

# EQUINE SALMONELLOSIS

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## Summary

Salmonellosis is a disease caused by an enteric or systemic infection with *Salmonella* spp. Clinically normal horses can transiently shed *Salmonella* organisms, but the prevalence of shedding is higher in horses presented to veterinary hospitals and horses with abdominal diseases. *Salmonella* infections can affect horses of all ages and range in severity from asymptomatic colonisation to severe systemic illness. The clinical signs of salmonellosis are variable and may include fever, mild abdominal pain, anorexia and depression without diarrhoea in some horses, but most horses that are clinically affected have moderate to severe, watery diarrhoea. Foals may develop haemorrhagic diarrhoea, septicaemia, pneumonia, meningitis, and septic arthritis or physitis. Treatments are largely supportive, and include fluid and electrolyte therapy, anti-inflammatory drugs, anti-endotoxin treatments, probiotics, intestinal protectants and nutritional support. Antimicrobial therapy is controversial. Salmonellosis is an important zoonosis.

## Introduction

The Gram-negative bacteria of the species *Salmonella enterica* are facultative intracellular anaerobes that are responsible for infections in humans and animals worldwide. *Salmonella enterica* includes 6 subspecies with more than 2000 serovars. Horses are not considered to be carriers of these bacteria, as there are no known strains that are host-adapted to the horse. Clinically normal horses can transiently shed *Salmonella enterica* organisms, however, and the prevalence of shedding in horses

presented to veterinary hospitals is reported to range from 6–13% (Palmer *et al.* 1985; Traub-Dargatz *et al.* 1990; Cohen *et al.* 1994, 1995; Mainar-Jaime *et al.* 1998; Kim *et al.* 2001; Ernst *et al.* 2004; Ward *et al.* 2005). These organisms are of particular concern in equine hospitals due to the mixing of large numbers of susceptible individuals and the potential for the development of multidrug resistant strains. Outbreaks typically result in substantial adverse impact on patient wellbeing as well as economic losses to the patient's owner and the facility. *Salmonella* spp. infections affect horses of all ages and can range in severity from asymptomatic colonisation to severe systemic illness. Salmonellosis typically manifests in horses as an acute enterocolitis with severe diarrhoea, but soft tissue infections and bacteraemia can also occur. Salmonellosis presents a substantial biosecurity challenge as the organisms are highly infectious, especially in susceptible individuals, and horses suffering from *Salmonella*-associated diarrhoea shed large numbers of infectious organisms into the environment.

## Source of infection

The initial source of infection in individual horses or even in outbreaks of salmonellosis is frequently not identified. Potential sources of infection include consumption of contaminated food or water; contact with contaminated environmental surfaces, equipment or handlers; aerosol exposure; direct contact with shedding animals; and ingestion of contaminated bird/vermin faeces or dead insects (Traub-Dargatz *et al.* 1990; Traub-Dargatz and Besser 2007). The most frequently reported outbreaks of salmonellosis have been in hospitalised horses (Kim *et al.* 2001; Ward *et al.* 2005a; Traub-Dargatz and Besser 2007). Clinically normal horses and other

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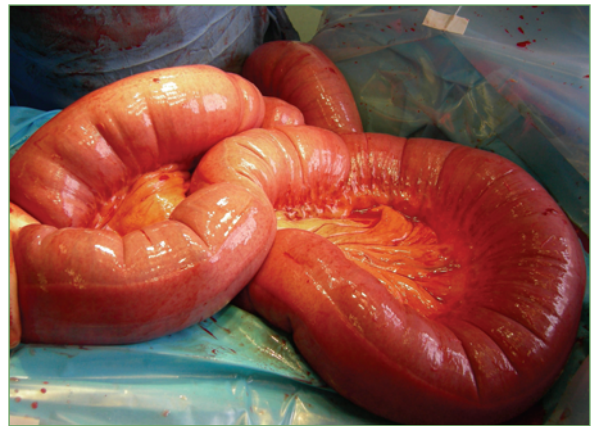
livestock species that shed the organism in their faeces are considered to be an important potential source of contamination of the environment. Horses with abdominal pain have increased shedding (5% identified via culture and up to 40% via polymerase chain reaction [PCR] techniques) suggesting that *Salmonella* spp. are common inhabitants of the gastrointestinal tract, but are generally shed in low numbers in the faeces unless there is an abdominal disorder (Cohen *et al.* 1995; Ernst *et al.* 2004; Ward *et al.* 2005b). Changes in intestinal motility and volatile fatty acid production by normal flora may increase the ability of *Salmonella* spp. to attach to the intestinal mucosa and to proliferate. The increased shedding of *Salmonella* spp. in horses with abdominal pain does not significantly affect mortality, but is undesirable because of the potential for colitis and increased environmental shedding. *Salmonella* organisms can persist in the environment for months to years depending on the serotype, moisture content, and temperature conditions.

### Pathophysiology

The development of equine salmonellosis represents the interplay of a number of factors, including the degree of bacterial exposure, the virulence of the *Salmonella* organisms, and the susceptibility of the host. Horses with impaction colic are particularly at risk (Fig 1). Outbreaks tend to be more common in large animal hospitals where these factors are common, on brood mare farms with a high-density population of mares and foals, or on farms where horses have been fed feed contaminated with *Salmonella* spp. Hot weather, increasing numbers of horses and foals on a farm, and wet flooring in barns or hospitals all seem to increase infection rates. Disease transmission is faecal-oral in nature and the severity of exposure is directly related to the number of bacteria that an individual horse ingests in contaminated feed or water, with the size of the infective dose being determined by the other factors of virulence and susceptibility. This infective dose may range from hundreds of organisms in particularly susceptible individuals to millions of organisms in a healthy animal (Murray 2002). The number of organisms shed by infected individuals can vary dramatically, with chronically infected cases often passing small numbers of organisms

intermittently, while acutely affected individuals may shed very large numbers of organisms. The virulence of any particular *Salmonella* organism is determined by its invasiveness, which depends upon the attachment of the organism to the mucosal epithelium and the production of enzymes and toxins (cytotoxins, endotoxin, and enterotoxins) that damage the epithelium and/or alter epithelial permeability and facilitate bacterial entry into the mucosal cells (Coburn *et al.* 2007) and infection of the *lamina propria*.

