 hours, they had not received equine plasma within the last 3 months and the serum total protein was greater than 4mg/dl. Horses were randomized to receive hyperimmunized plasma, control plasma (collected from non-immunized horses) or no plasma therapy. Clinical parameters were recorded and a fecal score (2 -14) assigned (every 6 hours) based upon diarrhea frequency, volume and consistency, for a total of 72 hours. A score less than 5 was considered normal. Fecal consistency was observed until resolution, discharge or death. Complete blood counts and biochemical profiles were collected at admission, 24 and 72 hours and at admission, 24 hours and 48 hours respectively.

Forty two horses were enrolled and 38 horses completed the study. At study admission clinical and clinicopathological parameters, other than fecal frequency score were comparable between the groups. Fecal frequency score was significantly different between the treatment groups ($p=0.003$). The mean duration of diarrhea was 40.7 ± 9.8 hours (mean \pm SEM), 119.2 ± 56.1 hours and 72.0 ± 24.5 hours for the hyperimmunized plasma, normal plasma and control groups respectively. This data confirms the hyperimmunized plasma used in this study decreased the time to resolution of diarrhea.

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Literature review

Chapter 1: Pathophysiology of acute colitis in the adult horse and discussion of the more common etiologies for this condition

Introduction

Acute colitis is a debilitating condition that can affect horses and ponies of any breed, age or gender. By definition, colitis is associated with inflammation of the colonic mucosa which leads to the development of diarrhea, although diarrhea may not be evident upon initial examination. The degree of colonic inflammation can be profound, leading to severe losses of fluid and electrolytes or permanent intestinal injury. Despite aggressive treatment the clinical status of an affected horse can deteriorate rapidly, leading to an overall fatality rate that has been reported to be as high as 70%.¹

There is a limited understanding of the prevalence and predisposing factors for acute colitis, predominantly due to the fact that determining the underlying etiology of acute colitis is challenging, and frequently not achieved. Few causes of acute colitis in adult horses have been documented compared with those in other animals and humans and a definitive diagnosis is reached in only approximately 35% of all acute equine colitis cases.² In addition, identification of a specific etiological diagnosis may be complicated by multiple potential pathogens.

Regardless of the cause, the clinical signs of diarrhea, abdominal pain, pyrexia, circulatory failure or even sudden death are similar and represent a derangement in the normal physiologic process of the large intestine i.e. colon and cecum. This chapter describes the normal physiology of the equine large intestine and the fundamental pathophysiology associated with acute colitis, and reviews the common etiological agents of acute equine colitis. (Table 1.1)

Normal Physiology

The equine large intestine is comprised of the cecum, large colon, transverse colon, small colon and rectum. The cecum has an average length of 1m and a fluid capacity of 33L, while the large colon is 3-4m in length and has a capacity of 130L. The small colon is narrow with a small

total capacity but it can be up to 4m in length.³ The large intestine is the principal site of digestion and water balance in the horse and on a normal daily basis it secretes and recovers a volume of fluid approximately equal to the total extracellular fluid volume of the animal, or about 100 liters/day.⁴ Up to 75% of the energy requirements in the horse are met by the products of microbial fermentation of carbohydrates in the cecum and colon, most importantly volatile fatty acids (VFA). A stable luminal environment is required for the efficient functioning of the cecum and colon, therefore, luminal pH is tightly maintained at pH 6.8 - 7.2 and the colonic and cecal luminal osmolality is kept at approximately 300mOsm.⁵

Passage of fluid and digesta through the cecum and large colon is relatively slow, in order to ensure adequate time for microbial digestion and fermentation and the absorption of the products of digestion. Fluid can take up to 50 hours to move through the large colon and digesta can take 2-3 days with times varying according to the type of digesta.⁶ The cecum and colon have 3 phases of motor activity: mixing, retention and retropulsion of ingesta. Motility in the cecum consists of mixing contractions in which the haustra alternately contract and relax. Additionally, every few minutes a strong, mass movement-type contraction occurs that forces some of the cecal contents through the cecocolic orifice into the colon. Within the colon, mixing and haustral contractions efficiently blend the ingesta and expose it to the mucosal surface for the absorption of water, electrolytes and the volatile fatty acids produced by bacterial fermentation.

Water moves into or out of the intestine until the osmotic pressure of the intestinal contents equals that of the plasma.⁶ The absorption of water depends on the absorption of nutrients, such as sugars and amino acids and on the absorption of ions. Absorption is mostly transcellular as tight junctions form intercellular contacts which regulate solute movement through the paracellular pathway. (Figure 1.1)

The ion shifts of primary importance in the equine colon include the net absorption of sodium and chloride ions and the net secretion of bicarbonate ions. (Figure 1.2) The transport mechanisms for these ions involve passive and active forces. The passive forces include the intrinsic permeability of the intestinal epithelial cells, the osmotic pressure gradient exerted by the contents of the intestinal lumen, the electrical potential difference across the intestinal

epithelial cells, the concentration gradient of solutes across the intestinal epithelial cells and the pH of the luminal contents.⁵ Active transport mechanisms include primary and secondary processes. Primary active transport involves movement of an ion against its electrochemical gradient using energy. In the equine colon sodium is actively transported by the sodium-potassium adenosinetriphosphatase ($\text{Na}^+\text{-K}^+\text{ATP-ase}$) pump present in the basolateral cell membranes of the colonic epithelial cells. This pump actively moves sodium out of the cells into the interstitium while moving potassium into the cells. This creates an electrochemical gradient of $\sim 35\text{mV}$ across the mucosal surface of the colonic epithelial cells, which facilitates movement of sodium from the colon lumen into the epithelial cells. The secondary active transport systems utilize the ‘free energy’ derived from passive diffusion of one ion down its electrochemical gradient to transport another ion against its electrochemical gradient. Sodium-hydrogen ion and chloride-bicarbonate exchange systems of this type have been identified in the mucosal surface of the equine colonic epithelial cells.⁵

Digestion in the colon is primarily by bacterial fermentation, with the normal large intestinal flora in horses being primarily comprised of anaerobes and streptococci, in conjunction with cellulolytic bacteria in the equine colon which appear to be similar to those found in the rumen of ruminants.⁷ The fermentation of soluble and insoluble carbohydrates yield volatile fatty acids (acetic, propionic and butyric acid), carbon dioxide, methane and lactate. The VFAs, which are the primary energy source in the horse, are passively absorbed through the mucosa of the colon and cecum into the blood where they are transported to the liver to be metabolized. Their absorption is tightly regulated by the luminal environment of the large intestine. At the normal pH of 6.8-7.2 the large intestinal contents 99% of the VFAs are ionized (dissociated).⁵ This form is poorly absorbed compared with the un-ionized (undissociated) form. The VFAs become un-ionized by transfer of hydrogen ions primarily from carbon dioxide. This carbon dioxide diffuses into the cells of the cecal and colon wall, hydrates to form carbonic acid and then dissociates into bicarbonate and hydrogen ions.⁵ The bicarbonate ions left behind accumulate in the luminal fluid. High concentrations of VFAs inhibit the $\text{Na}^+\text{-H}^+$ exchange but as the VFAs become un-ionized and are absorbed this pump activity increases and the H^+ ions enter the colon and cecal lumen to buffer the increased level of bicarbonate ions.⁵ (Figure 1.3)

Tight regulation of the resident gastrointestinal microbial population is important, as the normal flora protect the host from pathogenic bacteria by means of colonization resistance, whereby competing for space and nutrients the normal flora inhibits the colonization and proliferation of pathogenic bacteria.⁸ The normal flora also produce bacteriocins that inhibit growth of potential pathogens.⁸ Specific host defenses that further preclude the growth of pathogenic bacteria include gastric pH, gastrointestinal motility, the mucosal barrier and mucosal immunity. In addition, VFAs produced by the normal flora inhibit growth and block bacterial attachment of pathogenic bacteria to the mucosal surface. Any disturbance in the normal flora impairs these normal defense mechanisms and increases the susceptibility of the intestine to colonization by pathogenic organisms.

Pathophysiology

The pathophysiologic mechanisms of acute colitis can be subdivided into inflammation, abnormal passive and active secretion and decreased transit time. The inflammatory process is complex and is comprised of a multitude of different components. The equine large intestine is poised to mount an inflammatory response to antigenic stimuli, as lymphoid follicles and mast cells are plentifully distributed throughout the mucosa of the cecum and colon, and neutrophils and macrophages are normally present within the colonic mucosa and submucosa.⁹ Unfortunately, the process by which inflammatory cells attack foreign antigens is not always specific or well regulated, and inflammation can cause secondary damage to host tissues. Neutrophil, eosinophil, mast cell and mononuclear cell responses and the production of inflammatory mediators such as prostaglandins and leukotrienes can all result in cellular and tissue damage. Proinflammatory cytokine production also plays a significant role in the development of inflammation within the colonic mucosa, as murine acute colitis models have demonstrated elevated concentrations of IL-1alpha/beta, IL-6, IL-18, and granulocyte colony-stimulating factor within the colonic mucosa.¹⁰ Bradykinin and histamine are released by neutrophils and mast cells during inflammation and studies have shown that they increase secretion and impair absorption by the colonic mucosa.¹¹ The production and liberation of oxygen free radicals, which are directly cytotoxic, leads to further injury of the colonic mucosal

epithelium. Oxygen free radicals may also potentiate the activity of proteolytic enzymes released by phagocytic cells during the inflammatory process and they can inhibit antiproteases that are naturally found in the colonic mucosa which function to prevent protease-induced cellular damage. When the tissue injury becomes severe the mucosal epithelial cells are lost, leading to the formation of erosions and ulcers.¹¹ Overall, disruption of the colonic mucosa leads to a reduction in epithelial surface area, loss of absorptive cells and failure of the tight junctions, with the net result of increased fecal water secondary to impaired reabsorption and increased passive secretion. In recent studies which tried to correlate cytokine concentrations and macroscopic colonic lesions it was found that there was increase in both IFN-gamma and IL-6 related to the presence of necrosis of the colonic mucosa.¹²

Passive fluid loss from the vasculature is minimized in the normal horse because the capillary endothelium is relatively impermeable to macromolecules such as albumin. In acute colitis the endothelium is often damaged, resulting in increased capillary permeability to macromolecules and movement of albumin from the capillary to the interstitium. This decreases plasma oncotic pressure. Normally, small fluctuations in the driving forces for fluid movement do not cause interstitial edema because of factors that resist expansion of the matrix (edema safety factors). However, with diminished plasma oncotic pressure across the capillary wall, the water is lost from the vascular to the interstitial space. With increased protein loss the hypoproteinemia worsens and the development of interstitial edema becomes a self perpetuating process.¹³ Progressive tissue edema and plasma protein loss into the interstitium and eventually into the intestinal lumen occurs. Agents that increase capillary permeability in equine colitis include endotoxin, enterotoxins, oxygen radicals, histamine and prostaglandins.⁹

In many horses with colitis there is also active secretion of solutes and water by the inflamed colonic mucosa.¹⁴ As discussed earlier, the fluid and electrolyte transport processes in the colonic epithelial cells are tightly governed by a multitude of different processes. Many of these processes have not been studied in detail in the horse but it has been shown in many species that probably the single most important secretory event leading to an increase in fecal water/diarrhea and electrolyte loss is an increase in the secretion of chloride ion, which is primarily mediated by an increase in intracellular cyclic AMP (cAMP). Increasing chloride

secretion directly increases water secretion since water passively follows the chloride ions. A compensatory increase in water reabsorption is prevented by the inhibitory effect of increasing intracellular cAMP on the functioning of the Na^+H^+ pump which leads to a decrease in the reabsorption of sodium and the associated reabsorption of water. Increased cAMP also increases the transport of potassium out of the intestinal cells via a basolateral potassium channel which increases the intracellular chloride level, thereby further enhancing chloride secretion down the concentration gradient. Endogenous agents that can increase intracellular cAMP include prostaglandins E_1 and E_2 , calcium and vasoactive intestinal peptide (VIP).⁵ (Figure 1.4)

Intestinal motor function is intrinsic to the process of normal digestion and absorption. Fluid will not traverse the intestinal tract unless it is propelled by contractile activity.⁶ Propulsive and segmental activity of the muscle layers within the intestinal tract mixes the food with intestinal secretions, alters the surface area of intestine exposed to luminal contents and regulates the rate of intestinal transit and contact time during which mucosal absorption can occur. Abnormal motor patterns have been demonstrated to occur in acute colitis cases and may be a response of the bowel to irritation and/or increased intraluminal volume.¹⁵ Typically the abnormal motor pattern leads to an increased rate of transit, secondary to either an increase in propulsive activity or a decrease in the contractions normally impeding intestinal flow.¹⁶ Increasing rate of transit leads to a decrease in the contact time for fluid absorption, and results in an increase in fecal water and in the frequency of defecation (i.e. diarrhea).

Causes of Equine Acute Colitis

Salmonellosis

Salmonellosis is reported to be the most frequently diagnosed infectious cause of diarrhea in the horse.¹ *Salmonella sp.* belong to a genus of gram-negative facultatively anaerobic bacteria. Many different serotypes having been reported to infect the horse with those in Group B, including *Salmonella typhimurium* and *Salmonella agona*, appearing to be associated more commonly with disease than those in other groups.¹ Four syndromes of *Salmonella* infection have been described clinically and reproduced experimentally in horses: (1) inapparent infections

with latent or active carrier states, (2) acute colitis, (3) depression, fever, anorexia and neutropenia without diarrhea or colic, (4) septicemia with or without diarrhea.

In acute cases of *Salmonella* enterocolitis disruption of the host's defenses and colonization of the distal small intestine and colon is the first step in the pathogenesis.¹ The *Salmonella* bacteria attach to and then enter the mucosal epithelial cells in the ileum, cecum and proximal large colon. The common portal of entry into the intestinal cells is through the brush border but the bacteria can also penetrate via the tight junctions.¹ The bacteria enter the lamina propria, are phagocytosed by the mucosa-associated macrophages then multiply intracellularly and initiate local inflammation. Recruitment and activation of leukocytes occurs with subsequent production of prostaglandins, leukotrienes, reactive oxygen metabolites and histamine.⁸ Damaged mucosal epithelial cells and activated macrophages produce pro-inflammatory cytokines, such as interleukin-1 and tumor necrosis factor-alpha, which further upregulate the local inflammatory response and upon reaching the circulation initiate the systemic inflammatory response (fever, tachycardia, tachypnea, etc.). While *Salmonella* organisms which have crossed the mucosal barrier may enter the bloodstream, either directly or via the lymphatics, in most cases the bacteria do not disseminate beyond the intestinal mucosa and the mesenteric lymph nodes.¹

Additional pathogenic mechanisms demonstrated by *Salmonella* bacteria include toxin production and increased secretion. *Salmonella*-associated cytotoxin inhibits protein synthesis in mucosal cells causing morphologic damage and altered permeability.⁸ Virulent salmonellae also produce enterotoxin similar to the heat-labile toxin (LT) produced by *Escherichia coli*.⁸ Studies have shown a difference in the effect of enterotoxin in the small intestine to that in the large intestine and considerable species variation. Enterotoxin increases secretion of chloride and water by colonic mucosal cells via a prostaglandin-mediated increase in intracellular cAMP but the process is more complex than that of cholera toxin action, which is classically used for research models and is antigenically similar to *Salmonella* spp enterotoxin.¹⁷ In addition to the increase in prostaglandins and cAMP, a marked host inflammatory response occurs which consists of humoral and cellular components.¹⁸ The acute enterocolitis caused by *Salmonella* organisms is characterized by severe fibrinonecrotic typhlocolitis with interstitial edema and

variable degrees of intramural vascular thrombosis that may progress to infarction.¹⁹ Recovery of normal large intestinal function typically takes at least 5-7 days, but the severity of mucosal injury may be severe enough that normal function will not return and the diarrhea will become chronic in nature.

Clostridium difficile

Clostridium difficile is an obligate anaerobic spore-forming gram positive rod that is ubiquitous in the environment in the spore form. It was first identified in human neonatal feces in 1935 and research has shown that *C. difficile* bacteria are among the first bacteria acquired after birth and are a component of the normal gastrointestinal flora of foals and adult horses.^{20,21} *Clostridium difficile* has been reported to cause acute enterocolitis in humans and horses and is now recognized as the primary cause of nosocomial and antibiotic associated diarrhea and colitis in humans.²² The involvement of *C. difficile* in equine enteric disease was first described by Jones *et al* (1987) where it was associated with diarrhea in 27/43 foals in an outbreak of enterocolitis.²³ Disruption of the gastrointestinal flora by stress, antimicrobial therapy or other factors allows for overgrowth of *C. difficile* and the associated release of toxins leads to the development of acute diarrhea.

