Complications and prognostic factors in equine surgical colic

Sara Torfs

COMPLICATIONS AND PROGNOSTIC FACTORS IN EQUINE SURGICAL COLIC

Sara Torfs

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Promoter

Prof. Dr. P. Deprez

Co-promoters

Prof. Dr. C. Delesalle

Prof. Dr. G. van Loon

Department of Large Animal Internal Medicine and Clinical Biology

Salisburylaan 133, B-9820 Merelbeke

THE ONLY TRUE WISDOM IS KNOWING YOU KNOW NOTHING

SOCRATES

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LIST OF ABBREVIATIONS

5-HT	5-hydroxytryptamine
5-HT-d4	deuterated serotonin
BID	bis in die (twice a day)
β-TG	β-thromboglobulin
CFU	colony forming units
CI	confidence interval
CNS	central nervous system
COX	cyclooxygenase
CRI	constant rate infusion
CRT	capillary refill time
DIC	disseminated intravascular coagulation
EC	enterochromaffin
EDTA	ethylenediamine tetra-acetic acid
ELISA	enzyme linked immunosorbent assay
ESBL	extended spectrum β-lactamase
GI	gastrointestinal
HPLC	high-performance liquid chromatography
IM	intramuscular
IV	intravenous
LC-MS/MS	liquid chromatography tandem mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
LPS	lipopolysaccharide
MAP	mean arterial pressure
MRSA	methicillin resistant *Staphylococcus aureus*
MMC	migrating myoelectric complex *or* migrating motility complex
NF	nuclear factor
NSAID	non-steroidal anti-inflammatory drug
NO	nitric oxide
OD	optical density
OR	odds ratio
PCV	packed cell volume
PF4	platelet factor 4
PaO_2	partial arterial oxygen tension
POI	postoperative ileus
PPP	platelet poor plasma
PRP	platelet rich plasma
QID	quater in die (4 times a day)
RIA	radioimmunoassay
ROC	receiver operating characteristic
RSD	relative standard deviation
SERT	serotonin transporter
SI	small intestinal
S/N	signal-to-noise
SSI	surgical site infection
VC	vasoconstriction
VIP	vasoactive intestinal peptide

PREFACE

Colic or abdominal pain is still an important and feared cause of morbidity in the horse. Even though the majority of cases recover spontaneously or with medical treatment, a small percentage of equine colic cases require abdominal surgery. Although colic surgery has nowadays become a routine procedure, horses seem to be more susceptible to postoperative complications, in comparison to other species. These complications may vary from minor problems that mainly prolong the hospitalization period and increase the associated costs, to major problems demanding repeated surgery or leading to death of the horse.

This PhD thesis explores two important complications that veterinarians are confronted with after colic surgery. The first, postoperative ileus, is a highly fatal condition that is mostly encountered after surgery for a small intestinal problem. A risk factor analysis was performed and treatment options evaluated. Plasma changes of serotonin, an important mediator of intestinal motility, were also examined. The second complication, incisional wound infection, usually develops much later in the postoperative period. Different factors were analyzed that could influence its occurrence.

CHAPTER 1:

GENERAL INTRODUCTION ON EQUINE SURGICAL COLIC AND ASSOCIATED COMPLICATIONS

1.1. EQUINE SURGICAL COLIC

Colic or visceral abdominal pain is one of the most notorious diseases in the horse, feared by many horse owners. It accounts for almost one third of the veterinary exams (Knubben et al., 2008) and for 20 to 30 % of all deaths in horses (Leblond et al. 2000; Tinker et al. 1997), which equals to 0.24 to 0.7 colic deaths per 100 horses per year (Egenvall et al. 2008; Hillyer et al. 2001; Kaneene et al. 1997, Tinker et al. 1997). The term colic refers to abdominal pain originating from the gastrointestinal (GI) tract (true colic) or from other abdominal structures such as the urinary system, the reproductive system and the liver (false colic). This thesis however, focusses on colic originating specifically from the GI system.

The incidence of colic varies among regions and is influenced by several management and individual horse factors. Reported incidences vary between approximately 1 and 19 colic events per 100 horses per year (Egenvall et al. 2008; Hillyer et al. 2001; Kaneene et al. 1997; Mehdi and Mohammad 2006; Tinker et al. 1997; Traub-Dargatz et al. 2001; Uhlinger 1992). Although most cases are mild and resolve spontaneously or with limited medical care, there is a number of severe cases that require extensive treatment and may lead to death of the horse. Overall, the case fatality rate in colic horses is estimated at 6.7 to 15.6% (Kaneene et al. 1997; Traub-Dargatz et al. 2001; Tinker et al. 1997). The reported long-term fatality is even higher, about 25-30% (Egenvall et al. 2008; Van der Linden et al. 2003).

Compared to the high number of fatalities associated with colic, only a small percentage of colic events results into surgery. A large study monitoring 21,820 horses in the United States reported surgical treatment in only 1.4% of cases (Traub-Dargatz et al. 2001). Other studies report somewhat higher percentages, up to 6.3% surgical cases (Cohen et al. 1999; Tinker et al. 1997). It can be concluded that, even though an exploratory laparotomy may be lifesaving, an important number of severe colic cases does not receive surgical treatment. In some cases, this could be explained by the type of colic. For certain etiologies, e.g. equine grass sickness or inflammatory conditions such as colitis, typhlitis or duodenitis-proximal jejunitis, surgery is not

indicated. Other horses might not be suitable candidates for surgery because of the progress of their illness. Horses with symptoms of endotoxemia and severe cardiovascular compromise, such as tachycardia, increased packed cell volume (PCV) and congested or cyanotic mucous membranes, have increased risk of intra- or postoperative mortality (Ihler et al. 2004; Proudman et al. 2006). Finally, there is a group of horses that are in theory suitable candidates for surgery. In this group, the horse owner will make a decision for or against surgery based upon both economic and sentimental grounds. Apart from the value of the horse, the expected costs of surgery and postoperative care and the estimated prognosis are often decisive. Optimizing these factors can facilitate the owner's choice for a potentially lifesaving laparotomy. Although vast improvements in both surgical and postoperative care have led to a clear increase in survival rates during the last decades, colic surgery is still associated with a high complication rate (Hackett and Hassel, 2009; Klohnen 2009; Mair and Smith 2005a; Proudman et al. 2002). Postoperative complications can have a huge impact on both treatment costs and survival rates. Different types of complications and their impact on patient recovery are discussed below.

1.2. COMPLICATIONS

The complications of colic surgery can be divided into intraoperative and postoperative complications. Intraoperative complications can relate to general anesthesia and recovery, abdominal distention, hemorrhage, mesenteric tearing or intestinal rupture (Dukti and White 2009). This thesis however, focusses on the complications that develop in the postoperative period after recovery from anesthesia.

These complications may result from the surgery or from the colic itself. The consequences vary from a need for increased medical care or a prolonged hospitalization period to relaparotomy or death of the horse. The most important postoperative complications, reported in over 10% of horses, are postoperative colic or pain, surgical site infection, postoperative ileus and endotoxemic shock. After

surgery for a small intestinal problem, ileus or postoperative pain develop even more frequently, in up to 50% of the horses. Jugular thrombophlebitis, peritonitis, colitis/diarrhea, hyperlipemia, laminitis, hemoperitoneum and pneumonia are less consistently reported. Long-term complications, observed after discharge from the clinic, are mainly colic and ventral hernia formation. An overview of reported incidences of the different complications is presented in Table 1 and 2.

Table 1. Incidence of postoperative ileus and surgical site infections after equine colic surgery.

Complication	Reference	Colic type	Incidence (%)
Postoperative ileus	Hunt et al. 1986	SI	14
	MacDonald et al. 1989	SI resection	16
	Van der Velden and Klein 1993	SI	27
	Blikslager et al. 1994	All	21
	Vachon and Fischer 1995	SI strangulation	16
	Freeman et al. 2000	SI	10
	Roussel et al. 2001	All	18
	Morton and Blikslager 2002	SI resection	47
	Proudman et al. 2002	All	10
	Semevolos et al. 2002	SI resection	53
	Cohen et al. 2004	All	19
	Cohen et al. 2004	SI	50
	Mair and Smith 2005a	All	18
	Mair and Smith 2005a	SI	34
	Garcia-Seco et al. 2005	SI strangulation	34
	Holcombe et al. 2009	SI	27
Surgical site infection	Kobluk et al. 1989	All	29
	Phillips and Walmsley 1993	All	37
	Honnas and Cohen 1997	All	25
	Ingle-Fehr et al. 1997	All	24
	Galuppo et al. 1999	All	26
	Freeman et al. 2000	SI	7
	Van den Boom, Van der Velden 2001	SI strangulation	14
	Morton and Blikslager 2002	SI resection	37
	Proudman et al. 2002	All	16
	Cohen et al. 2004	All	19
	Mair and Smith 2005a	All	29
	Smith et al. 2007	All	25
	Freeman et al. 2012	All	20

SI = small intestinal

Table 2. Incidence of other complications after equine colic surgery.