In order to reach their sites of colonisation within the lower intestinal tract *Salmonella* spp. must first survive passage through the stomach, where they are exposed to a number of antimicrobial factors including the inherently low pH and the presence of hydrochloric acid. Following gastric passage the organisms must attach to the intestinal epithelium, and this is mediated by *fimbriae* or *pili* on the bacterial surface (Foley and Lynne 2008). Successful infection requires that the *Salmonella* organisms invade the epithelial cells and establish intracellular infection. After the bacteria initially attach to the epithelium they express a type III secretion system (T3SS), which facilitates epithelial invasion by allowing the direct transfer of virulence factors into the host cells. It performs this feat using a needle-like structure that penetrates the epithelial cell membrane and forms a conduit by which these factors are delivered into the epithelial cell (Foley



**FIGURE 1:** Small colon impaction as seen at exploratory celiotomy. The small colon is diffusely inflamed as well as distended; *Salmonella enterica* ssp. *enterica* serovar typhimurium was cultured from the colonic contents.

and Lynne 2008). Several different virulence factors can be involved, including *Salmonella* invasion proteins (Sips), endotoxin (LPS) and flagellin (Grassl and Finlay 2008). These factors are all potent inflammatory agents, and it appears that *Salmonella* organisms actually foster and utilise the host inflammatory response to facilitate their invasion of the intestinal epithelium, as their ability to establish infection is correlated with their ability to attract neutrophils to the epithelium (Coburn *et al.* 2007). These virulence factors act to stimulate local proinflammatory cytokine production, particularly of interleukin-1 beta and the neutrophil chemoattractant factor interleukin-8, and also activate cyclooxygenase within the epithelium.

The next step in the establishment of intracellular infection is the movement of the bacterium from the epithelial cell surface into the host cell. This process is also mediated by the T3SS, as several Sips (A, B and C) interact with the actin cytoskeleton of the epithelial cell resulting in the internalisation of the bacterium within a membrane-bound vacuole (Foley and Lynne 2008). This *Salmonella*-containing vacuole (SCV) does not fuse with lysosomes within the cell, and the organism is thus protected from the normal phago-lysosomal fusion process that is necessary for bacterial killing (Foley and Lynne 2008). The SCV moves from the luminal border of the epithelial cell to the basal membrane where the bacteria then interact with and enter macrophages in the submucosa (Foley and Lynne 2008). The gut associated lymphoid tissues, such as the Peyer's patches and mucosa-associated lymphoid tissue, appear to represent a primary target during the initiation of *Salmonella* infection (Grassl and Finlay 2008). The macrophages within these structures play a key role in the initial production of tumour necrosis factor-alpha (TNF- $\alpha$ ) and inducible nitric oxide synthase (iNOS), and these mediators play an important role in the up-regulation of the inflammatory response (Grassl and Finlay 2008). This inflammatory response contributes to the development of the diarrhoea that is characteristic of enteric *Salmonella* infections, and increased production of prostaglandin E2 by iNOS appears to be a major contributor to intestinal hypersecretion (Bertelsen *et al.* 2003).

Host susceptibility is increased in the presence of stress, such as that associated with prolonged

transport or surgery, or due to the presence of concurrent diseases resulting in impaired immune function. Altered diet, feed withdrawal prior to anaesthesia and treatment with antimicrobial drugs are other potential predisposing factors. Many of these factors will be present in hospitalised horses, and most of the published reports of outbreaks of salmonellosis have originated from veterinary teaching hospitals. In the normal intestine there is a large resident microbial community, termed the 'microbiota', which functions in a symbiotic manner with the host tissues to optimise nutrient utilisation, foster maturation and function of intestinal tissues and enhance the function of the intestinal immune system (Stecher *et al.* 2007). In addition, this microbiota provides an efficient barrier against infection by enteric pathogens. This ability of the enteric population of commensal bacteria to resist the proliferation of pathogenic bacteria, termed colonisation resistance, is impaired in the face of antimicrobial administration or gastrointestinal dysfunction, and loss of this function increases the susceptibility of the host to *Salmonella* infection (Sekirov *et al.* 2008). A history of prior antimicrobial exposure (Baker and Leyland 1973; Smith *et al.* 1978; Hird *et al.* 1986; Ernst *et al.* 2004) and abdominal surgery during hospitalisation (Owen *et al.* 1983; Begg *et al.* 1988; Ernst *et al.* 2004) have been shown to be risk factors associated with shedding of *Salmonella* in equine patients.

Following the establishment of a *Salmonella* infection a local and systemic inflammatory response develops in an effort to eliminate the organism. Mucosal inflammation results in increased mucosal permeability, increased secretion of water and electrolytes, and alterations in motility due to altered enteric nervous system function. The development of this secretory response, in combination with intestinal hypermotility and decreased intestinal transit times, may be beneficial by decreasing mucosal adherence of pathogenic organisms but may also interfere with the normal intestinal microbiota. Impairment of the normal barrier function of the intestinal mucosa, in combination with derangements in the normal flora, increases the pathogenicity of *Salmonella* organisms. This appears to result in part from the negative effects of these changes on the ability of the normal microbiota to effectively compete with the *Salmonella* organisms (Stecher *et al.* 2007).