In human beings *C. difficile* is considered to be the most common enteric pathogen in hospitalized patients.²⁴ Disease usually occurs in association with antimicrobial therapy and the risk of contracting the disease increases the longer antibiotic treatment continues.²⁵ Additional risk factors for *C. difficile* infection in human patients include severe underlying disease, presence of a nasogastric tube, change of diet, starvation, transportation and administration of anti-ulcer medication.²⁰ In the horse, a wide variety of antimicrobials have been associated with the development of *C. difficile* diarrhea but orally administered antimicrobials, or those recycled via the enterohepatic system, are the most well documented to increase the number of clostridial colony forming units in equine feces.²²

The pathogenesis of *C. difficile* has not been studied in detail in horses. A horse can be infected by the vegetative cells of *C. difficile* or its spores from other infected horses, a contaminated environment, or human beings. The *C. difficile* spores are resilient and not easily

destroyed in the environment. Baverud *et al* showed that they can survive in inoculated feces for at least 4 years.²⁰ Another possible route of infection is the proliferation of the *C. difficile* spores which are present at low concentration in the gastrointestinal tract of subclinical carriers.²⁰ If the normal gastrointestinal flora is disrupted, for example by a change in diet, antibiotic administration or by gastrointestinal surgery, then proliferation of *C. difficile* is increased.

Further complicating the assessment of the role of *C. difficile* in equine diarrhea is the fact that not all strains of *C. difficile* can cause disease.²⁰ Pathogenic strains produce at least 5 different toxins, of which toxin A and B have been studied in most detail.²⁶ Adenosine diphosphate-ribosyltransferase (binary toxin) has been recognised recently but its role in disease has not been determined.²⁷ Following ingestion *C. difficile* spores survive the low pH of the stomach and upper small intestine, then germinate in the terminal ileum and multiply in the colonic lumen. In the absence of competition by the indigenous microflora the bacteria grow to large numbers and produce toxins that damage the intestinal tissue.²⁴ Toxin A is an enterotoxin and is thought to be the most significant toxin with regards to induction of fluid secretion, inflammation and the characteristic alterations in intestinal morphology. Toxin A also weakens the tight junctions between the epithelial cells that line the colon thereby helping toxin B to enter the epithelial cells.⁸ It induces neutrophil influx into the intestinal tissue as well as mast cell degranulation and secretion of prostaglandins, histamine, inflammatory cytokines and 5-hydroxytryptamine by the activated leucocytes which lead to vasodilatory and secretory responses in the enterocytes.⁸ Toxin B has profound cytotoxic effects *in vitro* and is more than 1000 times more cytotoxic than toxin A but it appears to have few apparent pathologic effects on intact intestinal mucosa.²⁸ That said, a toxin-A negative, toxin-B positive strain of *Clostridium difficile* has been implicated in humans suggesting that toxin B may also be pathogenic.²⁸

Clostridium perfringens

Clostridium perfringens is similar to *Clostridium difficile* in its appearance, physiological requirements and environmental behavior. It is considered a normal inhabitant of the gastrointestinal tract of horses with a positive fecal culture being found in 12-22% of adult healthy horses and 90% of 3 day old foals.²⁹ Dietary factors such as antibiotic treatment and

stressful stimuli can all induce intestinal dysbacteriosis characterized by an overgrowth of *C. perfringens*. However, unlike *C. difficile* which has been recognized as a cause of colitis in horses with no predisposing factors, there is debate as to whether *C. perfringens* can be a primary cause of colitis in the adult.¹ Experimental administration of *C. perfringens* enterotoxin to ponies has been documented to cause clinical signs of colitis, thereby suggesting that this bacterium has the potential to be pathogenic in adult horses.³⁰ Certainly if *C. perfringens* overgrowth does occur then large quantities of enterotoxin could be produced, which could have pathogenic effects on the colon leading to clinical signs of colitis.

There are many genetically distinct strains of *C. perfringens* of variable virulence which produce one or more of a large group of exotoxins. The pattern of exotoxin production is used to classify *C. perfringens* into five biotypes: A, B, C, D and E. All five biotypes of *Clostridium perfringens* produce alpha-toxin, which hydrolyses lecithin complexes in the membranes of capillary endothelium and other cells, as well as in mitochondria. This results in impaired glucose uptake and energy production and also results in activation of arachidonic acid metabolism and the protein kinase C signaling pathways in enterocytes, thereby resulting in cellular damage and potentially cell death.⁸ Oral administration of α -toxin to human subjects was associated with increased secretion by small intestinal mucosal cells, however no evidence of cytotoxicity was seen.³¹ The pathogenic significance of alpha toxin in horses remains poorly understood.

Clostridium perfringens type A is the most common clostridial isolate from healthy horses of all ages, but is also the most common isolate from adults and foals with diarrhea, and so it is possible that it may cause disease if overgrowth occurs. Type C has been the most commonly reported clostridial enteric pathogen in foals in North America.⁸ The identification of *C. perfringens* types B, D and E from clinical cases of colitis is rare.

Virulent strains of *C. perfringens* type A, and to a lesser extent *C. perfringens* type C, typically produce an enterotoxin. This is a cytotoxin which inserts into cell membranes and forms pores which alter cellular permeability to water and macromolecules, a process which ultimately leads to cellular necrosis.¹¹ Another toxin of likely pathogenic significance is the β 2 toxin. This is thought to be a pore forming toxin, similar to enterotoxin. It has been documented

to be produced by a type of *C. perfringens* found in horses suffering from colitis, thereby suggesting a pathogenic role for β_2 toxin in the etiology of *C. perfringens* associated colitis.³²

Potomac Horse Fever

Potomac horse fever (PHF, equine monocytic ehrlichiosis) is caused by the obligate intracellular rickettsial organism *Neorickettsia risticii*. The role of this organism in Potomac Horse Fever was first established over 20 years ago when inoculation of blood from an infected horse led to the development of clinical signs in a control horse.³³ However, until the development of reliable PCR tests and the completion of recent studies, the life cycle remained undefined. Studies have now shown that the causative rickettsial organisms live within trematodes. These trematodes are ingested by freshwater operculate snails and aquatic insects which act as intermediate hosts.^{34,35} Bats, and possibly birds, may act as the definitive host of the helminth vector and as a natural reservoir of *N. risticii* but the definitive host for *N. risticii* remains a subject of debate.³⁶ The mechanism of transmission of *N. risticii* organisms to horses has been clarified by challenge studies, however. Ingestion of numerous intermediate hosts, namely aquatic insects including stone flies, mayflies and aquatic water snails, will lead to the development of clinical signs consistent with Potomac Horse Fever, whereas percutaneous inoculation with *N. risticii* organisms did not result in clinical disease.³⁷

Having established entry into the horse the target organ for *N. risticii* is the gastrointestinal mucosa, with the resulting lesions being most severe in the large intestine. The organisms locate within the mucosal epithelium and in the macrophages and mast cells of the lamina propria. They survive within macrophages by inhibiting the production of reactive oxygen species and by blocking phagosome-lysosome fusion, thereby avoiding lysosomal digestion. Following intracellular infection an increase in intracellular cAMP occurs within the host cells. This leads to a decrease in luminal reabsorption of sodium ions, an increase in the secretion of chloride ions and a decrease in water reabsorption in the colon, which in turn results in the development of profuse watery diarrhea. In addition, once infected cells fill with the rickettsial organisms, cell lysis occurs. As the disease progresses fibrinous necrotizing typhlocolitis with severe mucosal ulceration and inflammation of the lamina propria may occur.

Vasculitis and intravascular coagulation with perivascular edema are consistent pathological features in the large intestine infected with *N. risticii*.

While the infectious organism has been identified in aquatic snails from many areas of the world, clinical Potomac Horse Fever is only recognized to occur in North America, South America and Europe. The disease is most common from late summer to early fall, with a peak incidence in July and August in the northern hemisphere.⁸ Potomac Horse Fever is characterized by fever, leukopenia, anorexia, depression and diarrhea.³⁸ When PHF is experimentally induced by oral inoculation the latent period is typically 1-3 weeks, with a biphasic pattern of fever preceding the development of diarrhea. Whilst the initial fever may be as high as 107°F, this is often not detected in horses in the field and there is no indication of infection until the onset of the second episode of fever, in conjunction with severe depression and diarrhea.³⁷ Moderate to severe diarrhea is known to occur in 75% of horses affected with Potomac Horse Fever and may persist for several days.³⁹ While laminitis is a complicating factor known to develop in 20-30% of PHF cases, the pathogenesis of this condition remains unclear to date.⁸ Other complications of PHF may include the abortion of infected fetuses, vascular thrombosis, renal failure and protein-losing enteropathy.

Parasite-Associated Diarrhea

While parasite-associated colitis is most often clinically associated with chronic diarrhea, sudden onset diarrhea has been reported.¹ Cyathostomes (small strongyles) and large strongyles are the major equine parasites associated with acute colitis. In the case of cyathostomes infestation injury to the colonic mucosa is thought to be related to the simultaneous maturation and release of hypobiotic cyathostome larvae from the cecal and colonic mucosa. This phenomenon is seasonal in nature and therefore the disease is expected to occur only in late winter and early spring, although the stimulus for larval emergence is not clear.¹ Emergence of the encysted larvae causes mucosal injury, ulceration and inflammation, all of which may be responsible for the development of clinical disease.⁸ Alternatively, diarrhea associated with large strongyle infection, most importantly *Strongylus vulgaris*, is typically acute and occurs within several days of infestation. Migration of the fourth stage larvae progresses from the lumen

through the mucosa and submucosa into the arterioles of the intestine, causing mural edema, hemorrhage and infiltration of the wall with inflammatory cells.⁴⁰ Increased secretion and decreased absorption of fluid and electrolytes stimulated by inflammatory mediators such as prostaglandins and histamine may also play a role in the colitis induced by large strongyles.

Antimicrobial-associated colitis

Antimicrobial administration is recognized to cause colitis in the horse.⁴¹ The resulting condition can be very severe and horses with antibiotic-induced diarrhea are reported to be 4.5 times less likely to survive than those with diarrhea from other causes.⁴¹ Antimicrobial administration can cause profound depletion of the normal anaerobic microbial population in the intestine, which decreases carbohydrate fermentation and the production of volatile fatty acids. This contributes to the pathogenesis of antibiotic-associated diarrhea by inhibiting the normal effect of the flora in colonization resistance and by decreasing absorption of sodium and water by the colonic mucosa.

Antimicrobials that are present at high concentrations within the gastrointestinal lumen exert a more profound effect on the gastrointestinal flora than other antimicrobials. Oral antibiotics and those which are excreted in the bile and undergo enterohepatic circulation such as oxytetracycline and doxycycline are of greatest concern. Broad spectrum antibiotics such as the tetracyclines and beta lactams are the most common antimicrobial agents associated with colitis in humans whereas in horses trimethoprim sulphamethoxazole, the macrolides and tetracyclines have all been reported to cause colitis.⁴²

Non-steroidal anti-inflammatory drug toxicity

Non-steroidal anti-inflammatory drugs (NSAIDs) are well recognized as having potentially toxic effects on the gastrointestinal tract which may lead to diarrhea. The prostaglandins PGE₂ and PGI₂ are critical for the maintenance of normal mucosal blood flow within the gastrointestinal tract, therefore inactivation of the cyclooxygenase enzymes by NSAIDs leads to a decrease in prostaglandin production which, in turn, impairs mucosal blood flow and leads to mucosal injury and inflammation.⁴³ NSAID toxicity manifests as two clinical

syndromes termed generalized NSAID toxicity and right dorsal colitis. With the generalized form mucosal ulceration occurs throughout the gastrointestinal tract, with oral and gastric lesions being very common, while the ulceration is focal and severe in cases of right dorsal colitis. Why the ulceration is expressed only in the right dorsal colon in some horses is unknown but both clinical syndromes are often associated with the development of diarrhea.

The detrimental effects of non steroidal agents are typically dose-dependent and in most reported cases of non-steroidal toxicity the affected horses were receiving higher than recommended doses, often over many days.⁴⁴ The toxic dose of phenylbutazone in a healthy horse has been reported to be 8-10mg/kg for several days and doses of 15mg/kg or greater, when given on multiple days were found to be lethal, with death occurring as early as day 4 of treatment.⁴⁵ Flunixin meglumine does appear to be less toxic than phenylbutazone but foals given 1.1mg/kg/day for 30 days developed signs of toxicosis.⁴⁶ In another study a dose of 6.6mg/kg/day IV for 5 days was necessary to produce clinical signs of toxicosis in a group of foals.⁴⁷ Combining non-steroidal therapies (commonly referred to as ‘stacking’) does not reduce the potential for toxicity. Gastrointestinal ulceration and protein losing enteropathy were reported when a combination of phenylbutazone and flunixin meglumine was utilized, even though each drug was administered at the published and seemingly appropriate dose.⁴⁸

Cantharidin toxicity

Cantharidin is the toxic principle found in beetles of the genus *Epicauta*, commonly known as blister beetles. These beetles feed on alfalfa flowers and can be incorporated into hay if the alfalfa is cut and processed simultaneously, as by crimping. Horses then ingest the beetles in the hay. Cantharidin is a potent gastrointestinal irritant and it causes acantholysis and vesicle formation when applied topically.⁴⁹ This leads to severe ulceration and inflammation of the gastrointestinal mucosa throughout the gastrointestinal tract, resulting in severe diarrhea, which is often fatal.

Carbohydrate overload

Overconsumption of soluble carbohydrates overwhelms the absorptive capabilities of the small intestine, leading to a high percentage of soluble carbohydrates entering the large intestine. The subsequent pathogenesis for acute colitis primarily involves the toxic effects on the microbial flora in the large intestine.⁸ An increased amount of soluble carbohydrates reaching the cecum and colon results in rapid fermentation by gram-positive lactic acid-producing bacteria and a sudden increase in organic acid production. The intestinal pH decreases rapidly and the buffering capacity of the large intestine is overwhelmed. The profoundly acidic conditions result in death of the resident microbial flora. In turn, the lactic acid increases the osmotic load within the large intestine leading to the development of secretory diarrhea. The acidity also results in necrosis, erosion and inflammation of the large intestine mucosa, exacerbating the loss of water, protein and electrolytes into the lumen. In turn, inflammatory cytokines and endotoxin are absorbed across the inflamed large intestinal mucosa leading to systemic disease, such as endotoxemia and laminitis.

Conclusion

Knowledge of the pathophysiology and etiology of acute colitis allows the clinician to formulate rational diagnostic and therapeutic plans, maximizing the potential for positive outcomes with these challenging cases. While treatment is fundamentally supportive in nature, therapies targeting the appropriate etiologic agent may aid in lessening the severity of mucosal injury, thereby diminishing the severity of diarrhea and/or the systemic response. Treatment modalities directed toward the underlying pathophysiologic mechanisms may have benefit even if the etiology is unknown.

Table 1.1: Most common differential diagnoses for acute colitis in the adult horse.

Category	Diagnosis	Etiological Factor
Infectious	Salmonellosis	Large number of <i>Salmonella</i> serotypes
	Intestinal clostridiosis	<i>Clostridium difficile</i> <i>Clostridium perfringens</i>
	Potomac Horse Fever	<i>Neorickettsia risticii</i>
Parasitic	Strongylosis	<i>Strongylus vulgaris</i>
	Cyathostomiasis	Small strongyles
Toxic	NSAIDs	Excessive dose or prolonged therapy
	Antibiotic induced	↑ risk with oral antibiotics and beta- lactams
	Cantharidin	Blister beetle ingestion
Miscellaneous	Carbohydrate overload	Excessive consumption of soluble carbohydrates

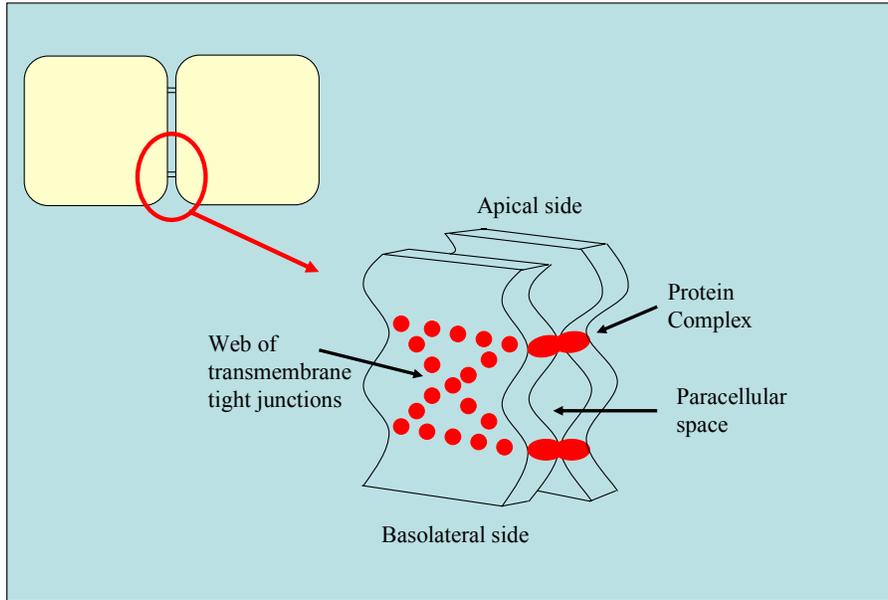


Figure 1.1: Tight junction.