Complication	Reference	Colic type	Incidence (%)
Postoperative pain	Van den Boom, Van der Velden 2001	SI strangulation	43
	Morton and Blikslager 2002	SI resection	61
	Proudman et al. 2002	All	32
	Van der Linden et al. 2003	All	31
	Mair and Smith 2005a[1]	All	32
	Mair and Smith 2005b[2]	All	35
Endotoxemic shock	Morton and Blikslager 2002[3]	SI resection	64
	Mair and Smith 2005a	All	14
Thrombophlebitis	Freeman et al. 2000	SI	1
	Van den Boom, Van der Velden 2001	SI strangulation	4
	Morton and Blikslager 2002	SI resection	13
	Proudman et al. 2002	All	10
	Mair and Smith 2005a	All	8
	Klohnen et al. 2009	All	2
Peritonitis	Phillips and Walmsley 1993	All	6
	Morton and Blikslager 2002	SI resection	11
	Mair and Smith 2005a	All	3
Diarrhea	Honnas and Cohen 1997	All	53
	Freeman et al. 2000	SI	3
	Morton and Blikslager 2002	SI resection	25
	Proudman et al. 2002	All	4
	Cohen et al. 2004	All	30
	Mair and Smith 2005a	All	3
Hyperlipemia	Mair and Smith 2005a	All	0.9
Laminitis	Morton and Blikslager 2002	SI resection	12
	Proudman et al. 2002	All	1
	Cohen et al. 2004	All	6
	Mair and Smith 2005a	All	0.4
	De la Rebière de Pouyade 2009	All	4
Hemorrhage	Freeman et al. 2000	SI	3
	Doyle et al. 2003	enterotomy/anastomosis	1.3
Pneumonia	Klohnen et al. 2009	All	0.5

SI = small intestinal; [1]Short-term; [2]Long-term; [3]Signs of endotoxemia

In this chapter all complications mentioned above will be discussed. Ileus and incisional complications, both widespread and with important prognostic or economic consequences, will be reviewed more extensively.

1.2.1. POSTOPERATIVE PAIN

Postoperative pain or colic is a very common complication, reported in about 30% of horses (Mair and Smith 2005a; Proudman et al. 2002). Pain developing shortly after surgery and improving over time and with analgesic medication is typically surgical or incisional pain. However, severe pain symptoms immediately postoperatively may indicate a recurrent, unresolved or new important GI problem that requires a repeat laparotomy (Hackett and Hassel 2009). Certain surgical procedures may involve specific problems. Intestinal resection and anastomosis may lead to an obstruction at the anastomosis site or ischemia adjacent to it. Anastomoses may also facilitate the development of intussusceptions (Dukti and White 2009; Mair and Smith 2005c). Internal herniation with intestinal strangulation may occur in apertures created during intestinal resection and anastomosis, such as defects in the recently closed mesentery after a jejunojejunostomy or the ileocecal fold created during a jejunocecostomy (Dukti and White 2009).

Colic events occurring after a period of hospitalization may be transitory and idiopathic, but might also be caused by mechanical or functional (ileus) intestinal obstruction or peritonitis, related or unrelated to the preceding surgery. Extensive examination including a clinical examination, rectal palpation, abdominal ultrasonography, blood work and diagnostic analgesic therapy can help the clinician to make a distinction. The treatment may be medical or, in case of severe mechanical problems or ongoing pain, surgical (Hackett and Hassel, 2009; Mair and Smith 2005a).

Colic symptoms may also display as a long-term complication. About 30% of all horses discharged from the clinic after colic surgery, suffer from sporadic or recurrent colic episodes (Mair and Smith 2005b; Proudman et al. 2002). Most episodes occur in the first months after surgery, but they are reported until up to a year postoperatively (Proudman et al. 2002). Surgery for a small intestinal problem, particularly an intestinal resection, puts horses at higher risk. It is likely that intra-abdominal adhesion formation accounts for a number of the recurrent colic cases.

However, in many cases the exact cause is never determined (Mair and Smith 2005b).

1.2.2. ENDOTOXEMIC SHOCK

Horses with severe colic, especially in case of ischemic intestinal lesions, are prone to endotoxemia (Senior et al. 2011). Even in the intestines of healthy horses, large populations of Gram-negative bacteria containing endotoxin (lipopolysaccharide; LPS) in their cell walls can be found. Colic associated intestinal stasis, ischemia or inflammation allow a massive proliferation of these bacteria. A concurrent loss of mucosal barrier integrity permits the migration of bacteria and endotoxin into the circulation (Werners et al. 2005). In sufficient quantities, endotoxin contributes to the systemic activation of the inflammatory cascade and may eventually result in circulatory shock. Endotoxemia is an important etiologic factor of the state of hypercoagulability and even fulminant disseminated intravascular coagulation (DIC) observed in severe colic cases (Dallap et al. 2004; Monreal et al. 2000).

Severe endotoxemic shock after colic surgery has been reported in 13.9% (Mair and Smith 2005a) of horses. Horses that are presented with signs of endotoxemia before surgery are predisposed, as well as horses with small intestinal lesions (Morton and Blikslager 2002). Endotoxemic shock can lead to organ damage and death (Werners et al. 2005). It is also associated with the occurrence of postoperative ileus (King and Gerring 1991; Mair and Smith 2005a; Morton and Blikslager 2002) and other complications like laminitis (Parsons et al. 2011; Werners et al. 2005). Although new therapeutic strategies for endotoxemia are currently being developed and tested in human medicine, treatment in horses still consists of conventional anti-inflammatory and supportive therapy, intensive fluid therapy in particular (Werners et al. 2005). Early detection and treatment of subclinical coagulopathies can help to prevent the development of fulminant DIC (Wada et al. 1995). Positive effects of the antibiotic drug polymyxin B have been reported, however, its use is limited due to its toxicity and lack of availability (Werners et al.

2005). The case fatality associated with endotoxemia is high, with only 37.1% survival to discharge in horses showing symptoms of endotoxemia after colic surgery, compared to 90.3% of horses without these symptoms (Mair and Smith 2005a).

1.2.3. THROMBOPHLEBITIS

Jugular thrombophlebitis is a complication typically associated with intravenous catheterization. Reported incidences after colic surgery vary widely from 1-13% (Table 2). A state of increased thrombogenesis, occurring in horses suffering from endotoxemia and DIC, puts horses at risk of developing jugular vein thrombosis. When infected, these thrombi may evolve to septic thrombophlebitis. Reported risk factors for thrombophlebitis include large intestinal disease, endotoxemia, a state of debilitation and various postoperative complications that result in a longer dwell time of the catheter (Dolente et al. 2005; Lankveld et al. 2001; Mair and Smith 2005a). Hygienic measures, e.g. wearing surgical gloves in patient handling, are important in the prevention (Klohnen 2009). Several other preventive measures are described. Prompt treatment of the underlying GI pathology will help to minimize the development of coagulation disturbances and systemic inflammatory reactions. Irritation of the endothelium can be limited by avoiding the administration of anisotonic fluids or undiluted irritating drugs, decreasing the number of venipunctures and proper catheter placement. Administration of non-steroidal anti-inflammatory drug (NSAID) and anticoagulant therapy may also be indicated (Divers 2003; Geraghty et al. 2009). Although there is no statistical evidence on catheter type as a risk factor for thrombophlebitis, it is known that the composition and stiffness of the catheter affect its thrombogenicity, whereby polyurethane over-the-wire catheters are considered the least thrombogenic (Divers, 2003).

Thrombophlebitis leads to symptoms of inflammation with local swelling, pain, discharge and fever. Complete thrombosis of the vein may cause head swelling. In some cases secondary complications like bacteremia or endocarditis may occur.