The loss of fluid, electrolytes and protein that result from the intestinal inflammation and hypersecretion induced by *Salmonella* infection may be severe, requiring aggressive supportive care. Profound intestinal inflammation can occur, leading to permanent dysfunction and overwhelming systemic inflammation, resulting in the death of the affected individual. Bacterial translocation can also occur, resulting in the spread of *Salmonella* organisms to the regional lymph nodes initially, with subsequent entry into the systemic circulation resulting in bacteraemia (Hollis *et al.* 2008).

### Clinical signs

The clinical signs of salmonellosis are variable and may include inapparent infections ('silent carriers') (Smith 1981) and a mild infection characterised by fever, mild abdominal pain, anorexia and depression without diarrhoea (Smith 1979). However, most horses that are clinically affected have moderate to severe, watery diarrhoea (Smith 1981) (**Fig 2**). Laminitis may be observed as a sequel to severe *Salmonella*-induced enterocolitis. Foals may develop haemorrhagic diarrhoea (rarely seen in adult horses), septicaemia, pneumonia, meningitis and lameness due to either septic arthritis or physitis. Small colon impactions in adult horses frequently have associated salmonellosis (**Fig 1**).

Most clinically affected horses have neutropenia, vacuolated neutrophils (toxic changes), hypochloraemia, hyponatraemia, elevated PCV and azotaemia. Acidosis will be present if the anion gap



**FIGURE 2:** Profuse diarrhoea associated with salmonellosis.

(lactate) is increased. Hypoproteinaemia generally occurs within a couple of days even in those horses without diarrhoea. A rebound neutrophilia may occur after the initial neutropenia. Coagulation abnormalities such as thrombocytopenia and low antithrombin III may occur in more severe cases resulting in colonic, pulmonary, and limb thrombosis.

In foals complete blood count (CBC), electrolyte, clinical chemistry, and coagulation markers are similar to those in the adult horses, although the number of band cells are often greater, and electrolyte abnormalities are generally more severe. Blood cultures, joint fluid, cerebrospinal fluid, or tracheal aspirates may be *Salmonella* positive in infected foals.

Abortion of mares can arise following infection by *Salmonella* serovar *abortus-equi*. Other clinical syndromes have also been associated with infection by this organism, including fistulous withers, orchitis, septicaemia and septic arthritis. Infection by this agent occurred in an endemic area in Japan (Akiba *et al.* 2003) and has occasionally been recorded in Europe in the past 20 years (Madic *et al.* 1997). Salmonellosis has also been associated with gastric dilation and ileus syndrome in adult horses (Merritt *et al.* 1982). Affected horses may present with fever and ileus with gastric reflux; *Salmonella* spp. may be isolated from the gastric reflux in these cases.

Chronic diarrhoea (i.e. diarrhoea that persists longer than 4 weeks) is not generally associated with salmonellosis (Smith *et al.* 1981). However, horses with chronic diarrhoea of other causes may shed *Salmonella* spp (Merritt 1994), and in some cases treatment with enrofloxacin may be beneficial (assuming that the underlying cause of the diarrhoea is also treated).

### Diagnosis

*Salmonella* is reported to be the most frequently diagnosed aetiological agent in equine infectious diarrhoea (Murray 1996). Thousands of serotypes of *Salmonella* have been identified, although the majority of equine cases of salmonellosis are typically associated with one of a few serotypes, including: *Salmonella enterica* ssp. *enterica* serovars *typhimurium*, *enteritidis*, *krefeld*, *saint-paul*, serovar *anatum*, *newport* and *infantis* (Hird *et al.* 1984; Benson *et al.* 1985; Carter *et al.* 1986; Donahue 1986;

Ikeda *et al.* 1986; Dargatz *et al.* 1990; Traub-Dargatz *et al.* 1990; Walker *et al.* 1991; van Duijkeren *et al.* 1994, 2002; Hartmann *et al.* 1996; Pare *et al.* 1996; Tillotson *et al.* 1997; Weese *et al.* 2001; Schott *et al.* 2001; Ernst *et al.* 2004). The most commonly implicated of these is *Salmonella enterica* ssp. *enterica* serovar typhimurium. Diagnostic testing for *Salmonella* organisms relies primarily on faecal culture, using selective enrichment media (selenite broth, tetrathionate broth, or Rappaport-Vassiliadis enrichment broth) to enhance the detection of *Salmonella* spp. by increasing the number of organisms, and selective isolation media (brilliant green agar, MacConkey agar or xylose lysine desoxycholate [XLD] agar) to decrease the interference of other enteric organisms in the isolation process. Suspected isolates should be cultured on lysine iron agar and triple sugar iron agar to aid in the differentiation of *Salmonella* colonies from other enteric bacteria. Once isolated in culture, *Salmonella* organisms should be further identified by means of standard biochemical techniques or using a biochemical identification kit (API 20E)<sup>1</sup>. All confirmed isolates should then be further characterised by means of antimicrobial sensitivity testing, serotyping and phage typing (Schott *et al.* 2001; van Duijkeren *et al.* 2002).

When performing faecal culture a minimum of 10 g of faecal material should be submitted (Larsen 1997). *Salmonella* organisms are more consistently shed in formed stool than in diarrhoeic stool (Larsen 1997), increasing the likelihood of isolating the organism in the early stages or as the animal recovers from clinical disease. The time required to isolate and identify *Salmonella* organisms from faecal samples using culture represents one of the primary limitations of this approach, as it may require 3–4 days to obtain a definitive result on any single faecal culture. In addition, faecal culture exhibits a low sensitivity for the detection of *Salmonella* shedders in the equine population, although the use of multiple cultures (5), combined with utilisation of selective media, allows for adequate sensitivity levels to be achieved (van Duijkeren *et al.* 1995). Culture of rectal mucosa with faecal material substantially increases the sensitivity of culture techniques (Palmer *et al.* 1985). Faecal culture remains the gold standard for clinical monitoring of equine patients, despite its limitations

and the recent development of more sensitive techniques, such as PCR.