Tight junctions seal the spaces between adjacent epithelial cells thereby preventing free movement of water and electrolytes between the gastrointestinal lumen and the interstitium.

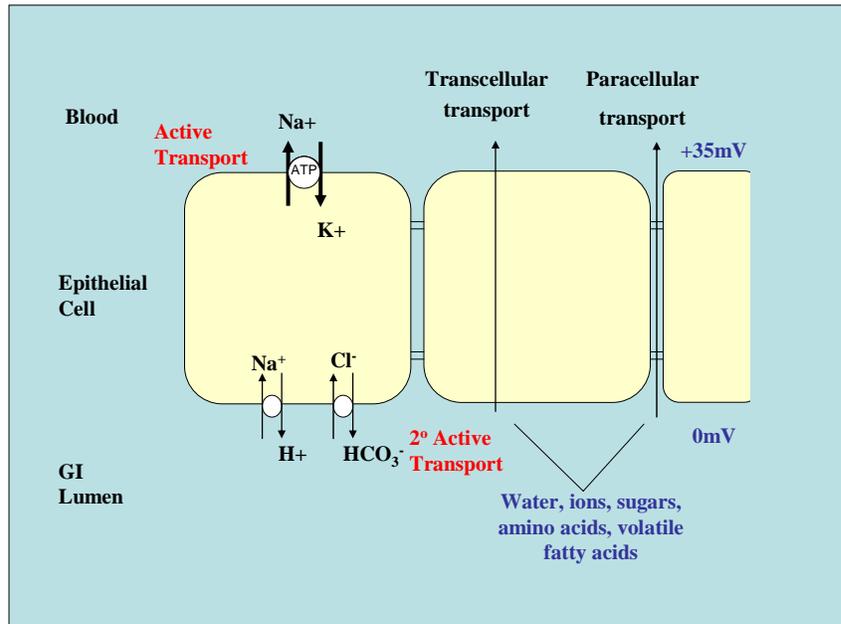


Figure 1.2: Transport mechanisms in the equine colon.

The $\text{Na}^+\text{K}^+\text{ATPase}$ pump utilizes ATP and Na^+ is pumped out of the gastrointestinal cell via primary active transport. The Na^+/H^+ pump and $\text{Cl}^-/\text{HCO}_3^-$ pumps utilize energy from other sources and are examples of secondary active transport. Tight junctions between the cells and the electrochemical gradient prevent free paracellular movement of ions.

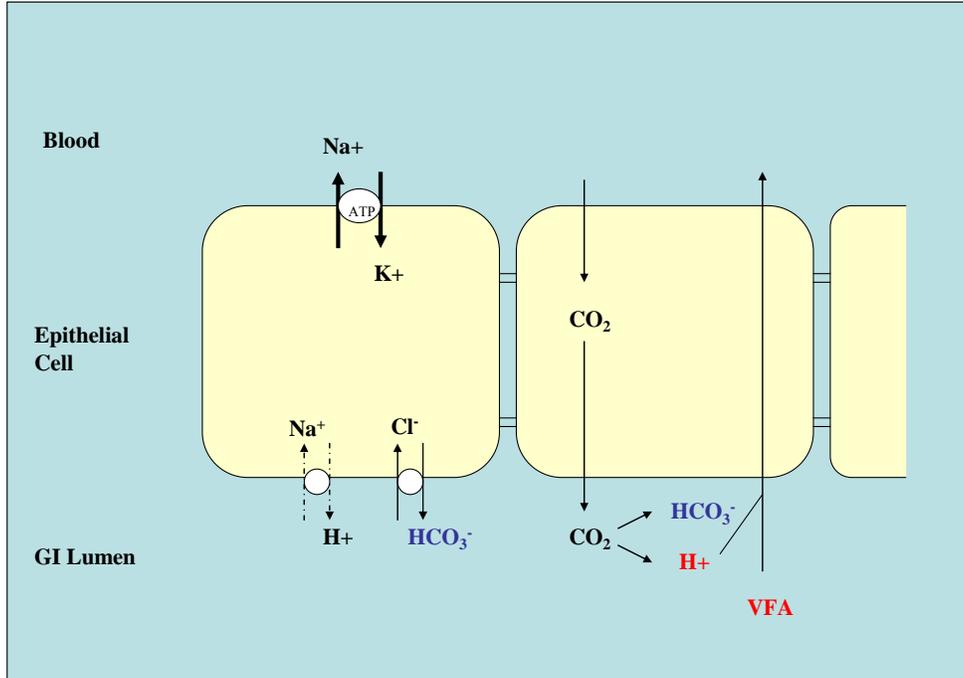


Figure 1.3: Mechanisms of volatile fatty acid absorption in the colon.

After ingestion of feed by the horse the VFA concentration increases in the lumen of the colon. Na⁺/H⁺ exchange is inhibited so therefore the HCO₃⁻ concentration increases. Once the VFAs have been absorbed the Na⁺/H⁺ exchange activity increases again and the H⁺ enters the lumen to buffer the HCO₃⁻

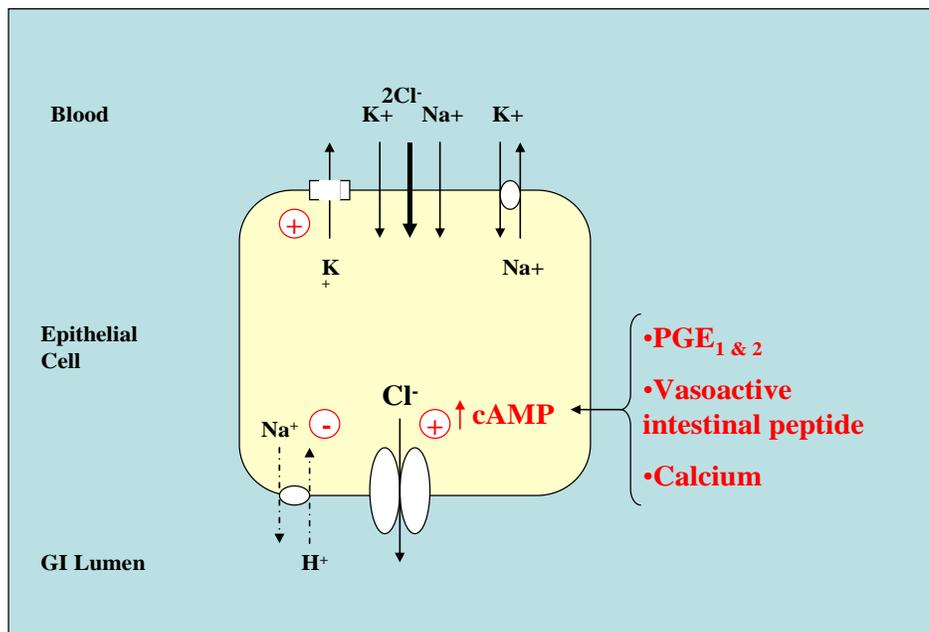


Figure 1.4: Increased chloride secretion as a result of increased cAMP.

Inflammatory mediators increase the intracellular cAMP which, in turn, leads to the opening of chloride channels. The secretion of chloride increases and water follows. Luminal sodium increases due to inhibition of the Na^+H^+ pump by the increased cAMP concentration

Chapter 2: Treatment of Acute Colitis in the Adult Horse

Introduction

For many diseases of the horse a goal of treatment is to target the underlying etiology, but reaching a definitive diagnosis in the acute colitis case is often not possible and is thought to occur in only 35% of all cases.² Appropriate diagnostic tests may identify specific infectious organisms but often not in a timely manner and since the condition has the potential to be fatal, treatment must be initiated prior to obtaining the results. Treatment goals for the acute colitis case include maintenance of fluid and electrolyte balance, maintenance of colloid oncotic pressure, replacement of plasma protein, control of local and systemic inflammation, promotion of tissue perfusion, promotion of mucosal repair, and nutritional management. Due to the potential for rapid progression colitis should be considered a medical emergency. Intensive care is required by all patients and, importantly, even if the primary treatment is effective, the sequelae associated with acute colitis may limit the horse's future ability to perform or be sufficiently severe as to require euthanasia. The cost of treatment can accumulate quickly and is often substantial.

Fluid Therapy

The water content of the gastrointestinal tract in ponies has been shown to be 6-10% of body weight, so when the integrity of the gastrointestinal barrier is compromised, fluid can shift from the intravascular to the intraluminal fluid compartment with potentially catastrophic effects.⁵⁰ When formulating an appropriate fluid therapy plan for the colitis patient an assessment of the patient's clinical status, estimation of the severity of ongoing losses and available laboratory information must all be considered. The degree of dehydration will vary depending on the time to onset of clinical assessment and the severity of disease but is generally estimated as a percentage of the patient's body weight. (Table 2.1) An accurate calculation of ongoing fluid loss in a diarrheic patient is more difficult. The frequency, consistency and volume of diarrhea per episode are useful subjective indicators.

With mild to moderate dehydration of short duration, and without continued gastrointestinal dysfunction and ongoing losses, replacement of the fluid deficit with an isotonic crystalloid solution may be adequate to restore fluid and electrolyte homeostasis. There are many commercially available fluids (Table 2.2) but a polyionic, isotonic, crystalloid fluid such as lactated Ringer's or an equivalent solution containing a bicarbonate precursor should be used as an initial replacement fluid.⁵⁰

The total volume of fluid to administer should be determined based upon the degree of dehydration/fluid deficit, maintenance requirements and anticipated ongoing losses. The rate of fluid replacement is determined by the animal's presenting condition, the required volume, body size and continued losses. A general guide for fluid replacement is 10-20ml/kg/hr but rates of 20-45ml/kg/hr have been reported to be well tolerated in adult horses.⁵⁰ Once the fluid deficit has been replaced most cases of acute colitis will require a fluid rate 2-3 times the maintenance rate to address ongoing fluid loss. (Table 2.3)

The biggest limitation to the use of crystalloid fluids is that in the patient with a systemic inflammatory response crystalloid fluids can rapidly exit the vasculature to equilibrate with the extracellular fluid, with only 30% of isotonic fluids and 10% of hypotonic fluids remaining in the intravascular space after 30 minutes.⁵¹ This means that their resuscitation effect may be short-lived and that they can cause edema. In severely dehydrated horses hypertonic saline (1-2 liters of 7% sodium chloride) can be used to quickly expand blood volume as fluid moves from the extracellular space into the vascular compartment in response to the hypertonic solution in the vascular space. Hypertonic saline can also increase myocardial contractility, reduce tissue and endothelial edema, improve microcirculation by hemodiluting the blood thereby improving blood viscosity and has been found to have immunomodulatory effects.⁵² In order to maximize the effects of hypertonic saline, polyionic fluids should be administered intravenously following the hypertonic saline treatment until the hydration deficit has been corrected. If ongoing fluid losses are significant, care must be taken to ensure adequate colloidal support and electrolyte supplementation are provided.⁵⁰

Electrolyte Supplementation and Correction of Acid Base Derangements

Horses with colitis often have marked electrolyte deficiencies which are exacerbated by the provision of aggressive fluid therapy. Diminished absorption and increased secretion lead to loss of plasma sodium, chloride, potassium, calcium and bicarbonate into the colon lumen. Clinical signs do not adequately predict the patient's need for electrolyte supplementation so this should be determined from measured plasma concentrations. Ongoing monitoring throughout the course of the condition is essential.

Clinical signs of low plasma sodium concentration include neurological disturbances such as reduced or absent menace response, intention tremor and hypermetric gait, however they are rarely seen until the plasma sodium concentration is less than 110mEq/l.⁵³ The fluid choice for hyponatremia depends on whether there is a concurrent hypochloremia. Sodium chloride is the best choice if the plasma chloride is also low. If the chloride concentration is normal or increased then sodium bicarbonate can be administered. If the hyponatremia is severe and the patient is not dehydrated hypertonic solutions may be indicated (7-7.5% sodium chloride and 5-8.4% sodium bicarbonate). The rate of sodium supplementation is controversial. In other species, the rapid correction of sodium deficits has been shown to cause demyelination of the pontine and extra pontine neurons resulting in severe neurological dysfunction.⁵⁴ It has not been established whether this is a risk in the horse, but as a guideline, sodium correction, using hypertonic saline or sodium bicarbonate, should be no faster than 1mEq/hr in the case of acute hyponatremia.⁵³

Hypokalemia is commonly seen in the acute colitis case due to enhanced glucocorticoid and mineralocorticoid release, decreased intake and absorption or excess loss secondary to the diarrhea and the infusion of large amounts of sodium-containing fluids which leads to increased renal potassium loss.⁵⁵ Clinical signs are chiefly related to electrical conduction across the cell membrane and most commonly include decreased gastrointestinal motility and muscle weakness.⁵⁶ Potassium is typically supplemented when the plasma concentration falls below 3.0mmol/l and intravenous potassium chloride is used at a rate of no more than 0.5-1mEq/kg/hour.⁵⁶ If a patient remains refractory to potassium replacement therapy then magnesium supplementation should be provided.⁵⁷

Clinical signs of hypocalcemia are often non specific and include muscle weakness, tetany, synchronous diaphragmatic flutter, tachypnea, tachycardia and ileus.⁵⁸ The normal ionized calcium level in a horse is 6.0-7.0 mg/dl and treatment for hypocalcemia is recommended if the level falls below 3.6mg/dl.⁵³ Intravenous 23% calcium gluconate solution is commonly used. The rate of supplementation is unclear but the effects of calcium administration wane rapidly so a constant rate infusion is often required and a safe rate of infusion would be 0.1-0.4mg/kg/min.⁵⁸

Metabolic acidosis frequently accompanies acute colitis due to colonic loss of bicarbonate and tissue lactic acid accumulation associated with endotoxemia and poor tissue perfusion. Acid-base disturbances are primarily corrected by addressing the underlying cause so a metabolic acidosis can be resolved by improving tissue perfusion by volume expansion, with fluids and colloidal support. The specific plasma constituent imbalance should also be considered. For example, hyponatremia leads to metabolic acidosis so sodium supplementation, without concurrent chloride supplementation, should resolve the acidosis. Similarly albumin is a weak acid so severe hypoalbuminemia may contribute to a metabolic alkalosis or mask a concurrent metabolic acidosis and protein supplementation should correct the acid-base imbalance.⁵⁹

Oral supplementation of fluids is an effective and economical adjunct to intravenous fluid administration. Oral fluids should be provided as long as colic signs or reflux are not present. Once severe electrolyte or acid base disturbances have been addressed most horses, if given the choice, will elect to drink a solution containing the electrolyte in which they are deficient. A typical 'water buffet' might include 1) a bucket with plain water, 2) water with 6-10g/l Lite salt (iodized salt and potassium chloride), 3) water with 10g/l baking soda (sodium bicarbonate) and 4) water with 6-10g/l plain salt (sodium chloride).⁶⁰

Colloidal support

Due to gastrointestinal losses and serum albumin catabolism, many horses with acute colitis are hypoproteinemic. Additionally, the absorption of bacterial products, such as endotoxin, may induce a systemic inflammatory response leading to increased vascular

permeability, further contributing to fluid and protein loss. Large volumes of crystalloid fluids cause hemodilution and contribute to a drop in the plasma oncotic pressure.⁶¹ In contrast, colloids preserve capillary oncotic pressure resulting in more effective volume expansion.⁵⁰ Commercially available colloids include plasma, human albumin, dextran-40, dextran-70, hydroxyethyl starch (Hetastarch) and polymerized bovine hemoglobin with fresh frozen plasma and Hetastarch being the two most commonly used for the acute equine colitis patient in the United States. As a rule, the higher the average molecular weight of the colloid solution the longer the colloid will persist in the circulation. The number of molecules in the solution determines the osmotic pressure, however, and colloids with a smaller average molecular weight exert a higher osmotic pressure and expand the plasma volume more rapidly. This results from drawing fluid from the interstitial space into the vasculature and thus increasing the circulating volume in excess of the amount of fluid infused.