Frequent monitoring of the catheter insertion site and the jugular vein is important for the timely discovery of cases. Treatments for thrombophlebitis aim at decreasing inflammation and eradicating infection. They include immediate removal of the catheter, followed by ultrasound guided aspiration for bacterial culture and, if necessary, drainage (Hackett and Hassel 2009; Klohnen 2009). Most cases resolve with local treatment including hot-packing or topical thrombolytic and anti-inflammatory therapy; some horses need systemic NSAIDs. Infected thrombi may require long-term antibiotic treatment. Septic cases refractory to therapy may be treated by thrombectomy under standing sedation. (Russell et al. 2010).

1.2.4. PERITONITIS

Generalized peritonitis is a potentially life-threatening but fortunately not very common complication. It is only diagnosed in a few percent (2.8-6%) of horses (Mair and Smith 2005a; Phillips and Walmsley 1993). After a small intestinal resection, the incidence increases to about 11% (Morton and Blikslager 2002). Probably a local, low grade peritonitis occurs more frequently. Although local peritonitis may pass by asymptomatically, it can be a cause of postoperative fever and might also lead to adhesion formation. Adhesions may remain asymptomatic or provoke recurrent colic symptoms in a later stage (Hacket and Hassel 2009; Klohnen 2009).

A generalized peritonitis provokes more apparent symptoms including fever, colic, weight loss, diarrhea, tachycardia, dehydration and ileus. It can be diagnosed by abdominal ultrasound and abdominal paracentesis including cytological examination (Dukti and White 2009; Hacket and Hassel 2009). The presence of bacteria in the peritoneal fluid is a risk factor for mortality (Hawkins et al. 1993). Treatment for peritonitis comprises broad spectrum antibiotic therapy, supportive therapy and correction of the inciting cause if known. The latter may include a repeat laparotomy (Dukti and White 2009). Abdominal lavage and drainage can be useful in the prevention of adhesion formation (Hague et al. 1998).

1.2.5. DIARRHEA

Postoperative colitis or diarrhea is currently observed in less than 5% of horses (Freeman et al. 2000; Mair and Smith 2005a; Proudman et al. 2002). However one large study reported the development of diarrhea in 30% of horses (Cohen et al. 2004), and in an earlier study an even higher incidence was reported, with diarrhea occurring in as much as 53.2% of horses and severe diarrhea in 27.5% (Cohen and Honnas 1996). The most important cause of diarrhea is a disturbance of the normal intestinal flora. Dysbacteriosis may be a sequel of the colic itself and the surgery, but also of postoperative feed restrictions and antibiotic treatments. Large intestinal colic and enterotomy, with evacuation of intestinal contents containing normal flora, are reported risk factors; early postoperative feeding of grass hay is protective (Cohen and Honnas 1996; Hackett and Hassel 2009). Options for the prevention and treatment of diarrhea in horses include fluid therapy, transfaunation and the administration of probiotics, adsorbents, NSAIDs, and, in specific cases, antibiotics. A comprehensive description can be found in a review article by Feary and Hassel (2006).

1.2.6. HYPERLIPEMIA

Anorexia or feed restriction in the perioperative period is associated with a temporary state of negative energy balance, which may provoke hypertriglyceridemia or hyperlipidemia (McKenzie 2011). However, the more severe and even potentially life-threatening hyperlipemia is a rare complication after colic surgery (Mair and Smith 2005a). It mainly affects obese or pregnant animals from certain breeds, such as ponies, miniature horses and donkeys. The excessive mobilization of lipids from fat depots leads to increased plasma concentrations of triglycerides, which may interfere with many physiological functions. Depending on the triglyceride concentration and the presence of gross lipemia, the consequences may vary from anorexia and depression to renal and hepatic failure and even death (McKenzie 2011).

1.2.7. LAMINITIS

An association has been demonstrated between the development of laminitis and surgical colic. Horses suffering from other postoperative complications, especially those related to endotoxemia like diarrhea or postoperative ileus, are more prone to developing laminitis. The presence of endotoxemia is an important underlying risk factor (Parsons et al. 2011).

Postoperative laminitis is a much dreaded complication due to its poor prognosis and the severe and chronic pain suffered by affected horses. Fortunately, it does not seem to occur very frequently. Reported incidences are low, 0.4 to 6% (Cohen et al. 2004; De la Rebière de Pouyade 2009; Mair and Smith 2005a; Proudman et al. 2002). However, it is observed more frequently after certain types of colic, with up to 12% of cases reported after a small intestinal resection (Morton and Blikslager 2002).

Multiple mechanisms are probably involved in the pathogenesis of laminitis; extensive research has led to two main hypotheses. The first focusses on hemodynamic changes within the foot, leading to edema and capillary hypoperfusion causing an ischemia-reperfusion related inflammatory response. Serotonin and other, gut-derived, amines probably play a role in the initiation of digital vasoconstriction (Elliot and Bailey 2006; Moor et al. 2004). The second hypothesis involves the activation of enzymatic events in the foot. A key point is the induction of matrix metalloproteinase (MMP) activity. MMPs can be activated by trigger factors from the GI tract reaching the laminae through circulation, but also by locally produced inflammatory mediators. Metabolic changes leading to a decreased glucose consumption by the laminae also play a role. Possibly, hemodynamic and enzymatic events coincide and contribute simultaneously to laminitis pathophysiology. Finally mechanical factors may add to the problem (Eades 2010; Elliot and Bailey 2006; Moore et al. 2004).

In surgical colic horses, prevention of laminitis consists mainly of anti-endotoxin and anti-inflammatory therapy, administration of low-molecular weight heparin and continuous cryotherapy (De la Rebière de Pouyade 2009; Hackett and Hassel 2009).

If a horse develops laminitis, the initial treatment consists of analgesic and anti-inflammatory therapy, minimizing mechanical trauma to the laminae and cryotherapy. In addition, every attempt should be made to control the inciting cause. Extensive reviews on the treatment of acute and chronic laminitis can be found in the literature (Morrison 2010; Reilly et al. 2010; Van Eps 2010).

1.2.8. HEMORRHAGE

Postoperative bleeding is a rare complication of colic surgery. Freeman et al. (2000) mention 2 cases of postoperative blood loss and anemia out of 74 horses that recovered from general anesthesia; Doyle et al. (2003) describe 7 cases of severe hemorrhage after colic surgery. Blood loss may originate from mesenteric blood vessels or from an intestinal enterotomy or anastomosis site. Decreased cardiac output and blood pressure during anesthesia can delay the onset of severe hemorrhage to the postoperative period (Dukti and White 2009). The first sign of intraluminal hemorrhage is usually melena, sometimes accompanied by colic signs (Doyle et al. 2003); while intraperitoneal hemorrhage (hemoperitoneum) typically provokes colic signs (Conwell et al. 2010; Dechant et al. 2006). Both types of blood loss frequently lead to hemorrhagic shock (Conwell et al. 2010; Doyle et al. 2003). A definitive diagnosis can be made by rectal palpation, abdominal ultrasonography and abdominal paracentesis. Advised treatments are fluid replacement with crystalloids and blood, IV formalin and repeat celiotomy (Conwell et al. 2010; Doyle et al. 2003).

1.2.9. PNEUMONIA

Pneumonia is not often mentioned as a complication of colic surgery. No cases were observed in two recent studies on large groups of horses (Mair and Smith 2005a; Proudman et al. 2002). However, one report describes four cases (4/747; 0.5%) of hemorrhagic pneumonia after colic surgery. All four horses had displayed intra-

operative nasogastric reflux (Klohnen 2009). In another publication four cases of pleuropneumonia after SI resection are mentioned (Morton and Blikslager 2002). Possibly, the aspiration of nasogastric reflux during induction or recovery from anesthesia is a causative factor. Since two out of four reported cases did not survive in spite of treatment, preventative measures are important. Preoperative nasogastric intubation with pre- and intraoperative emptying of the stomach and proper placement of a tracheal tube can help to avoid the aspiration of stomach contents.

1.2.10. POSTOPERATIVE ILEUS

Gastrointestinal ileus is defined as the functional inhibition of propulsive intestinal activity, irrespective of its pathophysiology (Koenig and Cote 2006). Some intestinal motility may still be present, but it is uncoordinated and does not enable the effective propulsion of chyme. This lack of propulsion causes the accumulation of intestinal contents, such as ingested food and water, but more importantly massive amounts of digestive secretions. The onset of ileus can be idiopathic in apparently healthy horses, but it is also a well-known postsurgical complication. Postoperative ileus (POI) can theoretically occur after all sorts of surgical interventions. However it most frequently develops after colic surgery, in particular small intestinal colic surgery. Reported incidences after unspecified colic surgery vary from 10-19%. Up to 50% of horses may be affected when only small intestinal colic cases are included (Table 1). Surprisingly, the incidence of POI has increased rather than decreased during the last decades (Table 1). According to Hunt et al. (1986) this might be explained by improvements in perioperative care, enabling more horses to survive the immediate postoperative period. Advances in surgical and postoperative care might also have led to changes in patient selection, with more severe cases being allowed to recover from surgery.