Polymerase chain reaction tests are available for the detection of *Salmonella* spp. DNA in faeces, and these offer a more rapid turnaround time and higher sensitivity than culture techniques, but do not allow for further identification of the organisms or for antibacterial susceptibility testing (Cohen *et al.* 1996). The PCR techniques that have been developed for the detection of *Salmonella* DNA in equine faeces have been demonstrated to be both highly sensitive and specific (Amavisit *et al.* 2001; Ewart *et al.* 2001; Gentry-Weeks *et al.* 2002; Kurowski *et al.* 2002; Ward *et al.* 2005). The high sensitivity of these PCR techniques results from the ability of these assays to detect even a single DNA fragment containing the targeted DNA sequence. As a result, PCR testing can result in much higher numbers of positive results than culture techniques, as seen in one study where 40% of clinical faecal samples were positive on PCR testing, as compared to 2% positive results with culture (Amavisit *et al.* 2001). An even more dramatic example of this phenomenon was observed in a study that revealed that 17% of horses presented to the outpatient service of a veterinary teaching hospital were positive for *Salmonella* DNA on faecal PCR testing, yet none of these animals were culture positive, and 65% of hospitalised horses were PCR positive, while only 10% were culture positive (Cohen *et al.* 1996). An even greater disparity was found between PCR and culture techniques when analysing environmental samples, with 0.001% (1/783) of the samples positive on culture and 14% (110/783) of the samples positive on PCR testing (Ewart *et al.* 2001). A recent study reported that 75% of horses hospitalised for problems other than gastrointestinal disease were positive on serial PCR for *Salmonella* DNA, while only 9.5% were positive on serial faecal culture (Ward *et al.* 2005). The wide disparity between the results of culture and PCR techniques likely reflects the ability of the PCR techniques to detect DNA from nonviable (dead or inactivated) organisms in the faeces or the environment. This possibility was supported by the findings of Amavisit *et al.* (2001), who reported that the use of enrichment culture techniques did not increase the detectability of *Salmonella* from clinical faecal samples (Amavisit *et al.* 2001). On the basis of these results it is apparent that PCR techniques are overly sensitive for routine clinical application.

Further characterisation of *Salmonella* organisms cultured from clinical cases is important epidemiologically, both for the equine population and human populations potentially exposed to these organisms, and this can be achieved by means of serotyping and phage typing after the organism has been isolated using culture techniques. Phage typing has recently revealed the emergence of *Salmonella enterica* subspecies *enterica* serovar typhimurium definitive type (DT) 104 as an increasingly common animal pathogen (van Duijkeren *et al.* 2002; Weese *et al.* 2001). Equine salmonellosis due to DT104

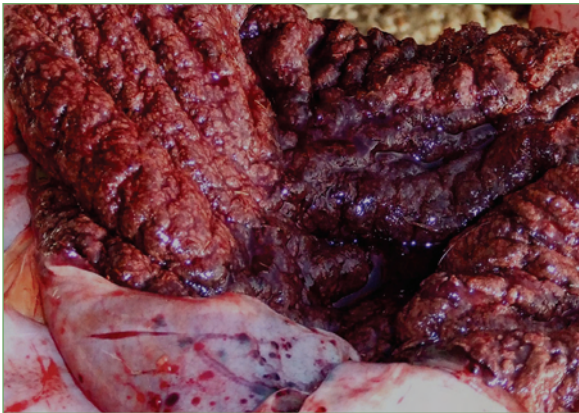
represents a serious concern, as the organism exhibits antimicrobial multiresistance and presents an increased risk of zoonosis (van Duijkeren *et al.* 2002; Weese *et al.* 2001). It has been recommended that the phage type distribution of *Salmonella* isolates should be monitored to ascertain if DT104 remains a common equine pathogen (Weese *et al.* 2001; van Duijkeren *et al.* 2002).

### Pathology

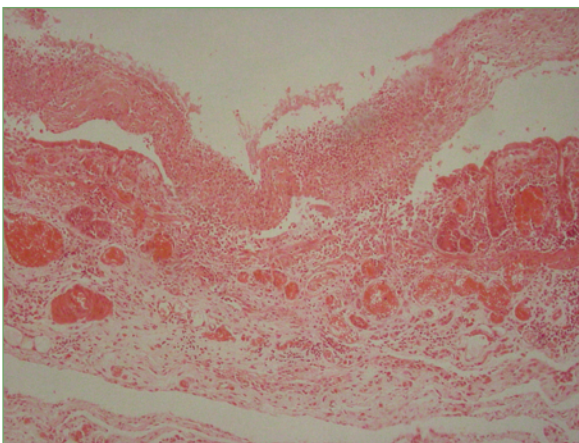
The gross pathological findings in horses with salmonellosis are those of enteritis and/or colitis. Typically, diffuse fibrinous or haemorrhagic inflammation of the caecum and large colon will be present. The mucosa may be ulcerated and there may be diphtheritic pseudomembranes adherent to the surface (**Fig 3**). Histologically, the caecum and colon show typhlitis/colitis with haemorrhage and coagulative necrosis (**Fig 4**). Fibrinocellular exudates may be attached to the necrotic epithelium. The capillaries of the *lamina propria* are frequently thrombosed. The mesenteric lymph nodes are typically swollen, haemorrhagic and oedematous (**Fig 5**). Small foci of hepatic necrosis ('paratyphoid nodules') may be observed in the liver.