Hydroxyethyl starch (up to 10ml/kg/day or ideally <20ml/kg cumulative dose) is comprised of modified polymers of amylopectin and is comprised of a mixture of molecules with a wide range of molecular sizes (10-3000kD). At a dose of 10ml/kg it was found to increase the plasma colloidal oncotic pressure of normal ponies from 24-27mmHg and a study in dogs showed that hetastarch expanded the plasma by 140% of the volume infused.⁵³ As for all colloids, the duration of action is dependent on the integrity of the capillary endothelium, but hetastarch has been shown to increase colloidal oncotic pressures for up to 24 hours in hypoproteinemic horses.⁶¹ Whilst generally considered a safe drug, doses of Hetastarch higher than 10 mg/kg/day may prolong bleeding by altering von Willebrand's factor function and by decreasing circulating Factor VIII coagulant activity.⁵⁰ Hetastarch is an appropriate therapy to use in the dehydrated colitis patient where rapid fluid resuscitation is required.

Plasma is extensively used in horses with colitis and is classically used for the treatment of hypoalbuminemia. Albumin, which has a molecular weight of 69kDa, is the primary component of plasma which exerts an oncotic effect. There is no published dose of plasma for the correction of hypoproteinemia but, as a guideline, the acute colitis case will require 10-15ml/kg plasma to raise the total protein approximately 1g/l. This will cost ~\$700 for a 500kg horse. Therefore plasma is not a cost effective therapy when the therapeutic goal is achieve rapid

improvement in oncotic pressure as is the case with the severely dehydrated patient. That said, plasma is a valuable therapeutic agent and in addition to supplementing albumin, it is a source of other potentially beneficial factors such as antibodies, complement, fibronectin and coagulation factors (II, VII, IX and X)⁵³.

Recently plasma has also come to be used as a means of providing passive immunity.⁶² Fresh frozen commercial plasma is now available, collected from horses immunized against *Clostridium difficile*, *Clostridium perfringens* and *Salmonella spp* (MG Biologics™, Ames, Iowa) and preliminary clinical studies have shown a decrease in the duration of diarrhea in horses treated intragastrically and intravenously with this ‘antidiarrheal’ plasma in comparison to diarrheic horses that received normal plasma.⁶² Adverse side effects of plasma administration in horses are uncommon and include immune-mediated reactions and changes in hemostatic variables.⁶³

Anti-inflammatory Therapy

Non-steroidal anti inflammatory drugs (NSAIDs) are used in the acute colitis case as anti-inflammatory agents and for their antiendotoxic effects. As inhibitors of cyclooxygenase 1 and 2 they break the self-perpetuating inflammatory cycle within the inflamed colonic mucosa and decrease the production of inflammatory cytokines such as TNF, thereby allowing the gastrointestinal mucosa to heal.⁶⁴ However, whilst their beneficial effects are likely to outweigh the negative effects, NSAIDs should be used with caution. Their use should obviously be avoided in the acute colitis case secondary to NSAID toxicity or in the severely dehydrated patient where administration can lead to renal medullary crest necrosis. Nonsteroidal anti-inflammatory drugs may cause injury to gastrointestinal mucosa in 2 ways: direct topical injury and inhibition of prostaglandin synthesis.⁶⁵ Prostaglandin I₂ and PGE₂ are cytoprotective to gastrointestinal mucosa and critical for mucosal repair so blocking prostaglandin production in these tissues using COX inhibitors may exacerbate gastrointestinal pathology.⁶⁶

Other anti-inflammatory treatment options for the acute equine colitis case include the free radical scavenger dimethyl sulfoxide (1g/kg IV q12-24hrs as a 10% solution), the antimicrobial, metronidazole (20mg/kg PO q8hrs), the prokinetic and analgesic drug lidocaine

(1.3mg/kg over 15 minutes then 0.05mg/kg/min), and the PGE₁ analogue misoprostol (5µg/kg q8hrs). Experimental evidence for each of these agents is limited but they may be of some clinical value.^{67,68}

Analgesic Therapy

Many horses with acute colitis develop mild to severe signs of abdominal discomfort from gas and fluid distention of the colon, colonic ischemia and infarction. NSAIDs are commonly used analgesics, in particular, flunixin meglumine (1.1mg/kg IV or PO q12 hrs). Alternative analgesics include, lidocaine, alpha 2 agonists, such as xylazine and detomidine, and opioids such as butorphanol. These are considered short-acting agents but constant rate infusions may be used to provide sustained clinical analgesia.⁶⁹ One must be aware that opioids and alpha 2 agonists decrease gastrointestinal motility and this may exacerbate colic signs in the acute colitis patient.

Bismuth Subsalicylate

Bismuth subsalicylate (BSS) is commonly administered enterally to horses with colitis in an effort to decrease inflammation and secretion in the colon. However, no empirical studies in horses have been reported to support its use. In a double blinded, placebo controlled study in children with acute diarrhea it was shown to significantly shorten the time to the last watery stool.⁷⁰ The precise mechanism of action of bismuth subsalicylate in any species is unclear but it may have an antisecretory action related to the salicylate moiety.⁷¹ Meanwhile, the drug and its intestinal reaction products, bismuth oxychloride and bismuth hydroxides appear to be bacteriocidal *in vivo* and *in vitro*.⁷¹

The dose required in adult horses to be beneficial, extrapolated from human medicine,⁷⁰ is large (3-4 liters q4-6hrs). This may limit its use in horses. In humans BSS is considered an extremely safe drug. A feeding trial whereby mice were fed 60 times the maximal recommended human dose did not result in any adverse effects and no histopathologic lesions were noted on post mortem.⁷² There appear to be no reports of BSS toxicity in the horse.

Di-tri-octahedral Smectite

Di-tri-octahedral smectite (Biosponge™, Platinum Performance, Inc) is a natural hydrated aluminomagnesium silicate of lamellar structure which binds to digestive mucus and increases intestinal resistance to bacterial damage.⁷³ It has been shown to increase water and electrolyte absorption in rabbit intestinal loops in the presence of *Escherichia coli* infection and a preliminary study in horses reported that administration of di-tri-octahedral smectite prevented the development of lincomycin-induced colitis in 4 horses while the 4 non-treated horses died or were subjected to euthanasia due to severe colitis.^{73,74} *In vitro* studies have shown that di-tri-octahedral smectite binds *Clostridium difficile* toxins A and B and *Clostridium perfringens* enterotoxin and endotoxin.⁷⁵ The current recommendations for a 1000 pound horse are 1lb Biosponge™ mixed with 3 liters of water and administered via nasogastric tube every 6-12 hours.

Probiotics

Restoration of the microbial ecology of the colon has attracted experimental interest in recent years, leading to the use of many different agents including commercially available probiotic pastes, live-culture yogurt, and techniques such as transfaunation. Transfaunation is the administration of either fresh colonic or cecal contents (5-6 liters) or a slurry made from fresh feces collected from a healthy horse, in an attempt to restore normal flora. There is reported clinical success of transfaunation in cattle but there are no reports in the literature evaluating its potential benefit in diarrhetic horses.⁷⁶ Disease transmission from the donor horse to the patient is a possible risk associated with this procedure. Bearing in mind that any microbial protein transfaunated to a horse has to survive the acidic environment of the stomach prior to reaching the alkaline environment of the cecum and colon, the recommendation for this procedure is that it is of questionable benefit and as such, should be reserved as a last resort treatment. Similarly, commercially available probiotic pastes are available to the equine clinician but there is little supportive evidence for their use in horses. One study in foals found a probiotic paste to be detrimental in that administration of the test product resulted in a longer mean duration of

diarrhea in the treated foals in comparison to the duration of diarrhea in foals treated with a placebo.⁷⁷

In comparison, *Saccharomyces boulardii* is a non-pathogenic yeast that has been used as an antidiarrheic agent both prophylactically and therapeutically in human medicine since 1962.⁷⁸ Experimentally, the yeast has been found to survive within the equine gastrointestinal tract and in acute equine enterocolitis the severity and duration of gastrointestinal disease was significantly decreased in horses receiving *S. boulardii*, compared with horses receiving a placebo.⁷⁸ *S. boulardii* has been found to release a protease that is able to digest *Clostridium difficile* toxins A and B. Additional mechanisms of action include an immunoprotective effect attributed to the promotion of the release of secretory immunoglobulins within the intestine or activation of the reticulendothelial and complement systems.^{79,80} Pharmacokinetic studies with *Saccharomyces boulardii* have not been performed in the horse and as such the dose used is extrapolated from the human literature. One study reported the use of 25g (10×10^9 yeast cells) q12hrs for 14 days in horses with acute colitis with no clinical adverse effects and a significant decrease in the severity and duration of gastrointestinal disease compared with a placebo group of horses.⁷⁸

Antimicrobial Therapy

The use of antimicrobials in horses with acute colitis is controversial since the potential detrimental effects associated with these drugs can outweigh the benefits. Negative effects attributed to antibiotics include the induction of antibiotic-induced diarrhea possibly due to disruption of the microbial population in the gastrointestinal tract. Additionally, oxytetracycline administration was found to prolong the excretion of *Salmonella sp* shedding in experimentally infected ponies so many clinicians recommend that no antimicrobial therapy is used if Salmonellosis is suspected.⁴²

That said there are many scenarios wherein antibiotics should be used. Firstly, empiric broad spectrum antibiotics should be considered in the horse with concurrent neutropenia and colonic inflammation.^{60,81} In these cases, transmural migration of bacteria can occur leading to septicemia and bacterial colonization of multiple organ systems and indeed, the authors have

seen cases of pneumonia and arthritis temporally associated with colitis and which were believed to be a result of this phenomenon. An appropriate therapeutic regime in these patients would include potassium penicillin (22,000 IU/kg q6hrs) in combination with gentamicin (6.6mg/kg q 24hrs). Care should be taken to ensure the patient is not dehydrated prior to administration of the aminoglycoside to avoid possible nephrotoxicity. Secondly, for certain infectious causes of acute colitis in the horse, such as Potomac Horse Fever (Equine Monocytic Ehrlichiosis) and *Clostridium sp*, specific antibiotic treatment is necessary to eradicate the known etiological agent. *Neorickettsia risticii*, the causative agent for Potomac Horse Fever, is highly sensitive to tetracyclines (e.g. oxytetracycline 6.6mg/kg IV q24 hours for 5 days).⁸² *Clostridium difficile* and *Clostridium perfringens* have been found to be eradicated using metronidazole (20mg/kg PO q 8hrs)²⁰ plus there is *in vitro* evidence to suggest good efficacy for chloramphenicol (20mg/kg PO q6hrs).⁸³

Nursing Care and Nutrition

Good nursing care and nutrition are essential to a successful outcome with the acute colitis case. Many horses with colitis exhibit partial or complete anorexia as a result of systemic inflammation and discomfort associated with dysmotility and ileus. Ideal diets for horses with colitis include low bulk and poorly soluble carbohydrate diets that are highly digestible.⁸⁴ Such feeds will decrease the amount of undigested concentrate that reaches the cecum where fermentation may exacerbate the diarrhea. Feeding small meals at frequent intervals of a predominantly pellet based feed is one way this can be achieved or alternatively hay can be fed. Care should be taken to ensure that it is good quality fresh green grass hay.⁸¹ Hay diets have the benefit, in comparison to pelleted feed, that they may permit a more normal production of volatile fatty acids in the colon thereby providing energy to the mucosal epithelial cells and promoting colonic healing.⁸¹ Lush grass and grain based feeds should be avoided due to their high sugar content and thereby fermentable nature. If the patient fails to voluntarily consume at least 70% of the calculated resting energy requirements then parenteral nutrition or, if the patient's gastrointestinal tract tolerates it, forced enteral feeding via a nasogastric tube is required.⁸⁴

Treatment of Common Sequelae to Acute Colitis

Due to the severity of the systemic illness associated with acute colitis complicating sequelae are frequently encountered; most importantly, endotoxemia, thrombophlebitis, organ thrombosis and laminitis. Understanding the pathophysiology associated with each of these conditions ensures that appropriate preventative or therapeutic measures can be taken to try and address these sequelae thereby ensuring the optimum chance of survival for the patient. At the current time laminitis is the primary reason for euthanasia in many colitis cases.⁸⁵

Response to Therapy

Response to therapy is determined by close monitoring of clinical signs, clinicopathological data and fecal water content. Signs that indicate improvement are decreased fever, stability of serum electrolyte concentrations and acid-base balance and improved appetite. Clinicopathologically, one of the earliest signs of improvement can be a decrease in the number of morphologically 'toxic' neutrophils. Decreased fecal water content and frequency of diarrhea all suggest clinical improvement. Importantly, acute colitis can have an infectious etiology so the affected horse may continue shedding the infectious agent even when the diarrhea has resolved thereby putting in-contact horses at risk if the patient mixes with other horses too soon. As such, the author recommends that any horse recovering from acute colitis be isolated for at least 7-14 days after complete resolution of the diarrhea.

Prognosis and Outcome

The fatality rate for acute colitis is highly variable, ranging from 10-70% among treated horses, and depends upon the virulence of the pathogen, the susceptibility of the host and the aggressiveness of the instigated therapy.¹ The potential for a substantial rate of mortality and the expense of treatment underscore the importance of identifying horses with a poor prognosis for survival. Horses that recover from acute colitis typically show clinical improvement 3-6 days after treatment begins whereas azotemia, clinicopathological findings consistent with hemoconcentration and hypoproteinemia, and failure to show demonstrable signs of improvement after 10 days of therapy suggest a more guarded prognosis.^{60,41} Certain types of

colitis, including necrotizing enterocolitis and antimicrobial associated diarrhea, have been associated with lower survival rates.⁴¹ If a horse survives acute colitis without developing sequelae such as laminitis, ongoing health issues are less likely.

Conclusion

Managing horses with acute colitis can be challenging. Many different treatments are available, some are of unclear efficacy, but they provide the clinician with the opportunity to explore different therapeutic approaches thereby making it a rewarding condition to treat. The frequent lack of a definitive diagnosis, the intensity of treatment and the overall cost of treatment are often factors that may deter the owner and the clinician from pursuing treatment, but it is important to remember that if the horse responds in the first few days to therapy the prognosis is favorable for a full recovery.

Table 2.1: Clinical assessment of dehydration.

* The normal PCV depends on the horse's breed and level of athletic training. Thoroughbreds and Standardbreds in training have normal PCVs up to 45%. The normal PCV of draft breeds can be as low as 25-30%. Splenic contraction and hypoproteinemia may affect the PCV and TP, respectively.

Parameter	Degree of Dehydration		
	Mild (4-6%)	Moderate (7-9%)	Severe (>9%)
Skin Turgor	Good to fair	Fair	Poor
Mucous membrane moisture	Good to fair	Tacky	Dry
Capillary refill time (sec)	1-2	2-4	>4
PCV (%)*	40-50	50-65	>65
Total plasma protein (g/dl)*	6.5-7.5	7.5-8.5	>8.5

Table 2.2: Composition of the commercially available polyionic fluids for both maintenance and resuscitation of the equine patient.