In addition to the presence of small intestinal pathology, pre- and intraoperative risk factors for the development of POI include the presence of reflux, signs of shock/endotoxemia or hypocalcemia at admission and the performance of a small

intestinal resection (Cohen et al. 2004; Delesalle et al. 2005; French et al. 2002; Roussel et al. 2001).

The course of ileus in horses is totally different from the pathology as known in human medicine. In human patients, a transient period of decreased motility, affecting mainly the colon, is frequently observed after abdominal surgery. A more severe form that may include the whole GI tract is reported after almost 10% of open laparotomies. It causes patient discomfort and brings about an increased risk of other complications, leading to longer hospitalization times and important economic losses. In spite of its importance, ileus in humans is normally not life-threatening (Senagore 2010).

Postoperative ileus in horses however, mainly affects the stomach and small intestines. A gradual accumulation of intestinal contents occurs, starting with the small intestinal region that was primarily affected by the hypomotility. Progression to the more proximal parts of the GI tract finally results in a dilation of the stomach and small intestines. Pancreatic secretion in horses is much more abundant than in other species: 10-12 liter per 100 kg body weight per 24h in ponies, compared to 1.3 liter in man and 0.6-1.1 liter in sheep (Stevens and Hume 1995). Failure to transport these secretions towards the colon in POI horses leads to a massive draining of fluids towards the stomach. Gastric rupture, sequestration of fluids and electrolytes within the GI tract and concurrent dehydration and shock make POI often fatal in horses. Postoperative ileus has been identified as the most common reason for euthanasia after colic surgery (Van der Linden et al. 2003). The associated case fatality ranges from 14-52% (Cohen et al. 2004; Freeman et al. 2000; Mair and Smith 2005a; Morton and Blikslager 2002).

Similar to the situation in human medicine, POI puts a horse at risk of developing other complications. It is significantly associated with the occurrence of postoperative pain and shock (Mair and Smith 2005a), with adhesion formation (Mair and Smith 2005b) and relaparotomy (French et al. 2002; Mair and Smith 2005c). Horses that recover from POI are more susceptible to subsequent development of jugular thrombophlebitis, laminitis and incisional infections (Cohen et al. 2004; Mair and Smith 2005a).

The onset of POI is usually within a day after surgery, but a delayed onset days later might also occur (Roussel et al. 2001). The associated symptoms are mainly provoked by gastric and small intestinal distention and by fluid and electrolyte loss. The principal clinical symptoms are tachycardia, depression and anorexia, sometimes accompanied by subtle signs of discomfort. Overt colic symptoms are less common. If present, they are usually associated with important gastric distention. Upon nasogastric intubation, several liters of reflux can be obtained. Spontaneous nasal discharge of stomach contents is rare. Upon abdominal ultrasound, the distended and fluid-filled stomach and small intestines can be easily recognized. Moderately distended small intestines are usually encountered at rectal palpation. Blood work shows signs of hemoconcentration: increased packed cell volume and sometimes hyperproteinemia. Electrolyte changes frequently occur, particularly hypocalcemia (Delesalle et al. 2005; Hackett and Hassel 2009; Klohnen 2009).

The further course of the disease is mainly defined by the degree of intestinal stasis. Mild cases can be limited to a delayed onset of motility persisting only a day postoperatively. On the other hand, a total loss of propulsive motility will lead to rapid GI distention and massive fluid sequestration with a high case fatality, even with intensive treatment. Reported nasogastric fluid losses may vary from a total of 15 up to 1500 liters. The reported median duration of POI is 3 days, ranging from 1 to 9 days (Roussel et al. 2001).

Both in humans and in horses, the multifactorial nature of POI makes it a sometimes unavoidable complication. However, the ever-growing knowledge of the pathophysiological processes that contribute to POI development has led to several strategies for the prevention and treatment of this complication. The normal intestinal physiology and the pathophysiology of ileus are discussed below.

NORMAL INTESTINAL PHYSIOLOGY

The most important function of the small intestines is to continue the enzymatic digestion, that has been started by the secretions produced by the stomach and, to a lesser extent, the salivary glands. There is also a significant resorption of nutrients. Water and most electrolytes can pass freely between the enterocytes, allowing both their absorption and secretion. At the same time, the intestinal wall has an important barrier function preventing the penetration of foodborne pathogens within the body (Herdt 2007).

Several properties enable the small intestine to fulfill these functions. The intestinal mucosa (Fig. 1) is lined with specialized cells: mainly enterocytes for resorption and enzymatic digestion and a lower number of protective, mucus producing goblet cells and regulatory entero-endocrine cells. The large contact surface essential for digestion and resorption is created by intestinal villi formed by the lamina propria mucosae and by microvilli, situated at the luminal side of the enterocytes (Herdt 2007, Salomon 2005).

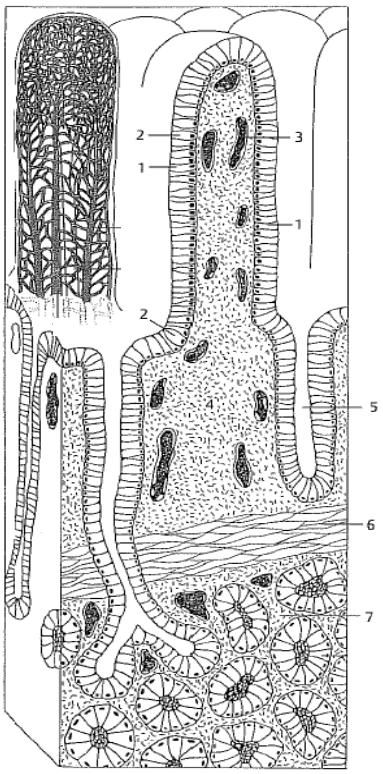


Fig. 1. Schematic histology of the duodenal mucosa and submucosa. Adapted from Salomon (2005).

1. Enterocyte
2. Goblet cell
3. Central blood vessel
4. Lamina propria mucosae
5. Intestinal glad or crypt of Lieberkühn
6. Lamina muscularis mucosae
7. Tela submucosae

Local immunity is provided by the gut associated lymphoid tissue (GALT) and by the Paneth cells, present in the crypts of Lieberkühn at the base of intestinal villi. The latter produce anti-bacterial enzymes and are capable of bacterial phagocytosis. Tight junctions connecting the apical sides of the enterocytes prevent the passage of harmful substances through the intercellular space (Herdt 2007, Salomon 2005).

An inner circular and outer longitudinal smooth muscular layer are responsible for the intestinal motility. This motility serves different purposes: the chyme has to be mixed with digestive secretions, held in place to provide contact time for digestion and resorption, and then gradually propulsed to a next intestinal segment. Therefore excellent coordination between secretion and motility is crucial. The regulation of small intestinal motility and secretion is discussed in the next section.

REGULATION OF INTESTINAL FUNCTION

The main regulatory system for the intestinal motility and secretion is the enteric nervous system or ENS (Fig. 2). It contains almost as many neurons as the spinal cord and can function independently. Its main function is to tailor the intestinal functions according to the local environment: the quantity and chemical composition of the chyme. The ENS consists of a network of highly connected receptors, sensory neurons, interneurons and motoneurons. The latter innervate the intestinal smooth muscle layers, but also the smooth muscle tissue present in glands and the walls of blood vessels. The ENS ganglia are arranged in two plexuses: the submucosal plexus of Meissner and the myenteric plexus of Auerbach, which is localized between the two intestinal muscular layers (Binder 2009; Herdt 2007).

The regulatory actions by the ENS take place via so-called entero-enteric reflexes. These reflexes may be initiated by neurons, hormones or both (see below). They enable a certain intestinal region to regulate the motility and secretion in more proximal regions (Herdt 2007; Lomax et al. 2010).