### Treatment

The treatment of salmonellosis is primarily supportive in nature, as the pathogenic bacteria may not respond to specific therapy. Substantial losses of



**FIGURE 3:** Post mortem appearance of salmonellosis. Severe colitis with extensive diphtheritic pseudomembranes over the mucosal surface.



**FIGURE 4:** Salmonellosis – photomicrograph of the large colon. An ulcer with overlying diphtheritic membrane is present. The mucosa is congested. Haematoxylin and eosin.



**FIGURE 5:** Post mortem appearance of salmonellosis. Oedematous and haemorrhagic colonic lymph nodes.

fluid from the circulating volume necessitate supportive fluid therapy in most cases, and accompanying losses of protein may also necessitate colloid therapy. Electrolyte derangements are often present, requiring that supplementation be provided either enterally or parenterally. Anti-inflammatory therapy is indicated in many of these conditions in order to address both the local and systemic components of the inflammatory response. Decreased voluntary feed intake or forced withholding of feed may necessitate nutritional support. Antimicrobial therapy may be indicated in some cases. The management of these cases can be quite intensive and is difficult to perform outside of a hospital environment.

### Fluid therapy

Horses with salmonellosis typically present with dehydration secondary to fluid losses in the form of diarrhoea alone or in combination with decreased voluntary fluid intake. The correction of dehydration requires fluid replacement therapy, as these patients are often unable to correct their fluid status by voluntary intake. The fluid therapy plan should address both the correction of existing deficits, and the provision of fluids to replace ongoing losses and provide for basal metabolic requirements. In most cases fluid replacement is best accomplished via the parenteral route, as this allows for the rapid administration of large volumes of fluid in acute cases, and also allows for the ready correction of any electrolyte deficits. Colloid therapy may also be indicated, as hypoproteinaemia can develop due to the loss of protein into the lumen of the intestine. Colloid administration is accomplished by the use of either equine plasma or a synthetic colloid such as hydroxyethyl starch (Hetastarch)<sup>2</sup>. Equine plasma is typically administered at doses ranging from 10–20 ml/kg bwt, with the therapeutic goal of correcting the hypoalbuminaemia. Repeated dosing may be required due to the severity of the presenting hypoalbuminaemia and ongoing losses due to the underlying enteropathy. The use of hydroxyethyl starches can be beneficial in providing additional colloidal support and may have a more prolonged duration of action, but care must be taken to avoid overdosage due to the possibility of haemorrhagic dysfunction. The recommended dosage range for

Hetastarch is typically 5–10 ml/kg bwt, but cumulative doses should not exceed a total of 20 ml/kg bwt. The use of hydroxyethyl starches will result in lowering of the measured total protein and albumin concentrations in the patient's serum due to dilution, which renders these values inaccurate as representations of colloid oncotic pressure. This requires that treatment be directed toward resolution of the clinical signs of hypoproteinaemia, rather than correction of the hypoproteinaemia itself.

Enteral fluid therapy has been proposed for cases of colitis, as small intestinal function is typically normal in these cases (Ecke *et al.* 1997; Schott 1998; Lopes *et al.* 2003). The enteral route is intrinsically more physiological, and has the additional advantages of reduced cost and simplicity (Lopes *et al.* 2003). Oral rehydration solutions are widely used in the treatment of human patients with diarrhoea, and the reported outcomes with oral rehydration are equivalent or superior to those reported with i.v. fluid therapy (Atherly-John *et al.* 2002; Nager and Wang 2002). The enteral route of administration can be used successfully in the treatment of horses with mild colitis, but more severely affected patients are usually unable to tolerate the administration of the volumes of fluids required to correct their deficits and replace their ongoing losses, and may exhibit increased discomfort, abdominal distension or even develop enterogastric reflux (Ecke *et al.* 1998; Lopes *et al.* 2003). Administration of enteral fluids in mild cases is easily accomplished using a large bore stomach tube or a smaller indwelling enteral feeding tube<sup>3</sup>. Enteral fluid solutions are easily prepared using tap water, and an isotonic solution can be formulated by combining 5 l of water with 1.5 tablespoons (28 g) of table salt, 0.5 teaspoons (3 g) of Lite salt<sup>4</sup> and 1.5 tablespoons (17 g) of baking soda (NaHCO<sub>3</sub>) (Lopes *et al.* 2003). Enteral fluids prepared as above are recommended to be administered as repeated bolus doses or as a continuous infusion at rates of up to 6–8 l/h (Ecke *et al.* 1997; Lopes *et al.* 2003). The authors' experience, however, suggests that such aggressive rates of administration can result in substantial worsening of diarrhoea and abdominal discomfort and should be avoided. Preferably the enteral fluids should be administered as smaller bolus doses or as a continuous rate infusion at a rate of 3–6 l/h (Lopes *et al.* 2003).

### Anti-inflammatory therapy

Anti-inflammatory therapy is important in the management of salmonellosis, primarily for the control of abdominal discomfort that can be present early in the disease process, but it is also indicated for the control of the systemic inflammatory response that accompanies this disease process. The most commonly utilised anti-inflammatory drug is flunixin meglumine, which is a potent visceral analgesic with a well-demonstrated ability to suppress abdominal pain in equine gastrointestinal diseases when administered at 1.1 mg/kg bwt *per os* or *i.v.* (Clark and Clark 1999). In addition, flunixin meglumine has been shown to have some 'anti-endotoxaemic' effects, as it suppress the systemic response to endotoxin when given as a pretreatment at doses as low as 0.25 mg/kg bwt, thereby minimising the severity of endotoxaemia-associated hypotension, hypovolaemia, haemoconcentration, pulmonary hypertension, tachypnoea, tachycardia and lactic acidosis (Bottoms *et al.* 1981; Dunkle *et al.* 1985; Ewert *et al.* 1985; Templeton *et al.* 1987). Additionally, flunixin meglumine has been shown to reduce the development of ileus following endotoxin exposure (King and Gerring 1989). It appears that nonsteroidal anti-inflammatory drug therapy may also provide a useful anti-secretory effect in salmonellosis by inhibiting the increased production of prostaglandin E<sub>2</sub> that accompanies *Salmonella* infection and which appears to be responsible, in part, for epithelial hypersecretion (Bertelsen *et al.* 2003). There is some evidence that nonsteroidal anti-inflammatory drug administration may impair the recovery of barrier function in equine intestinal mucosa, but this does not appear to be associated with increased absorption of LPS from the intestinal lumen *in vitro* (Tomlinson and Blikslager 2004, 2005).