Polyionic fluid	Na (mEq/l)	K (mEq/l)	Ca (mEq/l)	Mg (mEq/l)	Cl (mEq/l)	Base (mmol/l)
Maintenance fluids						
Normosol-M	40	13	0	3	40	16 (acetate)
Plasmalyte 56 (in 5% dextrose)	40	16	5	3	40	12 (actetate) 12 (lactate)
0.45% NaCl with dextrose	77	0	0	0	77	0
Resuscitation fluids						
Lactated Ringers Solution	130	4	3	0	109	28 (lactate)
Plasmalyte 148	140	5	0	3	98	27 (actetate) 23 (gluconate)
Normosol R	140	5	0	3	98	27 (actetate) 23 (gluconate)
0.9% NaCl	154	0	0	0	154	0

Table 2.3: Calculation of the appropriate volume of fluid to administer to a dehydrated horse and rate of fluid administration. (Note:* may need adjustment to take into account the effect of environmental temperature on insensible losses)

Replacement fluid volume (liters)	= Body weight (kg) x % dehydration
Rate of replacement fluid administration	= 10-20ml/kg/hr
Maintenance fluid volume	= 50-100ml/kg/24hr*
Rate of maintenance fluid administration	= 2-4ml/kg/hr

Example: 500kg horse that is 5% dehydrated

Replacement fluid volume	= 500 kg x 5% = 25 liters
Replacement fluid rate	= 5-10 l/hr
Maintenance fluid volume	= 25-50 l/day
Maintenance rate	= 1-2 l/hr

Chapter 3: Hyperimmune Plasma

Plasma

Plasma is the liquid component of blood and in horses it constitutes approximately 55% of the total blood volume. It is 95% water with the remaining 5% comprised of electrolytes, hormones, cytokines and proteins.⁸⁶ Over 500 different proteins have been found in mammalian blood of which albumin is the main type. Other proteins include immunoglobulins, complement and fibronectin. Plasma has a multitude of functions within the body. It acts as a reservoir that can either replenish insufficient water or absorb excess water from tissues. It maintains blood pressure and circulation throughout the body simply by filling and flowing through blood vessels and it has a thermoregulatory role, by carrying heat to and from body tissues. Also, each of the individual components has a separate role. Albumin functions primarily to maintain colloidal osmotic pressure and it is an important transport molecule. Fibronectin, a component of plasma, is essential for the monocyte-macrophage system to function normally in the processing of antigens and it may enhance opsonophagocytosis of circulating particulate debris and endotoxin.⁸⁷ Fibronectin may be severely depleted in septicemic patients and researchers have found that administration of fibronectin-rich cryoprecipitates may be life-saving in humans and animals.^{88,89} The quantity of fibronectin in commercial plasma is low but stable for at least 9 months in fresh frozen and stored equine plasma. Hence plasma administration may be beneficial to a patient by restoring circulating fibronectin. Antithrombin III provides 70% of the anticoagulation activity of plasma and may decrease the severity of coagulopathies associated with endotoxemia.⁹⁰ Other proteins, such as members of the complement cascade, act as a means of host defense against bacterial infection. Complement activation plays an important role in host defense against bacterial pathogens by enhancing phagocytosis through opsonization of both gram-positive and gram-negative bacteria. Immunoglobulins are important proteins that are a source of passive immunity to the recipient of the plasma. It is important to note that the quantity, type and quality of the immunoglobulins present in fresh frozen plasma are dependent upon the donor. The presence and role of other proteins, such as elastase and proteinase

inhibitors, complement inhibitors, and other inhibitors of hypercoagulability have not been completely evaluated but may offer further beneficial to the colitis patient.

Fresh frozen plasma is commonly used for protein replacement in cases of acute diarrhea in horses. Interestingly, a thorough review of the literature did not reveal any studies reporting the efficacy of plasma as a therapeutic agent for the equine colitis case. Administration should result in an increase in the patient's colloidal osmotic pressure. With enterocolitis cases inflammation within the gastrointestinal wall and increased vascular permeability commonly result in hypovolemia and hypoproteinemia. When plasma is administered intravenously, protein within the plasma pulls fluid from the interstitial tissue back into the circulation thereby increasing blood volume and improving cardiac preload. The dosage of plasma required as a transfusion to provide colloidal support is not documented for horses and is dependent on the patient's condition, owner finances and the clinician's decision. As a guideline 10-15ml/kg can be given as an initial dose and then the patient's serum total protein and clinical status should be reassessed. Typically plasma administered at this dose will increase the patient's total protein level by ~1g/dl.

Plasma is not routinely used in human medicine due to the potential risks, such as disease transmission and anaphylaxis. Side effects have been associated with 2.5% of infusions in humans and are not reportedly dose dependent.⁹¹ Similar side effects are occasionally seen in horses that receive plasma transfusions but are rarely uncontrollable or lead to a situation where the side effects outweigh the benefits of the plasma. A slow rate of administration at the start of a plasma transfusion can limit the risk of an anaphylactic reaction and allow time for the detection of adverse reactions. Disease transmission is obviously an important concern in human medicine and may be a valid concern in equine medicine. A case series by *Aleman et al* documented 4 horses infected with hepatitis post plasma transfusion.⁹² Product availability can also be a use-limiting factor.

Passive Immunization

Passive immunization is the administration of immunoglobulins as prophylaxis or for treatment of disease. The underlying scientific theory of ‘passive immunization’ has been reported in the human literature for centuries. As Samuel Pepys related shortly after the great fire of London, there may be a benefit in ‘mending of bad blood by borrowing from a better body.’⁹³ In 1890 von Behring and Kitasato recognized that serum contained soluble factors that could neutralize tetanus and diphtheria exotoxins. They developed their observations and in 1901 von Behring was awarded the first Noble Prize in Physiology and Medicine for his development of ‘serotherapy’ – the process of administering pathogen-specific animal sera for the prophylaxis or treatment of bacterial infections. In the 1930s protective antibodies were identified in the globulin fraction of serum and in 1940 Cohn’s laboratory identified gamma globulins, in particular immunoglobulins, extracted them and concentrated them for intramuscular injection for ‘passive immunization’ purposes against measles.⁹⁴

In the veterinary field one of the first practical employments of passive immunization was in 1907 when anti-hog cholera serum was tested by the BAI Field Station in Iowa. Dr. Dorset, Dr. McBryde, and W.B. Niles found that serum from the blood of immune hogs conferred immunity lasting only a few weeks to other hogs. At that time Hog Cholera, now more commonly referred to as Swine Fever, was thought to be caused by a bacterium but research by Dr. Dorset’s laboratory went on to prove that a virus was the etiological agent.⁹⁵

As knowledge of immunoglobulins increased over the next few years passive immunization with gamma globulins became a popular therapy, but difficulty in giving sufficient amounts to maintain adequate concentration of IgG in patients became a major problem. In the late 1960s attempts to solve this problem led to the use of fresh frozen plasma infusions rather than extracting the immunoglobulins from the serum. The results in adults were better than with IM gamma globulin therapy but volume restrictions and the concerns regarding the spread of nonA-nonB hepatitis limited further use.⁹¹ Furthermore, at this time, the antibiotic industry was rapidly developing, which led to ‘serotherapy’ being viewed as an outdated therapy and halting further research in this field.

Currently, with increasing bacterial resistance to antimicrobials, the recognition of new diseases, and the improved understanding of the pathogenesis and immunology of viral and bacterial infections, renewed interest in passive immunization has come about. Research in the human medical field has shown distinct benefits to the administration of immunoglobulins for the prevention and treatment of various diseases.⁹⁶⁻¹⁰⁰ In the veterinary literature the benefits of immunoglobulin therapy are poorly documented. What limited data there is tends to suggest more clinical application to the use of plasma rather than purified immunoglobulins. This is primarily because hyperimmune plasma is simpler and cheaper to obtain than purified immunoglobulins. Furthermore, the risk of disease transmission or adverse effects from plasma administration are more tolerable or at least less well documented for horses than in human medicine, although not unrecognized.⁹²

Hyperimmune plasma

Hyperimmune plasma is collected from donor animals with high concentration of circulating antibodies against a specific disease and then usually fresh frozen. There is currently no medical standard to regulate the amount of antibody that has to be present in the plasma to justify the term ‘hyperimmune’. Hyperimmune plasma does not appear to have any more side effects than normal plasma and doses can generally be increased up to the point of volume overload without increased risk to the patient.¹⁰¹ Since normal fresh frozen plasma contains gamma globulins, and side effects to gamma globulins are not dose dependent, it would seem logical to suggest that a horse is as likely to develop side effects on receipt of normal plasma as it is to hyperimmune fresh frozen plasma.⁹¹

Examples of prophylactic use of hyperimmune plasma in equine medicine are to treat failure of passive transfer in neonatal foals or to prevent Rhodococcal infection in young horses. Many foals are deprived of IgG at birth through inadequate colostrum, premature birth, premature lactation or failure to nurse. Supplementation with hyperimmune plasma has been found to increase serum IgG concentration in equine neonates and is consequently used to address the condition of failure of passive transfer.¹⁰² As for all commercially available hyperimmune plasma types the quantity of IgG in the hyperimmune plasma is not typically

stated but for the 'High Glo IgG for foals' supplied by MG Biologics™ (2366 270th St. Ames, Iowa 50014) the company claim the IgG concentration to be at least 2500mg/dl. Plasma from horses hyperimmunized against *Rhodococcus equi* is also frequently used in attempt to prevent the development of Rhodococcal pneumonia in foals.^{86,103,104} *R. equi* is one of the most important causes of disease in foals aged one to six months of age. Hyperimmune plasma prepared from plasma obtained from horses immunized with a suspension of clinical isolates of *R. equi* is commonly used on many farms in the first couple of weeks of the foal's life. Madigan *et al* demonstrated protection against naturally occurring *R. equi* in foals by administration of hyperimmune plasma and Hooper-McGrevy *et al* demonstrated that the degree of protection against *R. equi* pneumonia was similar between that provided by purified immunoglobulin specific for VapA and VapC and commercially available hyperimmune *R. equi* plasma, thereby confirming the protection to be antibody mediated rather than by other components of the plasma.^{86,105} Despite these findings, when hyperimmune plasma was compared directly with normal plasma, no significant difference in the prevention of *R. equi* was documented.¹⁰⁴

Examples of therapeutic use of hyperimmune plasma in equine medicine include: a means of treating endotoxemia in the horse, a therapy in the face of West Nile Fever (WNF) and to treat botulism. Endotoxin (LPS, lipopolysaccharide) is an integral part of the outer surface of gram negative bacteria. It is responsible for much of the morbidity and mortality associated with gram negative bacteremia. Passive immunotherapy focuses on the clinical efficacy of anti-lipopolysaccharide (anti-LPS) antibodies in equine plasma to bind and inactivate endotoxin released by the bacteria.¹⁰⁶ *In vitro* IgG antibodies in anti-LPS hyperimmune equine plasma also initiated the destruction of gram negative bacteria.¹⁰⁷ This action occurred within minutes of the treatment with anti-LPS and total bacterial cell disintegration and disruption occurred within 1-2 hours.¹⁰⁷ A wide spectrum of gram-negative bacteria appear to be destroyed by the actions of anti-LPS plasma, probably by means of complement activation.¹⁰⁷ An induced blinded clinical model with hyperimmune lipopolysaccharide core antigen plasma was performed and demonstrated that those horses that received the hyperimmune plasma had a 87% survival rate and those that received the preimmune plasma had a 47% survival rate hence there being a significant benefit to hyperimmune plasma in the reduction in the severity of endotoxemia.¹⁰⁸

Hyperimmune plasma as a treatment for equine West Nile Fever has not been studied, primarily due to the difficulties in reproducing the disease experimentally. However, hyperimmune plasma may have had a beneficial effect when used to treat an alpaca with WNF and when polyclonal anti-West Nile Virus antibodies were administered to mice post exposure with WNV mortality was reduced.^{109,110}

Hyperimmune plasma has been reported by Dr. Kinde reported as a successful treatment for *Clostridium botulinum* type-C intoxication.¹¹¹ This was an observational study and a purified type C anti-toxin was also used in the affected horse thereby confusing interpretation of the results.

Intravenous hyperimmune plasma therapy for acute colitis

Most infections that lead to the production of diarrhea begin in the mucosal surface of the gastrointestinal tract. The ability of serum antibodies to prevent enterotoxicity and mucosal damage is mechanistically less obvious with intravenous administration than with oral administration of antibodies. To be effective the antibody must leave the circulation and act on the etiological agent e.g. the *Clostridium difficile* toxins A and B within the colonic lamina propria or intestinal lumen. This may occur as a result of the exudation of serum proteins across an inflamed colonic mucosa.¹¹³ Supportive of this theory, intravenously-administered anti-toxin A monoclonal antibody was detected in the cecal contents of gnotobiotic mice following oral challenge with toxigenic *C. difficile* but not in unchallenged mice.¹¹⁴ However, interestingly, the 'leakage' of serum proteins into the intestinal lumen can occur in the absence of fluid loss (diarrhea) or gross changes in the epithelium, as described for hamsters protected by parenteral immunization with different toxoid vaccine preparations.¹¹⁵ Whatever the mechanism, intravenous immunoglobulin therapy has been reported to be efficacious in the prevention and treatment of several causes of acute diarrhea in humans.^{113,116} A rapid response to intravenously administered pooled immunoglobulins in two cases of severe *Clostridium difficile* diarrhea was reported and not only was there a rapid increase in serum antitoxin levels but there was also resolution of the diarrhea which had failed to respond to standard antimicrobial therapy of metronidazole and vancomycin.¹¹³ This paper utilized a pooled immunoglobulin source but did

document the presence of immunoglobulins against *C. difficile* toxins A and B in the healthy donor humans. A single study did report the use of intravenous anticlostridial/antitrotaviral hyperimmune plasma in a small number of neonatal foals with *Clostridium perfringens* diarrhea but the results were observational and the study not controlled.¹¹²

The dosage of hyperimmune intravenous plasma is empiric. An intravenous dose of 150mg/kg of *C. difficile* immunoglobulin was reported in humans and this resulted in serum IgG concentration greater than 5mg/dl whereas *C. difficile* toxin neutralization activity is evident *in vitro* at IgG concentrations of approximately 1mg/ml.^{113,117} Thus, neutralizing concentration of IgG against *C. difficile* toxins are likely achieved following immunoglobulin infusion. However, Pirofsky *et al* showed in a six year study that the serum level of IgG directly reflected the dosage of IgG administered intravenously and that there was marked individual patient variation present in the kinetics of attaining and maintaining serum concentration of IgG.¹¹⁷ In this study the serum IgG concentration of a majority of subjects (64%) returned to preinfusion IgG concentration by four weeks, although, 29% had serum IgG concentration higher than pre-infusion concentration at four weeks.

The duration of effect of intravenous immunoglobulin therapy is highly variable between patients.¹¹⁷ Typically in humans the dosage and frequency of immunoglobulin administration has been determined by the patient's response to therapy. As technology has advanced in recent times the precise dose the patient is receiving can be ascertained and dosing has become more controlled, however substantial kinetic variations are still seen.

Enteral hyperimmune plasma therapy for acute colitis

Enteral administration of antibodies delivers protective concentration of antibodies immediately and directly to the susceptible mucosal surface. Antibodies have many different mechanisms of action on and within the gastrointestinal mucosa. They facilitate antigen presentation, balance the cytokine network, recruit pathogens into lymphoid organs, neutralize microbial toxins, modulate T cell function and further antibody production, block cellular receptors for pathogens and bind to antigens directly, leading to cellular cytotoxicity. The

protective effects of antibody administration are immediate and unlike antibiotic administration there use does not generate drug-resistant organisms.

Due to their relatively short half life the presence of antibodies in the gastrointestinal mucosa is maintained via constant replacement. The regulation of antibody production in the gastrointestinal mucosa is by CD4⁺ lymphocytes in the sub-mucosa and by the mucosal epithelial cells themselves. The CD4⁺ lymphocytes produce many cytokines including those that are critical for the maturation of B cells such as TGF- β and IL-6, IL-2 and IL-10.¹¹⁸ Different types of antibodies are naturally present in the gastrointestinal tract. Secretory IgA is the predominant immunoglobulin class present at mucosal surfaces and it exerts a protective effect on the intestinal mucosa primarily by inhibiting the attachment of pathogens to the mucosal surface.¹¹⁹ Meanwhile, IgG has well established opsonizing and antitoxic properties. The predominant class of immunoglobulin in plasma, or more specifically hyperimmune plasma, is IgG. This is the most versatile group of immunoglobulins and is capable of performing all the different functions of immunoglobulin molecules. That said, it remains to be determined which antibody class is optimal in treating gastrointestinal disease in any mammalian species and as research continues this should be more carefully investigated.

Although less well studied than systemic passive immunization, the prophylactic use of mucosal antibodies is inherent in the ingestion of colostral antibodies. Supplementing the antibody repertoire in the mucus secretion offers an effective method for protecting a mucosal surface against pathogens to which the host has not been exposed or become immune.