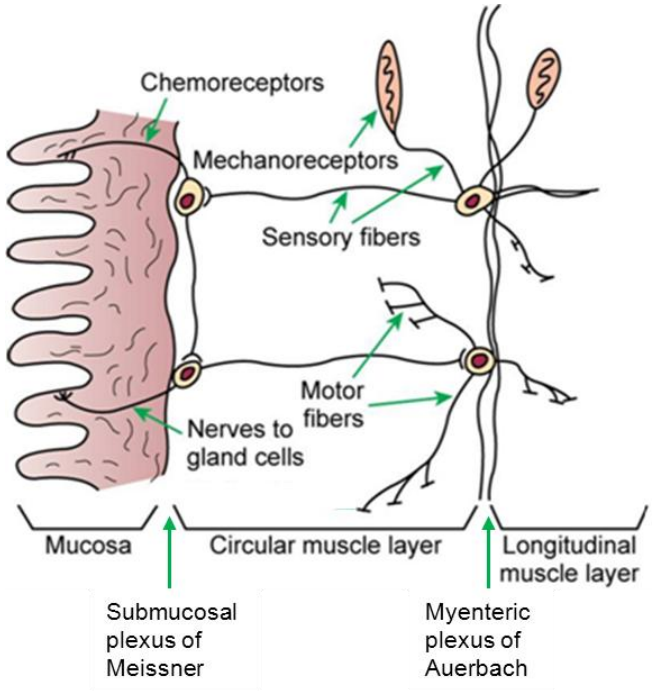


Fig. 2. Arrangement of nerve fibers and receptors within the enteric nervous system. Adapted from Herdt (2007).

Communication between the ENS and the central nervous system allows the adaptation of GI activity to the needs of the rest of the body. Information from intestinal mechano- and chemoreceptors reaches the brain via nerves of the autonomous nervous system. Afferent impulses from the parasympathetic system are integrated in the brain stem and signal back to the ENS as efferent vagal stimuli; this circuit is called the vagovagal reflex. Sympathetic afferent impulses are mainly pain signals, reaching conscious areas in the brain. There is also efferent communication from the brain towards the intestines. Parasympathetic activity generally increases intestinal function, whereas sympathetic stimulation inhibits it (Binder 2009; Herdt 2007; Lomax et al. 2010).

The GI tract does not only have its own nervous system. It also possesses an endocrine system, which consists of endocrine and paracrine cells spread between the enterocytes. Endocrine cells produce hormones, that reach and influence their distant target through the blood, while paracrine secretions spread by diffusion, affecting cells in

their direct surroundings. Although the different types of endo- and paracrine cells have an identical morphology, they produce a wide range of substances. Every cell type has its specific distribution throughout the GI tract, enabling the adaption of an intestinal region to its special needs. Examples are the gastrin producing G-cells, mainly located in the pyloric antrum of the stomach, and serotonin and somatostatin producing cells that can be found all over the small intestine (Herdt 2007; Salomon 2005).

Both the neurological and endocrine regulating system make use of messenger molecules. It is noticeable that the same substance may have a neurotransmitter function as well as an endo- or paracrine action, depending on where it was produced and how it is distributed. This class of messengers is therefore called the neurohumoral regulating molecules. Most of them are peptides, and currently at least 28 different types, involved in intestinal function, are known. Their target cells are not only intestinal smooth muscle cells but also ENS neurons and endo- and paracrine cells, generating a highly integrated system with an extensive feedback mechanism (Herdt 2007).

A major messenger molecule is acetylcholine, the most important messenger of the parasympathetic neurons and therefore a stimulatory molecule. It is counteracted by noradrenalin, the main neurotransmitter produced by the sympathetic system at intestinal level (Herdt 2007). A third class of messengers is the nonadrenergic-noncholinergic (NANC) system (Malone et al. 1999). Messengers belonging to the NANC system function in the ENS as neuromodulators: secondary neurotransmitters that influence the neuron in addition to the cholinergic or adrenergic effects exerted by the primary neurotransmitter (Freytag et al. 2008). Major NANC peptides that usually enhance motility are substance P and motilin; important inhibiting messengers are the peptides somatostatin and vasoactive intestinal peptide (VIP) and non-peptide molecules like nitric oxide (Binder 2009; Herdt 2007; Freytag et al. 2008; Malone et al. 2000). A very important neurocrine messenger is the amine serotonin. It is released by a specific type of entero-endocrine cells, the enterochromaffin cells, upon stimulation of mechano- of chemoreceptors in the intestinal mucosa. Serotonin may exert inhibiting or stimulating effects, dependent on the receptor type (Table 3). By targeting the mucosal projections of

primary afferent neurons, it initiates the entero-enteric reflex (Freeman 2008; Gershon and Tack 2007). The regulation of GI motility by the cholinergic, adrenergic and serotonergic system, including points of action of some prokinetic drugs (see below) is illustrated in Figure 3.

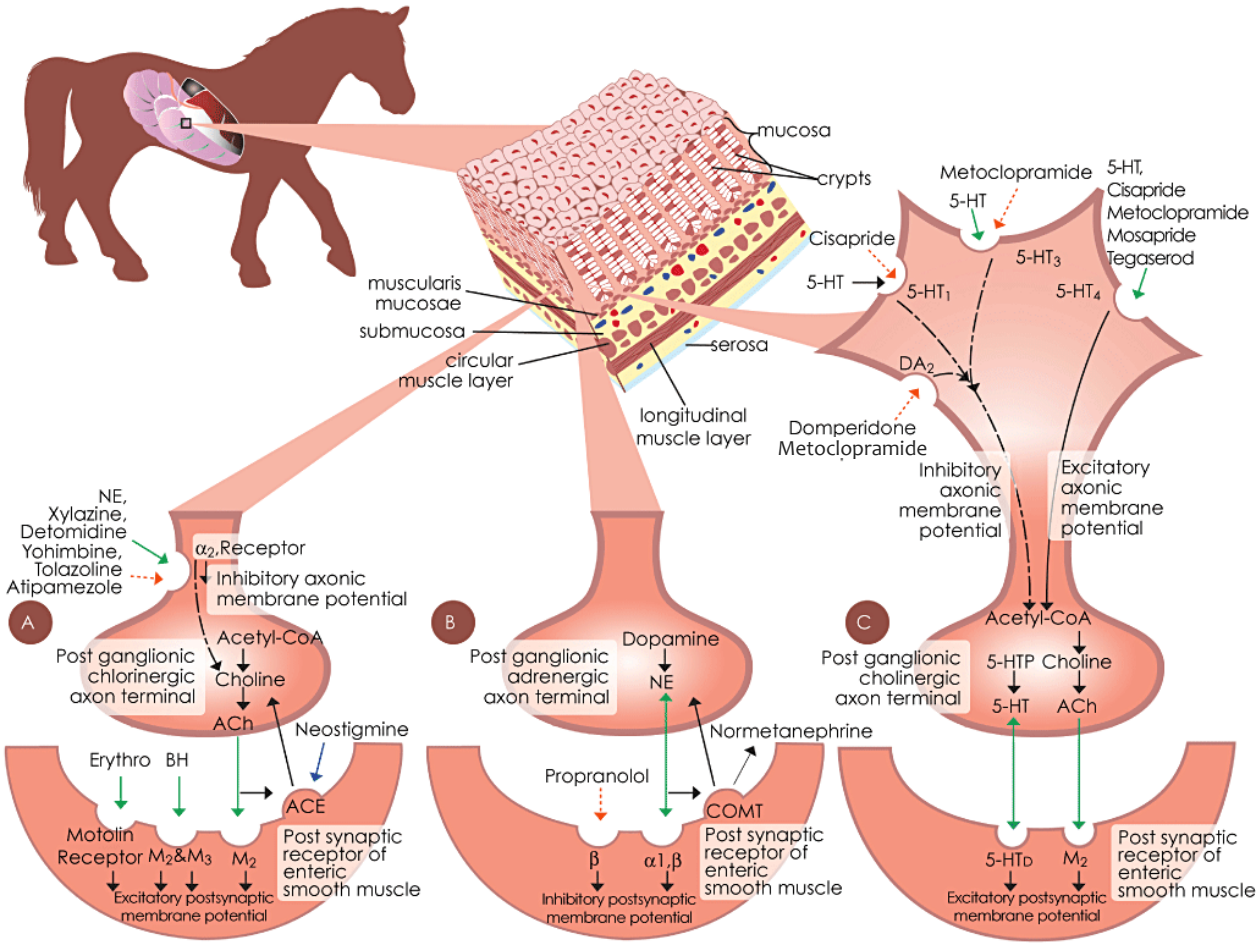


Fig. 3. Schematic representation of postganglionic cholinergic (A), postganglionic adrenergic (B) and pre- and postganglionic serotonergic (C) control of enteric smooth muscle. Adapted from Wong et al. (2011). 5-HT = serotonin; ACh = acetylcholine; NE = noradrenalin; DA = dopamine receptor; M = muscarinic receptor; $5\text{-HT}_{1,3,4,D}$ = 5-HT receptor families; Erythro = erythromycin; Green arrow = agonistic effect; Red dashed arrow = inhibitory effect.