### Anti-endotoxin therapies

As bacterial endotoxin has been shown to play an important role in the development of severe systemic inflammation (endotoxaemia) associated with gastrointestinal disease there has been significant interest in finding ways to inhibit the activity of endotoxin in the systemic circulation. Two basic approaches have been utilised in the attempt to neutralise endotoxin: the administration of anti-endotoxin antibodies and the use of chemical

substances that bind to endotoxin (Moore and Barton 2003). The development of antibodies to bacterial endotoxin has been challenging due to the antigenic variation of endotoxin between species of Gram-negative bacteria, and for this reason antibodies have been targeted against the more conserved core and lipid A regions of the endotoxin molecule (Moore and Barton 2003). Studies regarding the efficacy of anti-endotoxin antibodies in experimental equine endotoxaemia, and in horses presenting with colic, have also yielded conflicting results, leading to uncertainty regarding the clinical application of this type of therapy (Morris *et al.* 1986; Garner *et al.* 1988; Spier *et al.* 1989; Durando *et al.* 1994). Furthermore, worsened clinical signs of endotoxaemia and increased systemic inflammation associated with the administration of anti-endotoxin antiserum in a foal model of endotoxaemia has been reported (Durando *et al.* 1994). Serum and plasma products containing anti-endotoxin antibodies are commercially available for use in the horse and are widely used, but the uncertainty from published reports regarding this therapy needs to be resolved before specific recommendations can be made.

The use of the anti-endotoxin agent polymyxin B has been extensively examined in a variety of animal species and in man, and there is good evidence that this substance binds endotoxin and prevents it from initiating or potentiating the systemic inflammatory response. Polymyxin B has been examined in several equine endotoxaemia models. It has been demonstrated to decrease the severity of both the clinical signs of endotoxaemia and the severity of the systemic inflammatory response, even when administered before or after endotoxin exposure, although the best effects were associated with pretreatment (Durando *et al.* 1994; Barton 2000; Parviainen *et al.* 2001; Barton *et al.* 2004). The current recommendation for the clinical use of polymyxin B is to initiate therapy as early as possible using a dosage of 6000 iu/kg bwt (1 mg/kg bwt) diluted in 1 l of 5% dextrose given *i.v.* over 15 min every 8 h (Morresey and Mackay 2006). Due to the potential for nephrotoxicity it is recommended that horses administered this drug have adequate hydration and that serum creatinine be monitored (Moore and Barton 2003). Prolonged administration should also be avoided to minimise the risk of nephrotoxicity, and a maximum of 3–5 doses should be administered.

### Nutritional support

Horses suffering from salmonellosis should ideally have free access to roughage and supplemental feeding *ad libitum* with concentrates to meet at least their maintenance metabolic energy requirements (roughly 67 MJ for a 500 kg horse) (Magdesian 2003). Unfortunately, some horses suffering from salmonellosis may be anorexic due to the illness, impairing the patient's ability to meet their metabolic needs through voluntary intake. Most adult horses can reasonably be maintained without nutritional support for several days, as they will mobilise their endogenous energy reserves (fat, muscle) to meet their metabolic needs. However, some horses and ponies appear predisposed to excessive fat mobilisation and they should not be maintained without nutritional support due to the risks of hypertriglyceridaemia and hyperlipaemia (Dunkel and McKenzie 2003). Nutritional supplementation is most readily accomplished using the parenteral route as these patients are typically already receiving i.v. fluid therapy. Parenteral nutrition can be characterised as partial or complete, based upon whether or not it meets the animal's entire nutritional needs. Total parenteral nutrition requires the use of both carbohydrate and lipid energy sources, in combination with amino acids and strives to supply all of the patient's nutritional requirements. This degree of support is rarely required in adult patients, where partial caloric supplementation is adequate for short term support. Partial parenteral nutrition can be accomplished with carbohydrate or carbohydrate/amino acid solutions. Supplementation of the i.v. fluids with dextrose at a moderate rate of 21–42 kJ/kg bwt/day (1.5–3 l of 50% dextrose per day) appears to be beneficial in clinically ill horses with decreased or absent appetite as it minimises the degree of fat mobilisation secondary to a negative energy balance, and has been shown to correct hypertriglyceridaemia (Dunkel and McKenzie 2003; Magdesian 2003). If the patient requires support beyond a few days then amino acid supplementation should be provided.