Experimentally, topical passive immunization of mucosa has been found to effectively block transmission of bacteria (*Clostridium difficile*, *E. coli*, *Vibrio cholerae*), fungi (*Candida albicans*), viruses (Rotavirus, Respiratory syncytial virus and influenza) and parasites (*Cryptosporidium parvum*) that infect humans.¹²⁰ Predominantly the described effects have been prophylactic but therapeutic benefits have been reported. For example, Guarino *et al* evaluated 98 children with acute rotaviral gastroenteritis and found that children who received oral antirotaviral immunoglobulins exhibited significantly faster improvement of their clinical condition. The mean total duration of rotaviral diarrhea was 76 hours in the treatment group and 131 hours in the placebo group ($p < 0.01$).¹¹⁹ Similarly Tsubokura *et al* demonstrated a

therapeutic benefit of orally administered immunoglobulins in *Campylobacter jejuni*-infected chickens with a 80-95% reduction in fecal bacterial counts.¹²¹

Being proteins, one would expect that orally administered immunoglobulins would undergo rapid degradation in the gastrointestinal tract. However there is evidence in human medicine and animal research, albeit limited, to suggest that this is not the case. Increased stool IgG was measured in patients receiving orally administered immunoglobulins and when concentrated anti-rotavirus hyperimmune bovine immunoglobulin G was administered to 164 children suffering from diarrhea ~10% of the orally administered antibody was detectable in the patient's stools.^{123,124} In one report up to 12% of an oral dose of IgG administered to 6 immature human infants was recovered undigested or partially digested in the stools while another experiment showed that antirotavirus antibodies survived passage through the gut and that the amount of rotavirus antibody detected in the feces was directly proportional to the titer of rotavirus antibody administered to the subjects.^{125,126} Losonsky *et al* demonstrated that not only is immunoglobulin recoverable in the feces post oral administration but it also retains some immunologic activity and maintains the ability to modify the immune response in immunodeficient patients.¹²⁵ It is important to note that the horse's digestive tract, with its hind gut as the predominant site for fermentation, is anatomically different from that of the hamster or human gastrointestinal models discussed here. That said, passive immunization is recognized to have beneficial effects in ruminants (e.g. lambs with rotavirus) and hence the antibodies must be capable of surviving the conditions associated with fermentation.¹²⁷

Due to the limitations associated with measuring the fecal antibody content, the duration of action of orally administered immunoglobulins is unclear. One study reported fecal recovery of antibody up to 72 hours post consumption thereby suggesting a prolonged effect.¹²⁶ However, in the diarrheic patient a variety of factors differ from those in the healthy individual. Destruction of gastrointestinal mucosa, high gastric pH, limited oral intake and antibacterial therapy reducing bowel flora all potentially contribute to difficulty in digestion of immunoglobulin. In addition, a rapid transit time would suggest that the antibodies pass quickly through the intestinal tract of a diarrheic patient. As such, treatment with oral antibodies should probably be frequent.¹²³

Conclusion

Plasma is a commonly used therapeutic agent in the acute colitis case. It has many benefits but is primarily used intravenously for colloidal support. Passive immunization is not a novel concept but the use of hyperimmunized plasma for the treatment of acute colitis has not previously been investigated in horses. There is evidence in the human literature to support both the intravenous and topical provision of high concentration of antibodies against common gastrointestinal pathogens and whilst one would consider antibodies to be destroyed whilst passing through the acidic environment of the proximal intestinal tract it appears that this is not the case, and they are likely functional at the level of the large colon.

Chapter 4: Efficacy of hyperimmunized plasma in the treatment of horses with acute colitis

Introduction

Acute colitis is a debilitating disease that can affect horses and ponies of any breed, age or gender. By definition, colitis is associated with inflammation of the colonic mucosa which leads to the development of diarrhea. Gastrointestinal loss of fluid and electrolytes results, in addition to the absorption of bacterial products that induce a systemic inflammatory response leading to fever, persistent diarrhea and morbidity. Despite aggressive therapy the clinical status of an affected horse can deteriorate rapidly leading to an overall fatality rate that has been reported to be as high as 70%.¹

Treatment goals for the acute colitis case primarily consist of maintenance of fluid and electrolyte balance, preservation of colloid oncotic pressure and replacement of plasma protein, control of local and systemic inflammation, promotion of tissue perfusion, promotion of mucosal repair and nutritional management. A positive response to therapy, most importantly, is reflected clinically by cessation of the horse's diarrhea. Due to the systemic nature of the condition, normalization of clinical and clinicopathologic data is also indicative of clinical improvement.

Cortisol is a stress hormone produced by the adrenal cortex which initiates many of the biological responses that permit the horse to cope with adverse psychological, physiological and environmental stressors. Although plasma cortisol concentrations have not been studied in the equine colitis case, it is well recognised that concentrations of this stress hormone increase in the sick horse and remain elevated until resolution of the underlying disease.¹²⁸⁻¹³⁰ Therefore, elevations in cortisol concentration can be used as a guide to indicate improvement in the health status of a patient.

Plasma is a natural colloid commonly used therapeutically in the acute colitis case, for volume expansion and as a source of albumin. In addition to its recognised colloidal benefits there is a theoretical benefit to supplying the affected horse with plasma containing antibodies to possible etiological agents of diarrhea in horses. Passive immunization is a principal regularly employed in the equine industry to protect horses from the development of different diseases. Examples include protection against the development of tetanus in the unvaccinated horse,

prevention of Rhodococcal pneumonia in foals, to limit the development of clinical signs of West Nile Fever or botulism and finally as a means of preventing the clinical signs of endotoxemia.^{103,108,110,111} The intent of this study was to determine whether hyperimmunized plasma, more specifically plasma with higher concentration of antibodies to *Salmonella spp*, *Clostridium difficile* and *Clostridium perfringens* than normal plasma, is beneficial to horses with diarrhea compared to treatment with normal or no plasma.

Materials and Method

Animals

Client owned horses which were referred to the Marion DuPont Scott Equine Medical Center between November 2004 and November 2006 were used for this study. Owner permission was obtained prior to enrollment. Horses were enrolled in the study if they had diarrhea, as defined by the presence of watery feces with a frequency of greater than 3 episodes per 6 hour period for at least 6 hours duration. Further inclusion criteria included age over one year, duration of diarrhea less than 72 hours, serum total protein greater than 4mg/dl after clinical rehydration and no treatment with equine plasma or any other blood product within the last 3 months. Exclusion criteria included the presence of severe concomitant disease, such as laminitis, at the time of admission. If these conditions developed after enrollment, then the horse remained in the study. The inclusion criteria of a total protein >4mg/dl ensured that no horse was sufficiently sick that assignment to the control group would mean it did not receive appropriate therapy.

Treatment groups

Horses were randomly assigned to treatment group using a random number table. The hyperimmunized plasma group received the test article which was plasma collected from horses which had been immunized against the common equine diarrheal pathogens, *Salmonella sp*, *Clostridium difficile* and *Clostridium perfringens*. A second group, the normal plasma group, received the control article which was normal fresh frozen equine plasma produced from horses that had not been hyperimmunized. The third group of horses served as a further control group,

with horses receiving no plasma. The specific titers of antibody against diarrheal pathogens in the two plasma products was not tested, however the nature of the products were verified by the manufacturer.

Clinicians and hospital staff involved in patient care and documentation were unaware of the type of plasma given, although it was not possible to blind study evaluators to whether plasma had or had not been given due to its characteristic appearance. The hospital pharmacy served as the dosing coordinator, providing the plasma products in identical bags with no identifier as to their type (e.g. normal or hyperimmunized). The dose of plasma given was 12 ml/kg (rounded up to the nearest liter). (Table 4.1) Half was administered orally via a nasogastric tube and half was administered intravenously. Other treatment provided was at the clinicians' discretion, with the exception of di-tri-octahedral smectite (Biosponge™, probiotic pastes and *Saccharomyces boulardii*, which were not allowed.

Sampling

The study began at time 0 (T0 hours) which was immediately prior to administration of plasma for the two treatment groups or at a clinically comparable time for the non-treatment control group. Clinical observations were documented every 6 hours for a total of 72 hours (T72). These consisted of temperature, pulse, respiratory rate, fecal consistency, frequency of defecation and volume of feces per episode of diarrhea. A “fecal score” was constructed for use during the study. Fecal consistency, frequency and volume were each assigned a value and summed to provide a single ‘fecal score’ (2-14). (Table 4.2) Normal fecal score was considered to be less than 5. The fecal scoring system was developed empirically, with a weighted emphasis on fecal volume, based upon clinical experience. Fecal score was recorded until resolution of diarrhea, discharge or death and the duration of diarrhea after enrollment in the study was documented for each horse. Resolution of diarrhea was determined clinically by normalization of the fecal consistency and frequency for 3 consecutive defecations. Success or failure of resolution of diarrhea within the 72 hour study period was recorded. Duration of hospitalization and outcome were documented for each horse included in the study.

At study entry a fecal sample was submitted for *Salmonella* culture, *Clostridium difficile* and *Clostridium perfringens* toxin ELISA and a fecal parasites flotation assay. *Salmonella* culture was repeated prior to completion of the study using a fecal sample collected at T72. All additional diagnostic testing, most importantly that for Potomac Horse Fever (IgM ELISA, UC Davis, CA), was performed at the clinician's discretion.

Further clinicopathologic evaluations included a complete blood count (CBC) using whole blood collected at T0, T24 and T72 (hours), biochemical profiles using serum collected at T0, T24 and T48 and cortisol assay using serum collected at T0, T24, T48 and T72. Additional blood samples were collected for the measurement of plasma total protein at T12 and total protein and plasma globulin and albumin concentrations at T72. As such total protein was documented for each horse at T0, T12, T24, T48 and T72 hours. Blood was collected by jugular venipuncture into sterile evacuated tubes (Vacutainer, Becton Dickenson). Plain or lithium heparin tubes were used for serum collection and EDTA tubes were used for collection of plasma samples. For the CBC the whole blood was analyzed using a Pentra 60 C+ cell counter (ABX Montpellier, France). Each CBC provided measurement of packed cell volume, fibrinogen, total protein, total white blood cell count, segmented neutrophil count, band neutrophil count, lymphocyte count, hemoglobin, mean corpuscular hemoglobin level, mean corpuscular hemoglobin concentration and mean corpuscular volume. A manual white blood cell differential cell count was performed on each sample to verify the machine-generated values. For the biochemical profiles, total protein and individual albumin and globulin concentrations, each blood sample was centrifuged at 300g for 3 minutes and the plasma pipetted off prior to analysis using an Ace[®] Clinical Chemistry System (Alfa Wasserman, NJ 07006). Each biochemical profile measured plasma sodium, potassium, chloride, glucose, blood urea nitrogen, creatinine, osmolarity, total protein, albumin, globulin, albumin/globulin ratio, calcium, phosphorus, magnesium, total bilirubin, direct bilirubin, indirect bilirubin, lactate dehydrogenase (LDH), alkaline phosphatase (ALP), aspartate aminotransferase (AST), creatine kinase (CK), gamma glutamyltransferase (GGT) and triglycerides. For the cortisol assays, blood was centrifuged (300g x 10 minutes) after clotting and the serum harvested and frozen at -20°C within 3 hours of

blood collection. Cortisol concentrations were measured by radioimmunoassay (^{123}I) at a later date.¹³¹

Statistical Analysis

All data were entered into a desktop computer and following data entry validation were analyzed using commercial statistical software (SAS 9.0, SAS Institute, Cary, NC). Descriptive statistics were performed for continuous data at each time point. To determine if treatment with hyperimmunized plasma had an effect upon clinical or clinicopathologic variables, data were analyzed using repeated measures ANOVA, with Tukey-Kramer correction for post-hoc comparisons. Main effects of time and treatment group, as well as time/treatment interaction were analyzed. All continuous data were evaluated by observation of a plot of the residuals to ensure that the data met the assumptions of the ANOVA. Those data not meeting the assumptions of the ANOVA were transformed as necessary prior to final analysis. This applied only to the data for observed duration of diarrhea.

Analysis of duration of diarrhea by treatment group as well as duration of hospitalization was performed using one way ANOVA, after confirmation of the assumptions of the ANOVA by observation of a plot of the residuals. Success, namely resolution of the patient's diarrhea, or failure of resolution of each patient's diarrhea within the 72 hour study period was analyzed non-parametrically using a Fishers exact test. Group analysis of the survival of each patient to dismissal was assessed using a Fishers exact test.

Mean duration of hospitalization was analyzed between the three treatment groups using a one way ANOVA with Tukey-Kramer correction for *post hoc* comparisons. A one way ANOVA with a Tukey-Kramer *post hoc* correction factor was used to assess the mean time to resolution of diarrhea by diagnosis rather than by treatment group. Within each different diagnosis group pairwise comparison was used to assess if there was a statistical difference between the treatment groups. A P value of <0.05 was pre-set as significant.

Results

Forty-two horses met the criteria for inclusion in the study. Of this number, 15 received hyperimmunized plasma, 13 received normal plasma and 14 did not receive any plasma. Twenty seven of the horses were geldings, 2 were colts and 13 were mares. Nineteen were Thoroughbreds, 9 were Quarter Horses, 4 were pony breeds, 3 were warmbloods, 3 were Tennessee Walking horses, two were draft breeds and 2 were Arabians.

At admission, no significant difference was found between the three treatment groups for any of the clinical parameters measured except for frequency of diarrhea. (Table 4.3) A significant difference between the mean frequency of diarrhea for the hyperimmunized group (mean score = 2.5 ± 0.1) and control group (mean score = 1.7 ± 0.2) and control group and the normal plasma group (mean score = 2.4 ± 0.2) was found ($P=0.0003$). (Figure 4.1) Over the course of the study period no significant difference for any of the clinical parameters was found between the three treatment groups. (Figure 4.2) (Table 4.4)

At admission and over the course of the study period, no significant difference was found between the three treatment groups for any of the clinicopathological parameters measured. (Tables 4.5 and 4.6)

The total protein concentration was higher over time for the horses that received hyperimmunized plasma in comparison to those horses that received normal plasma and the control group but the difference between the groups over time did not reach significance. ($P=0.0594$). (Figure 4.3) Cortisol concentrations decreased over time for all three groups but were lower at all time points for the normal plasma group in comparison to the hyperimmunized plasma group. (Figure 4.4) There was no statistical difference between treatment groups, however.

Thirty eight out of the 42 horses completed the 72 hour study, 13 were in the hyperimmunized plasma group, 12 were in the normal plasma group and 13 were in the control group. Thirty three of the 42 horses had resolution of the diarrhea within the 72 hour study period. Eleven of these horses were in the hyperimmunized group, 12 in the normal plasma group and 10 horses in the control group ($P=0.0797$).

All horses that completed the study had resolution of diarrhea prior to either discharge or euthanasia. The observed duration of diarrhea revealed that horses in the hyperimmunized group had a shorter duration of diarrhea in comparison to horses in the other two treatment groups. The mean duration of diarrhea for these horses, excluding those that failed to survive to completion of the study, was 40.7 ± 9.8 hours for the hyperimmunized group, 119.2 ± 56.1 hours for the normal plasma group and 72.0 ± 24.5 hours for the control group. A significant difference was found between the mean values for the hyperimmunized plasma group and normal plasma group ($P=0.0433$). (Figure 4.5)

Eight horses died or were euthanized prior to hospital discharge. Four of these 8 horses were in the hyperimmunized plasma group, 2 were in the normal plasma group and 2 were in the control group. (Table 4.7) Four horses died or were euthanized prior to completion of the study. 2 of these 4 horses were within the hyperimmunized group, 1 was in the normal plasma group and 1 was in the control group. There was not a significant difference in outcome (i.e. survival or death) between the three treatment groups ($P=0.0821$). Of the surviving horses, 1 developed thrombophlebitis, 1 had abdominal surgery and 2 developed incisional infections. These complications occurred after completion of the study and resolution of the respective patient's diarrhea.

Of the horses that survived to hospital discharge, the mean duration of stay was 9.11 ± 1.26 days for all horses included in the study and 9.77 ± 2.91 days, 8.73 ± 1.43 days and 8.75 ± 1.86 days for the hyperimmunized plasma group, normal plasma group and control group accordingly. No significant difference between the treatment groups for the duration of hospitalization was found ($P=0.9302$). (Figure 4.6)

Ten of the 42 horses were diagnosed as having clostridiosis (*Clostridium difficile* and/or *Clostridium perfringens*), 3 had Potomac horse fever (PHF), 2 had salmonellosis and 27 had an undetermined or 'open' diagnosis. (Table 4.8) There was no evidence of parasite mediated diarrhea in any of the horses in the study. The mean observed duration of diarrhea for each diagnosis group was 133.8 ± 83.9 , 162 ± 99 , 96.0 ± 24 and 45.9 ± 7.9 hours for the clostridiosis, PHF, salmonellosis and open diagnosis groups respectively. A statistical difference between the four

groups was found ($p=0.0282$). (Figure 4.7) Pairwise comparison revealed the difference to be between the hyperimmunized and the normal plasma groups in the open diagnosis group.