Table 3. Overview of serotonin receptors with their locations, functions and clinically relevant agonists and antagonists.

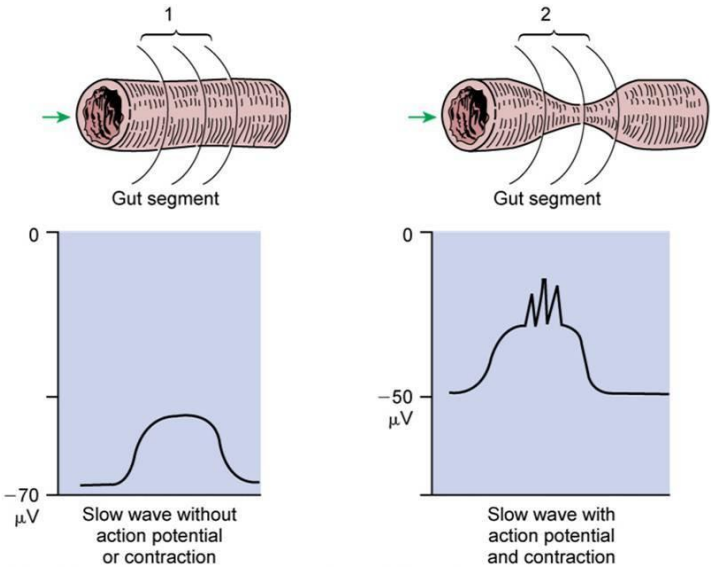
Receptor	Location	Function	Agonist	Antagonist	Reference
5-HT_1	digital vein*	VC	sumatriptan buspirone		Gershon and Tack 2007; Zerpa et al. 2007
5-HT_{1A}	CNS	neuronal inhibition, behavior	buspirone	yohimbine	Mohammad-Zadeh et al. 2008
5-HT_{1A}-like	jejunal circular muscle*	GI contraction			Delesalle et al. 2008b
5-HT_{1B}	CNS; vascular	presynaptic inhibition, behavior, pulmonary VC	sumatriptan		Mohammad-Zadeh et al. 2008
5-HT_{1P}	myenteric neuron	stomach relaxation	sumatriptan		Gershon and Tack 2007
5-HT_M	CNS; vascular	locomotion, cerebral VC	sumatriptan	yohimbine	Mohammad-Zadeh et al. 2008
5-HT_2	jejunal circular muscle*		cisapride		Nieto et al. 2000
5-HT_{2A}	CNS; PLT	neuronal excitation, behavior, learning, PLT aggregation		ketanserin cyproheptadine chlorpromazine LSD	Mohammad-Zadeh et al. 2008
5-HT_{2B}	gastric fundus			chlorpromazine yohimbine	Mohammad-Zadeh et al. 2008
5-HT_{2C}	CNS	cerebrospinal fluid secretion			Mohammad-Zadeh et al. 2008
5-HT_3	jejunal circular muscle,* ileum,* pelvic flexure,* sensory and enteric nerves	visceral sensation, emesis, delayed gastric emptying, MMC stimulation		metoclopramide cisapride ondansetron dolasetron tropisetron	Gershon and Tack 2007; Mohammad-Zadeh et al. 2008; Nieto et al. 2000; Weiss et al. 2002; Wong et al. 2011
5-HT_4	duodenal,* ileal* and pelvic flexure* smooth muscle, CNS	GI contraction	metoclopra-mide cisapride tegaserod mosapride		Delco et al. 2007; Mohammad-Zadeh et al. 2008; Okamura et al. 2009; Prause et al. 2010
5-HT_{5A}	CNS	unknown			Mohammad-Zadeh et al. 2008
5-HT_6	CNS	unknown			Mohammad-Zadeh et al. 2008
5-HT_7	duodenal,* ileal,* and pelvic flexure* smooth muscle layers and myenteric plexus, CNS, vascular	GI relaxation			Mohammad-Zadeh et al. 2008; Prause et al. 2009

CNS = central nervous system; GI = gastrointestinal; MMC = migrating motility complex; PLT = platelet; VC = vasoconstriction
*Specifically identified in equine tissue

MOTILITY

Intestinal motility patterns are coordinated by the interstitial cells of Cajal (ICC). The ICC are mesenchymal cells with characteristics of both smooth muscle cells and neurons, that form extensive networks throughout the intestinal tract. They possess the ability for spontaneous depolarization and act as intestinal pacemaker cells. Certain intestinal regions with a higher concentration of ICC, like the proximal duodenum, are viewed as pacemaker sites (Fintl and Hudson 2010; Hudson et al. 1999; Pavone and Mandara 2010).

The ICC membrane potential changes give rise to waves of partial electric depolarization that migrate along the intestine, called the slow waves. In the equine small intestine they occur about 12 times per minute (Wong et al. 2011). A slow wave alone is not sufficient to cause a complete depolarization -an action potential- and contraction; an additional stimulus from the ENS is needed (Fig. 4). However, to obtain an intestinal contraction upon ENS stimulation, the concurrent passage of a slow wave is necessary as well. In this way, the slow waves generated by the ICC provide the necessary coordination of intestinal contractions (Fintl and Hudson 2010).



1. Passage of a slow wave provokes partial depolarization without contraction of the intestinal segment.
2. ENS stimulation increased the membrane potential to -50 mV. Passage of a slow wave causes an action potential and contraction.

Fig. 4. Slow waves and action potentials. After Herdt (2007).

Two different motility phases occur in the small intestine. The digestive phase is characterized by propulsive contractions and non-propulsive, mixing contractions and logically serves for the digestion of food. During the interdigestive phase, powerful peristaltic contractions, propagating over a great length of the small intestine, remove indigestible residues and help to control the intestinal bacterial population. These "housekeeping" contractions are called the migrating motility complex or migrating myoelectric complex (MMC) (Herdt 2007). How exactly the MMC is regulated is still subject of research. However, complex interactions between the ENS, humoral factors and extrinsic innervation are involved. A major determinant of the MMC pattern is motilin, which is released just before the phase of peak electrical and mechanical activity (Binder 2009; Sasaki and Yoshihara 1999).

In horses, that are naturally continuous feeders, the MMC is not limited to the interdigestive phase; it is continuously present despite the intake of food. About 20 cycles occur throughout the day, mostly propagating all the way along the small intestine. Together with the rather high mean MMC velocity in horses, the MMC in horses helps to fasten the transportation of chyme towards the large intestine, allowing an efficient digestion of plant fibers (Baker and Gerring 1994; Koenig and Cote 2006; Ruckebusch et al. 1991; Sasaki and Yoshihara 1999).

A disadvantage of the complex system with slow waves and MMC patterns, is its fragility. Disturbance of motility patterns immediately affects intestinal functioning (Ruckebusch et al. 1991).

PATHOPHYSIOLOGY OF ILEUS

Normal intestinal motility demands a normally functioning smooth muscle layer that is normally stimulated and regulated. The motility may be disturbed at the level of the circular and longitudinal muscle layers, the ICC, the ENS or the autonomic connections with the central nervous system.

INFLAMMATION

Local inflammation is one of the major pathophysiological pathways in the pathogenesis of ileus. Intestinal distension, manipulation during surgery, endotoxin and especially ischemia and reperfusion can trigger the inflammatory reaction (Dabareiner et al. 2001; Doherty 2009; Kalff et al. 1999; Little et al. 2005). Intestinal ischemia in a certain bowel region can also affect adjacent bowel (Malone et al. 2001). Indeed, already at the time of surgery, distinct molecular changes are present in the apparently normal intestinal tissue at the oral border of strangulated intestine. Evidence was found for the activation of protective and degenerative pathways and the existence of an early inflammatory response in the intestinal mucosa, muscle layer and nervous tissue (De Ceulaer et al. 2011a). There were microscopic signs of degeneration and also a modification of structural smooth muscle proteins like myosin (De Ceulaer et al. 20011b). These early signs of degeneration and muscle damage could provide a molecular and morphological basis for the development of POI.