### Probiotics/prebiotics

Restoration of the microbial flora of the gastrointestinal tract has been shown in many species to aid in the resolution of colitis and this is most readily accomplished by the administration of live

beneficial enteric organisms. These organisms are termed probiotics, which have been defined as live microbial feed supplements that are beneficial to health (Fooks and Gibson 2002). A more recent, broader concept is that of 'biotherapeutic agents', which have been defined as living microorganisms used either to prevent or to treat diseases by interacting with the natural microecology of the host (Elmer and McFarland 2001). Much of the research regarding probiotics has been performed in other species, and the types of organism used in equine probiotics are generally the same as have been administered to human patients. As a result it is not clear that the organisms present in many equine probiotics (*Lactobacillus*, *Bifidobacterium*, *Enterococcus*) are necessarily the most relevant to the equine gastrointestinal flora (Weese *et al.* 2004). The fact that probiotics are marketed as feed supplements also means that there is no requirement regarding the demonstration of efficacy of these products, therefore any label claims of efficacy should be viewed with caution. This concern is reinforced by the disappointing results of the few trials that have looked at the effects of probiotics in equine salmonellosis (van Duijkeren *et al.* 1995; Parraga *et al.* 1997; Kim *et al.* 2001) and foal diarrhoea (Weese and Rousseau 2005). The yeast *S. boulardii* has been shown to be beneficial in equine clostridial enterocolitis (Desrochers *et al.* 2005), and could prove useful in the treatment of salmonellosis as it has been reported to have beneficial effects in an experimental salmonellosis model (Czerucka and Rampal 2002). Further work is clearly required in order to better define the types of probiotic organisms most likely to be beneficial in equine colitis.

An alternative means of restoring the normal gastrointestinal flora is the provision of nondigestible oligosaccharides as a 'prebiotic'. The concept behind prebiotics is that of an insoluble fibre that selects for, and stimulates the growth of, beneficial microorganisms in the large intestine that can alter the microbiota to a healthy composition and exert beneficial effects on the host (Bengmark 2001). The substance most studied as a prebiotic is germinated barley feedstuff (GBF), which is generated by the brewing industry as a by-product of the brewing process. GBF has been shown to have anti-inflammatory effects in animal models of colitis, with one study reporting decreased gastrointestinal and

systemic inflammation as well as decreased mucosal injury in association with increased levels of the beneficial short-chain fatty acid butyrate (Kanauchi *et al.* 2008). A similar study demonstrated a superior effect of GBF as compared to a probiotic consisting of *Lactobacillus* and *Cl. butyricum* organisms that demonstrated no effect (Fukuda *et al.* 2002). Dried GBF is widely used in dairy cattle feeds and is a component of some commercial horse feeds and appears to be a safe feed supplement, although no specific reports are available regarding GBF administration in the horse. The first author has utilised fresh and frozen GBF in horses with colitis at an empirical dosage rate of 0.2–0.4 kg 3–4 times daily, with some encouraging clinical results. Further work is required, however, to demonstrate efficacy of this treatment in equine enterocolitis and to determine the most appropriate dosage of GBF for feeding to horses with diarrhoea.

### Gastrointestinal protectants and adsorbents

An additional means of limiting gastrointestinal inflammation is the administration of products by the enteral route, which may exert anti-inflammatory effects on the mucosa or that impair the activity of the enteric pathogens or their toxins (Tillotson and Traub-Dargatz 2003). Bismuth subsalicylate has been used as an agent to protect the gastrointestinal mucosa and decrease mucosal inflammation, but there is not much evidence that it has a significant effect in secretory diarrhoea in any species (Aranda-Michel and Giannella 1999; Zaman *et al.* 2001). This compound has been reported to stimulate intestinal sodium and water absorption and to have anti-inflammatory and antibacterial effects, including direct binding of bacterial toxins (Aranda-Michel and Giannella 1999). Bismuth subsalicylate is widely regarded as a safe over-the-counter human antidiarrhoeal agent, but there are reports of toxicity associated with overdosage (Vernace *et al.* 1994; Gordon *et al.* 1995). Recommended dosages for bismuth subsalicylate in the horse range from 0.5–4 ml/kg bwt every 4–6 h (Tillotson and Traub-Dargatz 2003).

Recent work has examined the possible application of the adsorptive substance di-tri-octahedral smectite (DTO smectite; Biosponge<sup>5</sup>) in enterocolitis. This product and the related

dioctohedral smectite, have been shown to bind *Clostridium difficile* toxins A and B, and *Cl. perfringens* enterotoxins *in vitro* (Martirosian *et al.* 1998; Weese *et al.* 2003). These compounds are thought to act by several mechanisms, including direct binding of bacterial toxins, direct adsorption of bacteria, modification of the gastrointestinal mucus to inhibit toxin absorption and repair of mucosal integrity (Gonzalez *et al.* 2004). A recent study utilising an experimental model of inflammatory colitis in rats has demonstrated that this type of compound may also have direct anti-inflammatory effects within the intestinal mucosa (Gonzalez *et al.* 2004). A small study reported that outcome was substantially improved in horses suffering from clostridial enterocolitis with the use of DTO smectite (Herthel 2000). The recommended dosage is 1.4 kg of powder in water via nasogastric tube, followed by 0.4 kg every 4–6 h. While the indication for using this product in the horse suffering from salmonellosis is less clear than in the case of clostridial colitis, clinical experience suggests that this product may be of some benefit in salmonellosis.

### Antimicrobial therapy

The role of antimicrobial therapy in the treatment of salmonellosis is controversial, due to concerns regarding lack of efficacy and the potential development of antimicrobial resistance (Frye and Fedorka-Cray 2007; Vo *et al.* 2007). Often, antimicrobial therapy is used in patients suffering from gastrointestinal disease due to the presence of fever and leucopenia, which may indicate the presence of bacterial infection or may result from the effects of bacterial toxins such as endotoxin. There are additional concerns that severe gastrointestinal disease may be associated with impairment of the barrier function of the gastrointestinal mucosa, resulting in an increased risk of bacterial invasion leading to localised infection or septicaemia and infections distant to the intestine (pneumonia, endocarditis, meningitis etc.). The efficacy of systemic antimicrobial therapy in the prevention of bacterial invasion in gastrointestinal disease is not established (Koratzanis *et al.* 2002).