Discussion

This study assessed the effects of treatment of acute colitis with enteral and intravenous plasma containing high concentration of antibodies to specific equine diarrheal pathogens. The results showed that the use of hyperimmunized plasma with high concentration of antibodies against the common infectious causes of acute colitis, resulted in a shorter duration of diarrhea in clinical patients compared to the duration of diarrhea in patients that received no plasma or plasma from equine donors not immunized against *Clostridium difficile*, *Clostridium perfringens* or *Salmonella sp.* Resolution of diarrhea signifies improvement in the fluid regulatory mechanisms within the diseased colon which is usually secondary to healing of the damaged tissue. Therefore, a shorter duration of diarrhea suggests a response to treatment, improved clinical status and a reduction in the risk of clinical dehydration to the patient.

The administration of normal plasma does not specifically address the etiologic agents of colitis. It is a supportive therapy which provides colloidal support to the systemic circulation whilst the large colon heals. As such, when looking at values that represent the systemic abnormalities caused by an acute colitis one would not expect a difference in a patient's parameters if they were to receive no plasma or normal plasma. In comparison, the therapeutic goal of hyperimmunized plasma is as a treatment to target the underlying etiological agent for the colitis. A positive clinical response to administration of hyperimmunized plasma would be a decrease in the severity of the patient's diarrhea and potentially a significant difference in clinical and clinicopathological data from that of patient's receiving normal or no plasma.

A significant difference ($P=0.003$) was noted in fecal frequency score between the no plasma group and both the hyperimmunized plasma group and the normal plasma group at admission. Although this might suggest the diarrhea in the control group was less severe at admission than the other two groups, it is important to note that frequency of diarrhea is only one component of diarrhea and for that reason we derived the fecal scoring system. Fecal frequency, consistency and volume of diarrhea per episode are commonly evaluated factors when assessing

diarrhea in humans. However, even in the human literature the effect of dehydration and fluid therapy on the frequency of diarrhea is unclear.^{132,133} Similar to observations in humans, diarrheic episodes in horses can be in clustered episodes so scoring fecal frequency every 6 hours, as was done in this clinical trial, may have been too short a time frame and number of episodes per 12 or 24 hours may have been more appropriate. In humans it is possible to monitor the number of episodes over time but this can be challenging in the horse with profuse watery diarrhea in a bedded stall. Hence, it is not considered that the slight increase in frequency of diarrhea alone in one group, suggests a measurable difference in severity of diarrhea among groups.

Other than fecal frequency score, the treatment groups were of comparable clinical status with no difference in clinical, hematological or biochemical parameters at admission. That said, although significance was not reached, the group that received hyperimmunized plasma tended to have a higher cortisol level, lower total protein, higher fecal consistency score and total fecal score, higher fibrinogen level and lower white blood cell count at admission. This might imply that the hyperimmunized patients were ‘sicker’ than those in the other two groups. Certainly, the greater number of mortalities in the hyperimmunized group (4/8) in comparison to 2/8 for each of the normal plasma group and the control group may support this theory.

When each clinical, hematological and biochemical parameter measured during this study was assessed over time there was a significant difference in heart rate, total protein, albumin, globulin, cortisol, creatinine, sodium, potassium, chloride and fecal score. These changes reflected the clinical improvement in the horses over time as expected following treatment in most cases. When these parameters were analyzed over time by treatment group, however, there was no significant difference between the three groups.

Serum cortisol concentrations, while not well documented in diarrheic horses, are known to be increased in stressful situations such as colic and secondary to transportation.^{129,134} For all 3 treatment groups the mean cortisol concentrations were high at admission and decreased in accordance with a positive clinical response. However, many horses included in this study were transported to the referral hospital shortly prior to study inclusion. The randomization for the

treatment groups did not differentiate between those horses recently transported and those that developed diarrhea whilst in the hospital, a fact that may have influenced this trend.

Administration of hyperimmunized plasma is a specific ‘antidiarrheal’ therapy which targets the causative agent of the colitis. As mentioned earlier, a change in systemic parameters may have been expected in this study but this change would have been secondary to an associated clinical improvement and not because of the plasma itself. Significant change in the measured parameters was not found in the hyperimmunized group over time, presumably because each horse received treatments concurrent with the plasma therapy. Over the study period, systemic parameters were infrequently abnormal at admission to the study and the limited sample size all meant that the ability to interpret each parameter over time for the hyperimmunized group was restricted.

Mean total protein concentration for the hyperimmunized group was consistently above 6mg/dl. In the acute colitis case, protein is lost through the inflamed large colon wall into the gastrointestinal lumen.¹³ Plasma administration provides protein to the patient. If gastrointestinal inflammation is ongoing, there is protein loss until the primary etiology is resolved and the patient’s gastrointestinal mucosa heals. The results of this study showed that for the hyperimmunized plasma group the mean values for total protein concentration remained elevated but for the normal plasma group the mean value dropped at 24 hours and for the control group the mean value fell at 48 hours, presumably reflecting ongoing protein loss. For each of the latter groups the means then increased again presumably reflecting gastrointestinal healing and reduction in protein loss. The maintenance of a stable protein level in the hyperimmunized group could be explained by an expedited recovery of the gastrointestinal mucosa secondary to treatment of the underlying etiology for the acute colitis. This observation is consistent with the shorter mean duration of diarrhea documented for the horses that received hyperimmunized plasma.

Eight of the 42 horses (20%) included in this study died or were euthanized. Four were in the hyperimmunized plasma treated group and two were in each of the other two treatment groups. This difference in outcome between the treatment groups did not reach statistical significance but an overall survival rate of 80% is very similar to that reported in the literature.⁴¹

Of the eight horses in this study that died or were euthanized 4 died from secondary complications. Laminitis is the most frequently encountered secondary complication to acute colitis as found in this study whereby 3 of the 4 horses with secondary complications were euthanized due to the development of this crippling condition.⁸⁵ Although the pathophysiology of laminitis associated with diarrhea remains unclear it is thought to be linked to the absorption of gut derived toxins and vasoactive factors.¹³⁵

For 70-90% horses in the three treatment groups the diarrhea resolved within the 72 hour study period. This would suggest that the time period for the experiment was adequate. Clinical experience suggests that the longer the duration of acute diarrhea in this study, the more guarded the prognosis. This is supported by reports in the literature whereby acute diarrhea of longer than 3-6 days duration is said to carry a diminishing chance of a full recovery.^{41,136} Duration of hospitalization was not significantly different between the treatment groups. Several horses in this study had concurrent problems to the colitis. Therefore, further consideration of the duration of hospitalization had to be interpreted with the multifactorial nature of this parameter in mind.

Classification of the data by diagnosis rather than by treatment revealed that a definitive diagnosis was reached for only 40% of the horses. This is comparable to values reported in the literature of approximately 35%.² A significantly shorter duration of diarrhea was found in the open diagnosis group. There is no study in the equine literature that has found a single variable that permits accurate prediction of the horse's prognosis, other than response to treatment within the first three to four days, but the current study would suggest that undifferentiated colitis carries a better prognosis than those for which a specific diagnosis can be made.¹³⁶ Interestingly, the hyperimmunized plasma treated horses within the open group had a significantly shorter duration of diarrhea in comparison to the other treatment groups. This would suggest that the antibodies provided in the hyperimmunized plasma are not simply producing an antibody-dependent cellular cytotoxic response on the known etiological agents, *Clostridium difficile*, *Clostridium perfringens* and *Salmonella sp*, but have additional mechanisms of action. This is similar to the multiple actions of J5 hyperimmune plasma therapy as a means of treating endotoxemia. The antibodies in this plasma have been found to act on a wide variety of different gram negative bacteria.¹⁰⁷ Proposed mechanisms of action might be opsonization and

phagocytosis, neutralization of pathogenic toxins, modulation of host T-cell function and antibody production, balance of the cytokine network or even blockage of cellular receptors for pathogens. Many etiological agents for acute colitis in the horse such as viruses go undiagnosed but research continues and new agents are being discovered. For example, there is recent evidence to indicate that spirochete associated colitis can occur.¹³⁷

The results of this study did not allow us to differentiate between the effects of enteral or intravenous hyperimmunized plasma administration. Due to the clinical nature of this study each horse received multiple treatments concurrent to the plasma therapy. In such a situation it is impossible to determine the patient's response to a single treatment but a larger study population would have strengthened the power of the study. Hyperimmune plasma may be administered intravenously or enterally and there is evidence in the human literature to support efficacy for both routes.^{113,116,119} To be effective, the primary mechanism of action involves the intravenously administered antibody leaving the circulation to act directly on the etiological agent e.g. *Clostridium difficile* toxins A and B within the colonic lamina propria or intestinal lumen. This may occur as a result of the exudation of serum proteins across an inflamed colonic mucosa but has also been documented in the absence of pathophysiological change to the large intestinal mucosa.^{113,115} In contrast, enteral administration of plasma delivers protective concentrations of antibodies immediately and directly to the susceptible mucosal surface. Once there they may trap the pathogen in mucus and then act by a number of mechanisms to prevent penetration of the mucus layer and subsequent infection of target cells.¹²⁰ Being proteins, one would expect that orally administered immunoglobulins would undergo rapid degradation in the gastrointestinal tract, however, there is evidence in the human and animal literature to suggest that this is not the case.^{125,126} In order to determine the optimum route of hyperimmune plasma administration further investigation would be required. The dose and route of plasma administered in this study was decided upon with consideration of the manufacturers recommendations. There is no evidence in the literature to support an optimum dosage or route of administration.

The clinically effective dose of both enterally and intravenously administered hyperimmune plasma is unclear. Wide patient variability is reported in humans possibly due to the multitude of different actions of the immunoglobulins.¹¹⁷ Additionally, in the diarrheic

patient, intestinal motility can be increased so enteral administration of immunoglobulins may result in their passage through the gastrointestinal tract at an increased rate. With these considerations in mind, the recommendation for treatment of acute colitis with hyperimmune plasma would be that dosing should be frequent, possibly every 2-3 days.¹²³ However, no horses in this study developed diarrhea again after normalization of their feces had been documented. Further studies are indicated in the equine patient to determine the appropriate dosing of hyperimmune plasma in the treatment of acute colitis. No adverse effects associated with the hyperimmunized plasma administration were noted in this study thereby implying it is a safe product to use. A more specific study design would be required in order to ascertain appropriate product safety.

Overall, the study showed a promising response to the use of hyperimmunized plasma. The limited sample number decreased the power of the study but the preliminary results are promising and sufficient to advocate the use of this plasma in comparison to normal plasma or no plasma at all regardless of the underlying primary etiology for the acute colitis.

Table 4.1: Treatment groups in this study.

Group	N	Treatment	Dose
Hyperimmunized plasma	15	Hyperimmunized plasma	12ml/kg
Normal plasma	13	Normal plasma	12ml/kg
Control	14	No plasma	0ml/kg

Table 4.2: Classification method for fecal scoring.

Calculation of Fecal Score = (Consistency points + Frequency points) x Volume points

For example, the maximum possible score: $(4 + 3) \times 2 = 14$

Point Value	Consistency of Diarrhea
1	Normal, formed feces
2	Soft feces but formed
3	Loose feces, sitting on shavings
4	Watery feces
Volume of Diarrhea	
1	Low volume per episode of diarrhea
2	High volume per episode of diarrhea
Frequency of Diarrhea	
1	Low frequency, none or one episode every 6 hours
2	Medium frequency, 2-3 episodes per 6 hours
3	High frequency >3 episodes per 6 hours

Table 4.3: Summarized results for key clinical parameters at admission.

Parameter	Hyperimmunized (mean ± SEM)	Normal plasma (mean ± SEM)	Control (mean ± SEM)	P value
Temperature	100.24 (±0.35)	100.82 (±0.31)	100.16 (±0.36)	0.339
Pulse	51.1 (±4.9)	53.9 (±3.1)	52.0 (±4.3)	0.919
Respiratory rate	19.2 (±2.2)	18.7 (±1.7)	21.4 (±3.3)	0.727
Fecal consistency	3.7 (±0.1)	3.7 (±0.1)	3.4 (±0.2)	0.327
Fecal frequency	2.5 (±0.1)	2.4 (±0.2)	1.7 (±0.2)	0.003
Fecal volume	1.7 (±0.1)	1.5 (±0.1)	1.6 (±0.2)	0.639
Fecal score	11.0 (±0.9)	9.4 (±1.0)	8.4 (±0.9)	0.148

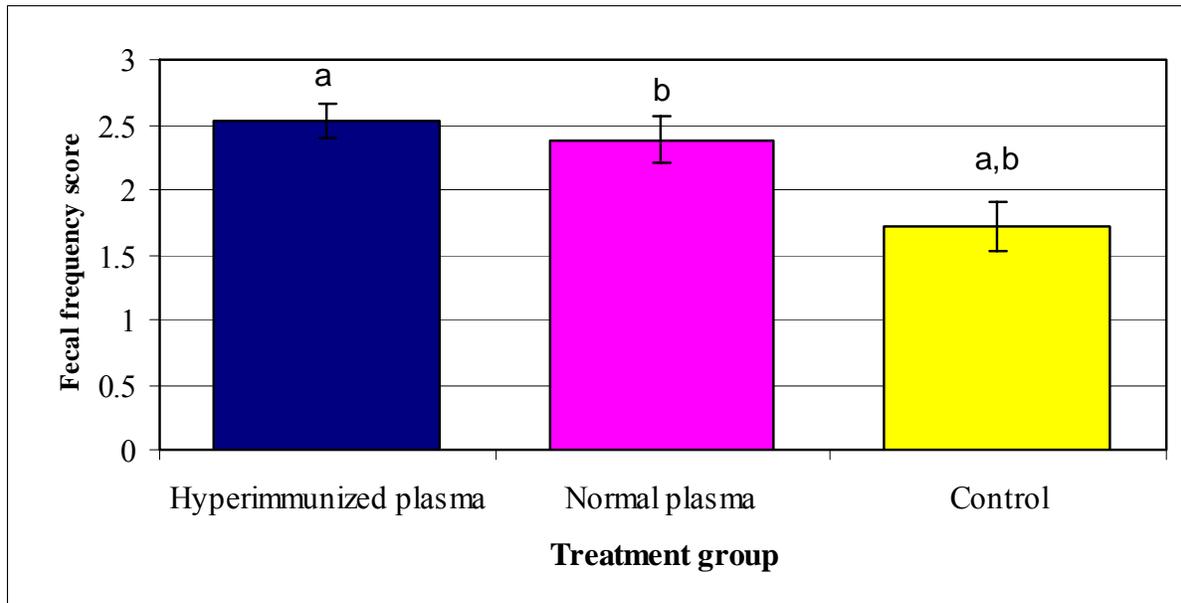


Figure 4.1: Mean fecal frequency score (mean \pm SEM) among the three treatment groups at admission. Different letters signify a difference between the treatment groups ($P < 0.05$).

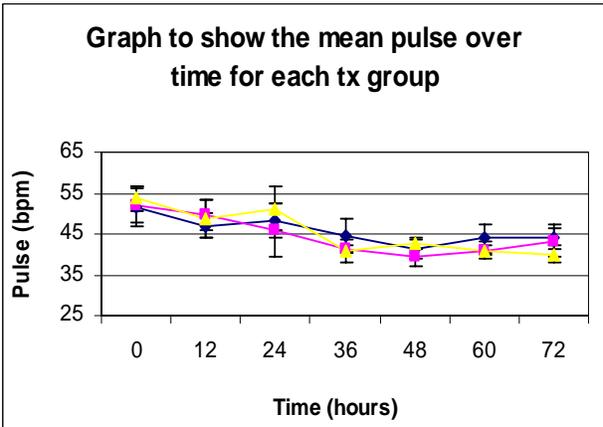
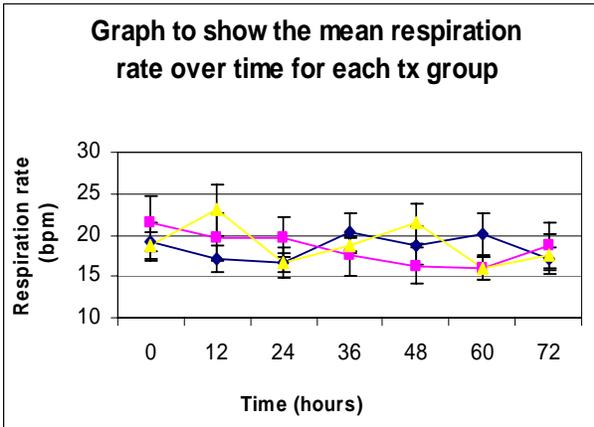
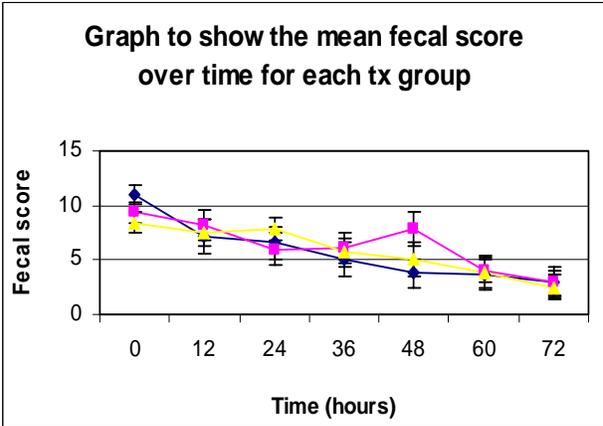
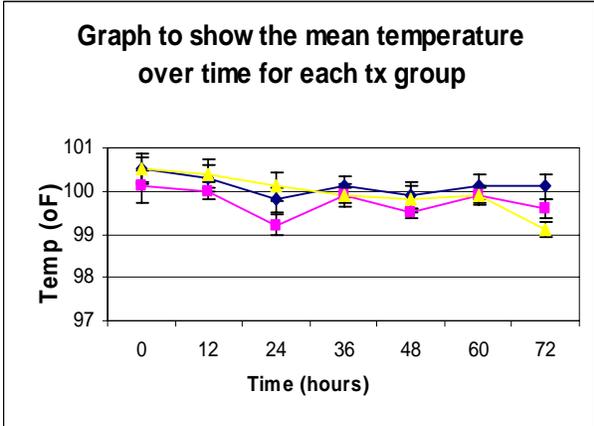


Figure 4.2: Summary of data for selected clinical parameters over time for each treatment group.