The subsequent inflammatory reaction is probably initiated by a population of resident macrophages in the intestinal muscular layers, that release pro-inflammatory mediators upon activation (Wehner et al. 2007). Alternatively or concurrently, mast cells are probably also important in initiating the inflammatory process (De Winter et al. 2012). There is induction of various cytokines, nitric oxide and prostaglandins, and increased expression of adhesion molecules, tumor necrosis factor α and interleukins 1 and 6 (Kalff et al. 1999). Neutrophils are attracted towards the site of inflammation and within 18 hours, they have infiltrated all layers of the intestinal wall. The most pronounced inflammatory reaction is observed at the level of the tunica serosa, muscular layer and myenteric plexus (Little et al. 2005). The neutrophils, a typical cell type in acute inflammatory processes, produce pro-inflammatory mediators that attract other inflammatory cells and cause local damage. Once intestinal inflammation is induced, it may be maintained for days (Kalff et al. 1999; Little et al. 2005; Rakestraw 2008).

Inflammation causes structural damage to smooth muscle cells, neurons and pacemaker cells, hampering a normal intestinal motility. Several inflammatory mediators, particularly nitric oxide, can also function as inhibiting neurotransmitters and cause additional neurogenic inhibition of motility. Inflammation can also change receptor populations on neurons and smooth muscle cells, favoring inhibitory populations (De Winter and De Man 2010; Rakestraw 2008). It was demonstrated that the number of stimulating motilin receptors is decreased in colitis cases (Depoortere et al. 2001) and after intestinal ischemia or dilation in horses (Koenig et al. 2006), while myenteric substance P receptors were increased in inflamed intestine (Mantyh et al. 1995). Finally, ischemia-reperfusion related inflammation may lead to a massive serotonin release from the enterochromaffin cells towards the intestinal lumen and surroundings (Galmarini et al. 1997; Kaihara et al. 1997; Matia et al. 2004). Increased plasma concentrations of serotonin have been measured in surgical colic cases (Delesalle et al. 2008a). Serotonin released by the intestinal EC cells may reach the plasma, or alternatively, increased concentrations may originate from the activation of platelets by e.g. endotoxemia (Bailey et al. 2000; Delesalle et al 2008a). Serotonin changes may inhibit intestinal motility by subsequent intestinal serotonin depletion or receptor desensitization, which is a known feature of serotonin receptors (Lalley et al. 1994). Increased plasma serotonin concentrations also enhance platelet activation (Li et al. 1997) and could therefore play a role in systemic reactions such as DIC. Serotonin changes may contribute to the pathophysiology of ileus and serotonin might also be a marker for intestinal compromise. Although a number of studies have been published on the levels of free serotonin in equine plasma, the lack of validated species-specific diagnostic tests has led to a wide range of reported normal values (Bailey and Elliot 1998; Delesalle et al. 2008a; Haritou et al. 2008).

The inflammation hypothesis is supported by the fact that the typical time of onset of POI in the horse corresponds with the development of neutrophilic infiltration (Little et al. 2005). However, in the very acute stages of ileus, neurogenic pathways probably also play a role.

NEUROGENIC INHIBITION

Neurogenic inhibition of intestinal motility may be mediated by the ENS or the autonomic nervous system. Local ENS inhibition is mainly caused by the presence of inhibiting entero-enteric reflexes. Distention of a small intestinal segment will provoke reflectory inhibition of the motility in proximally located segments (Lomax et al. 2010). This is useful in a physiological situation: it allows the distended segment to empty before new chyme arrives. In a situation of pathological distention however, it might contribute to the development of prolonged hypomotility and ileus. The entero-enteric reflexes may also be triggered by inflammatory reactions affecting the sensory afferent nerves (De Winter et al. 2009).

Neurogenic inhibition by the autonomic nervous system starts with mechano- or nocireceptors that stimulate afferent sympathetic nerves. Information from these receptors is communicated to the brain, but also activates sympathetic reflexes at spinal and supraspinal level (Herdt 2007; Doherty 2009, Wong et al. 2011). Upregulation of sympathetic efferent signaling will decrease the intestinal motility, secretion and blood supply (Lomax et al. 2010).

It is not possible to make a clear distinction between inflammatory and neurogenic mechanisms, because there are many interactions between the immune and nervous system. Not only can inflammation cause neurogenic inhibition as explained before, the autonomic nervous system also has immunomodulating effects: it influences the intensity of the inflammatory reaction. A strict control of the inflammatory process is indeed essential to allow a proper defense against pathogens with as little damage to the host tissues as possible (Van der Zanden et al. 2009). Sympathetic stimulation may have pro- or anti-inflammatory effects in different situations. An important example of a pro-inflammatory action is the initiation of nitric oxide production by adrenergic stimulation of intestinal macrophages (Lomax et al. 2010; Wong et al. 2011). Parasympathetic effects are typically anti-inflammatory. Vagal afferents from the intestine can activate the hypothalamus-pituitary-adrenal axis and increase corticosteroid production.

Macrophages also possess cholinergic receptors on their cell surface, that might be stimulated by vagal nerve fibers (Van der Zanden et al. 2009).

MISCELLANEOUS CONTRIBUTORS

Additional to intestinal inflammatory and neurological disturbances, surgical colic in the horse can have major effects on the body as a whole. Metabolic changes, shock and endotoxemia cause further disturbance of the intestinal homeostasis and may induce inflammation. Intestinal ischemia leads to a disturbed cellular calcium homeostasis with high intracellular calcium concentrations and hypocalcemia (Crouser and Dorinsky 1996). It was demonstrated that hypocalcemic horses are prone to postoperative ileus and death (Delesalle et al. 2005).

Other negative effects on intestinal motility are directly related to applied treatments. An intestinal resection logically causes the interruption of intracellular connections of the ICC network and between smooth muscle cells. It was demonstrated in dogs that the MMC stops at the anastomosis site. Continuity of the intestinal contraction has to be maintained by local pacemakers located aboral from the new connection (Arnold et al. 1991).

Another treatment related issue is the inadvertent inhibition of motility by medication. Drugs acting on α_2-adrenergic receptors, like xylazine and detomidine, are often administered to colic horses for their sedative and analgesic properties. However, they also interact with α_2 receptors on parasympathetic nerves and decrease acetylcholine release (Wong et al. 2011). They disrupt the MMC complex and cause a temporary but distinct decrease of intestinal motility (Merritt et al. 1998; Sutton et al. 2002; Zullian et al. 2011). Other analgesic drugs, the opioids, are notorious especially in human medicine for inhibiting large intestinal motility (Cali et al. 2000). Their negative effects on motility are also observed in horses (Boscan et al. 2006; Merritt et al. 1998; Sutton et al. 2002).

PREVENTION AND TREATMENT STRATEGIES

Due to the multifactorial nature of the complication, strategies for the prevention and treatment of ileus need to focus on different aspects. In general, prevention or treatment of ileus actually means prevention and treatment of intestinal inflammation and neurogenic inhibition. Once POI has developed, causative treatments must of course be combined with symptomatic treatments, aiming at the restoration of homeostasis.

First, risk factors for the development of ileus have to be determined and, if possible, avoided. Recently, the presence of reflux or signs of endotoxemia and shock at admission and the need for a small intestinal resection have been identified as major risk factors (Cohen et al. 2004; French et al. 2002; Roussel et al. 2001). They might be avoided or at least improved by timely referral and surgery, which emphasizes the important role of the referring veterinarian. During colic surgery, strategies for the prevention of ileus include correct surgical technique with limited intestinal manipulation and mesenterial traction (Doherty 2009). A pelvic flexure enterotomy may have a protective effect (Cohen et al. 2004; Mair and Smith 2005a; Roussel et al. 2001). Intraoperative administration of intravenous (IV) lidocaine also seems beneficial (Brianceau et al. 2002); this treatment will be discussed below. Proper fluid therapy, aimed at maintaining a normal hydration and correcting abnormalities in electrolytes and blood gasses, is essential both intra- and postoperatively (Doherty et al. 2009). For the management of ileus, supportive therapy has to be complemented by repeated gastric decompression by nasogastric intubation.

Different classes of drugs may be used both in the prevention and treatment of ileus. Non-steroidal anti-inflammatory drugs decrease the effects of endotoxemia and suppress intestinal inflammation. Their analgesic properties are useful in the prevention of sympathetic activation by pain. However, selective cyclooxygenase-2 (COX-2) inhibitors were shown to have negative effects on intestinal motility (Menozzi et al. 2009), while nonselective inhibitors have been reported to slow down the recovery of the epithelial barrier function (Little et al. 2007). At this moment the

best strategy seems the use of nonselective inhibitors, combined with IV lidocaine treatment. Indeed, IV lidocaine has been demonstrated to attenuate the negative effects of the nonselective COX inhibitor flunixin meglumin (Cook et al. 2008).