Antimicrobial resistance is common in the *Salmonella* organisms associated with enterocolitis, especially to the beta-lactams, tetracyclines,

trimethoprim, and the sulpha drugs (van Duijkeren *et al.* 2002; Randall *et al.* 2004). The intracellular localisation of *Salmonella* organisms limits their susceptibility to antimicrobials that exhibit a limited ability to penetrate the cell wall, such as the aminoglycosides, which decreases the utility of these drugs, even though many isolates are sensitive to amikacin. Increased *in vivo* susceptibility is seen to those antimicrobials that are able to reach therapeutic levels intracellularly, such as the fluoroquinolones, and these drugs are widely used in human salmonellosis patients (van Duijkeren and Houwers 2000). Cephalosporins are also frequently used in human salmonellosis patients, and the third generation cephalosporin ceftiofur has been reported to be effective in the treatment of calves with salmonellosis (Fecteau *et al.* 2003). Many equine and other domestic animal *Salmonella* isolates are reported to be sensitive to ceftiofur and the fluoroquinolones (Seyfarth *et al.* 1997; van Duijkeren *et al.* 2002), although ceftiofur resistance does appear to be increasing (Frye and Fedorka-Cray 2007). Multi-drug resistant strains from several equine nosocomial outbreaks have been reported to be sensitive to ciprofloxacin, which is the active metabolite of enrofloxacin (Dargatz and Traub-Dargatz 2004).

While the treatment of equine patients suffering from salmonellosis with appropriate antimicrobials is controversial it should be considered as it may result in an improved chance of survival. Given the presence of multiresistant strains of *Salmonella* it is important that one determines the antimicrobial sensitivity pattern of any equine isolates and utilise this as a guide to ongoing therapy in the individual patient or concurrently affected individuals. Based upon the available data, empirical treatment with enrofloxacin could represent a reasonable initial approach in the severely affected patient while sensitivity results are pending. Enrofloxacin is the most commonly used fluoroquinolone in the horse and it has a relatively broad spectrum, with excellent activity against Gram-negative organisms. Enrofloxacin is a concentration dependent antimicrobial, and exhibits peak concentration-dependent bactericidal effects with prolonged post antibiotic effects. As a result it can be given at relatively high doses at a decreased frequency. Toxicity is primarily due to adverse effects on cartilage maturation, resulting in a contraindication to its use in growing animals

(Beluche *et al.* 1999; Egerbacher *et al.* 2001). Enrofloxacin is administered at 7.5 mg/kg bwt once daily orally or 5 mg/kg bwt once daily i.v. (Giguere *et al.* 1996; Kaartinen *et al.* 1997).

## Control

The shedding of *Salmonella* organisms into the environment from horses as well as domestic and wild animals in the vicinity of the facility cannot be entirely prevented; therefore there is always a risk of exposure. As a result, the control of *Salmonella* infections is dependent upon the utilisation of effective biosecurity measures designed to minimise the risk of infection in susceptible individuals and biocontainment procedures to minimise the spread of disease when infection does occur. Segregation of horses likely to shed *Salmonella* organisms, such as those having suffered from intestinal impactions or having undergone colic surgery, can help to reduce the risk of exposure for susceptible individuals in the hospital population. Isolation of animals that develop diarrhoea and/or fever and leucopenia represents a first step in biocontainment, and can be accomplished using barrier procedures within the hospital ward or preferably by moving the individual to a separate housing facility used solely for this purpose. Barrier procedures must be tailored to suit the individual facility but include the wearing of gloves, gowns and boots when working with the affected individual, as well as using foot baths and hand washing and disinfection (Weese 2004). Manure from suspect or confirmed cases should be handled separately from the rest of the facilities waste stream and should never be spread on pastures. Faecal samples for *Salmonella* culture should be collected at the time the animal is isolated, both for surveillance and for the optimisation of patient therapy. Serial cultures should be performed in order to ensure that 3–5 cultures are negative for *Salmonella* prior to removing an animal from isolation and returning them to the hospital or farm population. The stall and any other potentially contaminated surfaces must be thoroughly cleaned and disinfected, and it is recommended that the surfaces be cultured prior to reuse in order to ensure that disinfection has been effective. When used after cleaning to remove organic debris, sodium hypochlorite is an effective disinfectant and is widely used to good effect.

## Outcome

Due to the severity of the local and systemic inflammatory responses induced by salmonellosis, the prognosis for survival is somewhat guarded in most cases. It is possible that the prognosis may be improved with aggressive supportive care and specific therapy, but this is difficult to predict given the variability of these organisms with regards to virulence and resistance to antimicrobials. Due to the widespread shedding of *Salmonella* organisms by clinically normal horses and the increased susceptibility to infection in hospitalised horses, surveillance and infection control will remain the mainstays for controlling equine salmonellosis.

## Public health risk

Salmonellosis is an important zoonotic disease that is considered to be responsible for more than one million human cases of diarrhoea, 15,000 hospitalisations and 400 deaths annually in the USA (Voetsch *et al.* 2004). Most cases of human infection arise from food-borne exposure, including contamination of horsemeat in parts of the world where horsemeat is used for human consumption (Espie and Weill 2003). Direct contact with infected horses is also an important risk factor for zoonotic transmission (Anon 2001). The emergence of multi-drug resistant strains, such as multidrug resistant *Salmonella* serovar Newport, causes particular concern about direct transmission between infected animals and their owners and attending veterinary staff (Traub-Dargatz and Besser 2007).

## Manufacturers' addresses

<sup>1</sup>bioMerieux, Hazelwood, Missouri, USA.

<sup>2</sup>Hospira, Lake Forest, Illinois, USA.

<sup>3</sup>MILA international Inc., Erlanger, Kentucky, USA.

<sup>4</sup>Morton Salt, Chicago, Illinois, USA.

<sup>5</sup>Platinum Performance, Los Olivos, California, USA.

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