Table 4.4: Summary of P values for each key parameter measured for each treatment group and by treatment group over time.

	P value (treatment)	P value (time)	P value (treatment x time)
Temp	0.3122	0.5497	0.377
Pulse	0.9427	<0.0001	0.7196
Respiration	0.8999	0.7864	0.6448
Fecal score	0.7672	<0.0001	0.1702

Table 4.5: Summary of key clinicopathological parameters at admission.

Parameter	Hyperimmunized (mean ± SEM)	Normal (mean ± SEM)	Control (mean ± SEM)	P value
Fibrinogen	440.0 (±45.6)	430.8 (±51.1)	378.6 (±56.6)	0.650
Total protein	6.3 (±0.2)	6.5 (±0.3)	6.7 (±0.3)	0.565
White blood cell count	6973.3 (±848.9)	9553.9 (±3548.7)	7235.7 (±982.0)	0.632
Band cell count	199.1 (±79.2)	438.0 (±174.7)	224.6 (±76.0)	0.291
Cortisol	9.59 (±1.51)	7.31 (±1.67)	7.00 (±1.83)	0.475

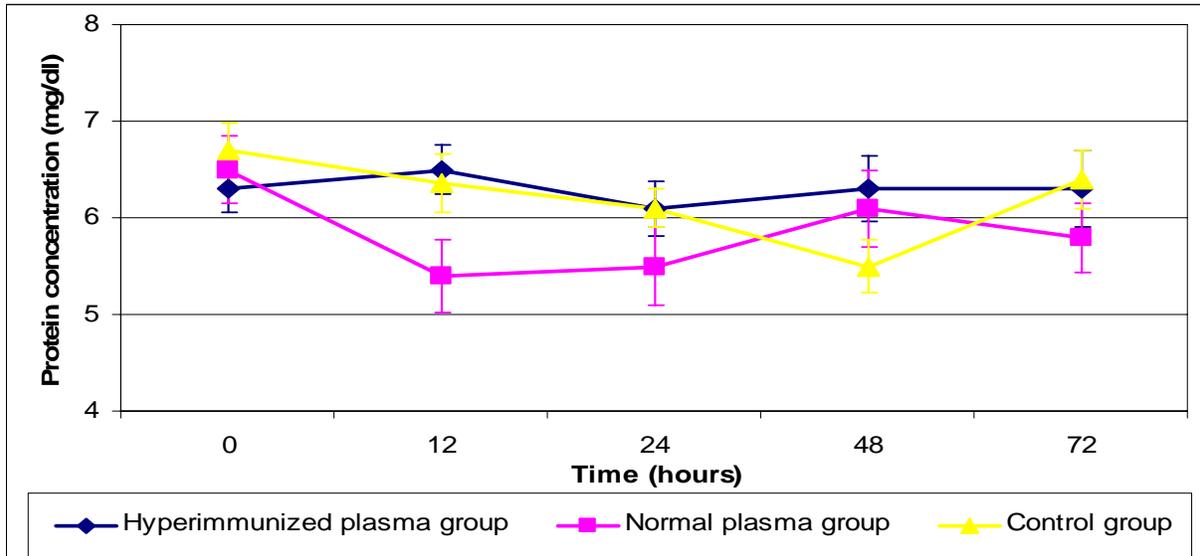


Figure 4.3: Changes in protein concentration (mg/dl) over time for each treatment group (mean \pm SEM).

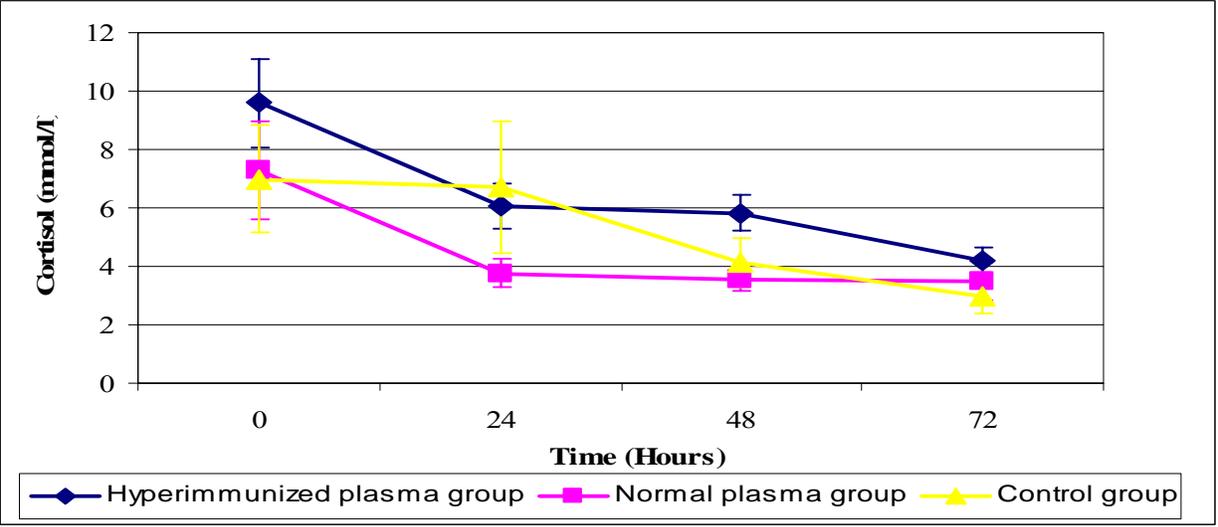


Figure 4.4: Cortisol concentration (mmol/l) (mean \pm SEM) for each treatment group over time.

Table 4.6: Summary of P values for clinicopathological parameters measured for each treatment group and by treatment group over time.

	P value (treatment)	P value (time)	P value (treatment x time)
Total Protein	0.4224	<0.0001	0.0594
Albumin	0.9918	0.0002	0.3167
Globulin	0.3974	0.0075	0.21
WBC	0.4515	0.3579	0.5495
Bands	0.9918	0.7767	0.3528
Segmented neutrophils	0.4943	0.4274	0.6957
Fibrinogen	0.4708	0.8155	0.9239
Cortisol	0.2863	<0.0001	0.4757
Creatinine	0.4281	<0.0001	0.3295
Sodium	0.0832	0.0012	0.6055
Potassium	0.5293	0.0428	0.2135
Chloride	0.1229	<0.0001	0.3642

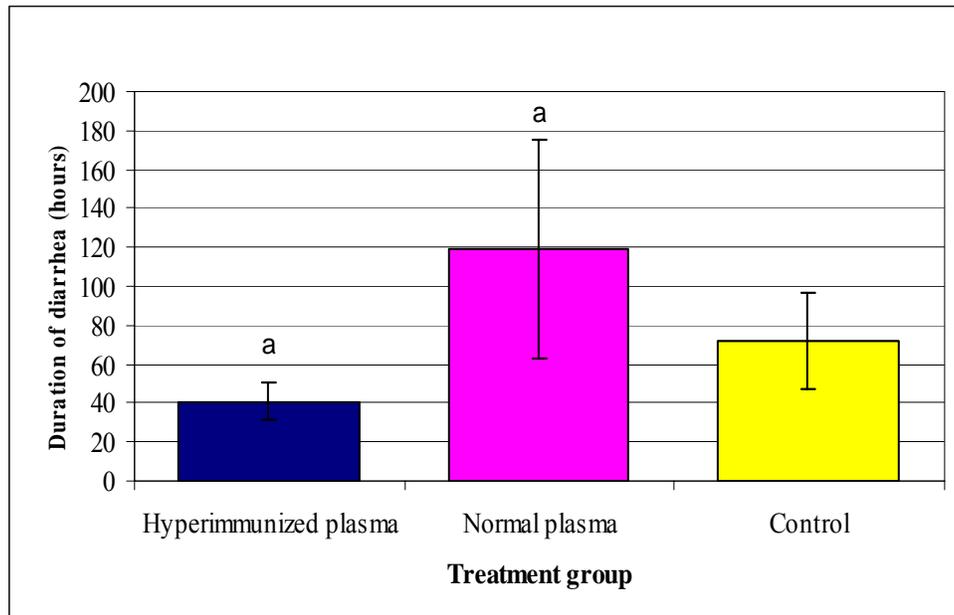


Figure 4.5: Mean duration of diarrhea (mean \pm SEM) (hours) for each treatment group. Letters indicate significant difference between the treatment groups ($P < 0.05$)

Table 4.7: Key points regarding the eight horses that did not survive to hospital discharge.

Horse	Treatment group	Completion of the study	Duration of hospitalization (days)	Primary reason for natural death or euthanasia
1	Hyperimmunized	No	1	<i>Clostridium difficile</i> colitis
2	Hyperimmunized	No	2	Intestinal rupture
3	Hyperimmunized	Yes	8	Endotoxemia
4	Hyperimmunized	Yes	16	Laminitis
5	Normal plasma	Yes	4	Laminitis
6	Normal plasma	No	1	Small colon impaction
7	Control group	No	1	Laminitis
8	Control group	Yes	12	Intestinal adhesions post colic surgery

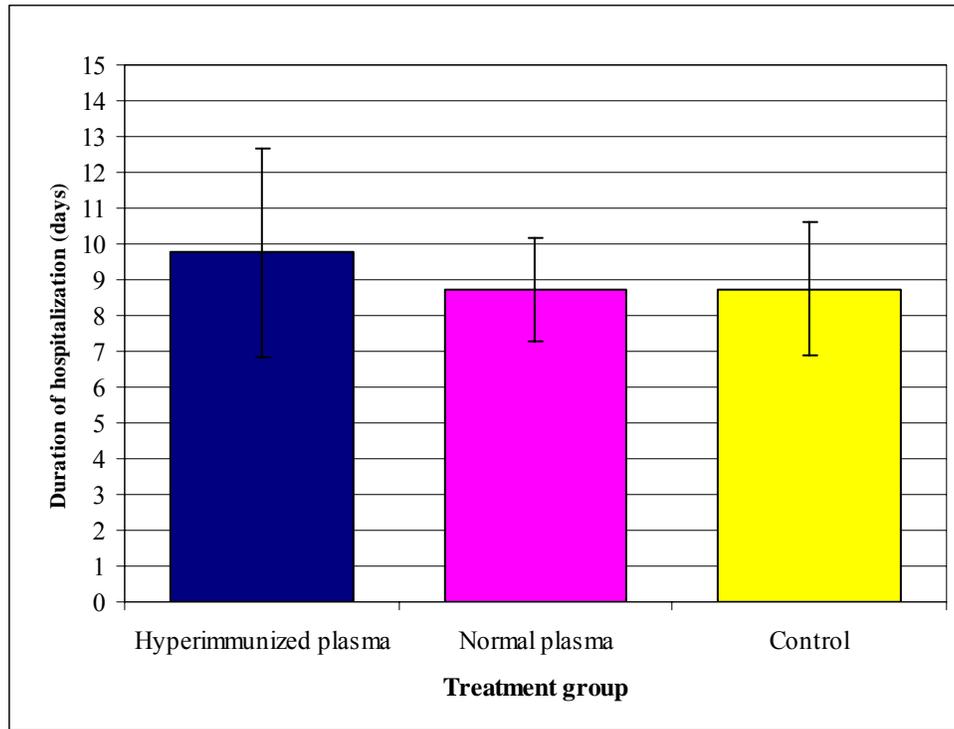


Figure 4.6: Mean duration of hospitalization (days) for each treatment group (mean \pm SEM).

Table 4.8: Morbidity within treatment group by diagnosis reached

Diagnosis	Hyperimmunized (n)	Normal (n)	Control (n)
Clostridiosis	3	3	4
Open	10	9	8
Potomac Horse Fever	0	1	2
Salmonellosis	2	0	0

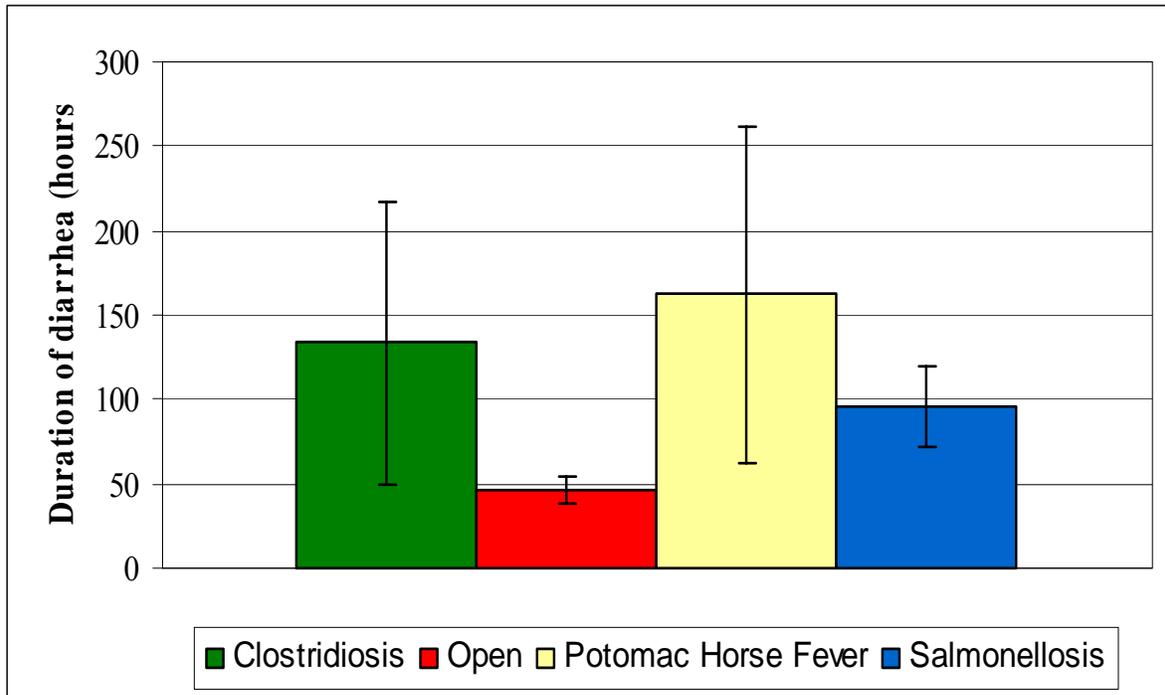


Figure 4.7: Duration of diarrhea (hours) by clinical diagnosis (mean \pm SEM).

Chapter 5: Conclusions

Hyperimmunized plasma is an effective means of treating acute colitis in the adult horse. Its use results in a reduction in the duration of the patient's diarrhea in direct comparison to a control group of horses that did not receive any plasma and those horses that received non-hyperimmune plasma. The mechanism of action for the hyperimmunized plasma is unclear but the results from this study would suggest that it may be of benefit to all cases of acute colitis and not simply *Clostridium difficile*, *Clostridium perfringens* and *Salmonella*-associated colitis. Further research is indicated to ascertain an optimal dosage for the hyperimmune plasma used in this study and clarify whether enteral or parenteral administration is more beneficial to the patient.

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