The local anesthetic lidocaine, administered intravenously at a loading dose of 1.3 mg/kg followed by a continuous rate infusion (CRI) of 0.05 mg/kg/min, is a very promising drug in the prevention and treatment of ileus. It exerts prokinetic effects through its anti-inflammatory properties. Lidocaine, a sodium channel blocker, has been demonstrated to inhibit neutrophil activity and cytokine response in humans. It also enhances the recovery of bowel function (Lan et al. 2004; Kuo et al. 2006). In horses, it has been shown to reduce mucosal COX-2 expression, decrease neutrophil counts (Cook et al. 2009) and enhance smooth muscle contractility (Guschlbauer et al. 2011) after ischemia-reperfusion injury. Horses that received IV lidocaine after exposure to endotoxin had better clinical scores and lower activity of pro-inflammatory TNF-α (Peiro et al. 2010). However, only limited evidence exists for its clinical efficacy in the treatment or prevention of ileus. Brianceau et al. (2002) found a positive effect on intestinal motility measured by ultrasonography, but no clinical differences, in colic horses after intra- and postoperative lidocaine treatment. Malone et al. (2006) described a faster recovery from ileus in a group of 32 horses that received lidocaine for 24 hours, but they did not observe positive effects on survival rates. In view of the popularity of this drug as a prokinetic agent (Van Hoogmoed et al. 2004), there is a need for more studies evaluating its effects in a clinical situation.

Two other prokinetic drugs popular for use in horses are metoclopramide and erythromycin. The first is a potent dopamine D_2 receptor antagonist and a moderate serotonin 5-HT_3 receptor antagonist and 5-HT_4 receptor agonist (Fig. 3). It also blocks α_2-adrenergic receptors (Hall and Washabau 1997; Tonini et al. 1995). In horses, it increased *in vitro* contractility of smooth muscle strips (Nieto et al. 2000) and it enhanced jejunal motility *in vivo* (Okamura et al. 2009). However, no effects on jejunal or pelvic flexure motility were found in an earlier study (Sojka et al. 1998). In an *in vivo* setting, metoclopramide attenuated the negative effect of IV endotoxin on gastric emptying (Doherty et al. 1999). A continuous infusion (0.04 mg/kg/h)

reduced the incidence and severity of postoperative ileus in one study (Dart et al. 1996). However, due to its possible extrapyramidal side effects it is often used by clinicians at lower rates or via different administration routes (Van Hoogmoed et al. 2004), without a lot of evidence on their effectiveness.

Erythromycin, a macrolide antibiotic, is used as a prokinetic drug in horses because of its effects on motilin receptors (Fig. 3; Koenig et al. 2002). Effects on motility have been observed in humans and dogs. However, prolonged treatment with this drug is thought to induce receptor down regulation and high doses were less efficient in dogs than low doses (Parkman et al. 1995; Van Hoogmoed et al. 2003). In horses, increases in gastric (Ringger et al. 1996) and cecal (Lester et al. 1998) emptying have been observed after erythromycin administration. However, in a myoelectric activity study variable effects were measured, including periods of decreased motility (Roussel et al. 2000). Koenig et al. (2006) observed a decreased number of motilin receptors after intestinal ischemia or distention, which may alter the response to erythromycin. No clinical studies conducted on POI horses have been published up to date and therefore, clinicians are using variable dosage and administration protocols (Van Hoogmoed et al. 2004).

Another class of prokinetic drugs act on serotonin receptors (Table 3). Cisapride (Fig. 3), a drug that mainly stimulates 5-HT_4 receptors, but also reported to antagonize 5-HT_3 and 5-HT_1 receptor activity and stimulate motilin production, was used frequently in horses. Unfortunately it became unavailable due to reported cardiac side effects in human medicine (Okamura et al. 2009; Van Hoogmoed et al. 2004; Wong et al. 2011). Although new 5-HT_4 agonists are being commercialized in human medicine, knowledge about their efficacy in horses is very limited. Even the presence of a motility stimulating intestinal 5-HT_4 receptor in horses is uncertain (Delesalle et al. 2006; Delesalle et al. 2008b). The 5-HT_4 agonist mosapride has been demonstrated to enhance gastric, jejunal and cecal motility in horses (Okamura et al. 2009). A disadvantage of the drug is its oral administration route, complicating its use in horses with POI. Another 5-HT_4 agonist, tegaserod, was found to stimulate equine 5-HT_4 receptors (Prause et al. 2010), stimulate *in vitro* pelvic flexure

contractility (Delco et al. 2007) and accelerate GI transit in healthy horses (Lippold et al. 2004). Unfortunately, the availability of this drug is also limited. Clinical studies in horses involving mosapride or tegaserod are lacking.

Other prokinetic drugs used in horses include α_2-adrenoreceptor antagonists, cholinergic stimulants and opioid antagonists (Van Hoogmoed et al. 2003; Wong et al. 2011). Within the scope of evidence based medicine and in view of the high morbidity and mortality of POI, there is an absolute necessity for clinical studies with prokinetics in horses.

1.2.11. SURGICAL SITE INFECTION

Incisional drainage is a common and important sequel of colic surgery. Reported incidences vary from 7.4-37% (Cohen et al. 2004; Freeman et al. 2000; Honnas and Cohen 1997; Mair and Smith 2005a; Phillips and Walmsley 1993; Proudman et al. 2002). An increased incidence has been reported in horses with large intestinal colic (Phillips and Walmsley 1993). Incisional drainage is often a sign of wound infection (suppuration) but it may also be aseptic. In practice, the distinction is not always clear and definitions used in literature are therefore divers. A draining wound that was or appeared aseptic at first, may later prove to be infected. Therefore generally the term surgical site infection (SSI) is used for both types of drainage.

Surgical site infections usually develop near the end of the hospitalization period, or even after discharge (Coomer et al. 2007; Galuppo et al. 1999; Smith et al. 2007). Their onset may be delayed by postoperative antibiotic treatment. An early symptom of SSI is fever, often without any localizing signs (Hardy and Rakestraw 2011). However, only pyrexia starting > 48h postoperatively, lasting > 48h or with a peak temperature > 39.2°C was associated with postoperative infection in a recent study (Freeman et al. 2012). Some horses show wound tenderness and edema. A definite diagnosis of SSI can be made by ultrasonographic examination of the wound (Wilson et al. 1989) or by the detection of wound drainage (Hardy and Rakestraw 2011).

Incisional wound infections are associated with an important prolongation of hospitalization and increased cost. They also represent a significant risk factor for hernia formation (Gibson et al. 1989; Honnas and Cohen 1997; Ingle-Fehr et al. 1997; Mair and Smith 2005b; Smith et al. 2007). Combined effects of bacterial activity and inflammation probably result in weakening of the tissue, allowing the suture material to tear through it. Herniation may occur until up to 100 days postoperatively (French et al. 2002). Another long-term consequence of SSI is the increased formation of intra-abdominal adhesions (Mair and Smith 2005b). Unfavorable cases of SSI may lead to wound dehiscence (Hardy and Rakestraw 2011; Smith et al. 2007).

PREVENTION

Several authors have examined the risk factors for SSI. In general, the risk for developing an SSI depends on the dose of bacterial contamination, the virulence of associated bacteria and the resistance of the host (Ahern and Richardson 2011). An increased risk for SSI development has been reported after a second laparotomy (Freeman et al. 2000; Mair and Smith 2005a; Mair and Smith 2005c; Phillips and Walmsley 1993). The importance of the innate host immunity is clearly demonstrated by the higher incidence of SSI in horses that were endotoxemic at admission (Smith et al. 2007) or developed other complications like POI (Cohen et al. 2004) or jugular vein thrombophlebitis (Mair and Smith 2005a).

Intraoperative risk factors mainly influence the dose of bacterial contamination. Some authors mention procedures with a risk of intraperitoneal contamination, e.g. intestinal resection or enterotomy, as a risk factor (Honnas and Cohen 1997; Mair and Smith 2005a; Phillips and Walmsley 1993), while others did not find an association (French et al. 2002; Smith et al. 2007). Prolonged anesthesia duration is another risk factor for SSI (Smith et al. 2007).

Risk factors related to wound suturing are mainly associated with the local resistance against infection. Important issues are the presence of suture material as a

nidus for bacterial growth, death space formation and ischemia due to suture tension. Reported risk factors for linea alba closure include its dissection prior to closure (Mair and Smith 2005a) and the use of polyglactin 910 (Honnas and Cohen 1997) or chromic gut (Gibson et al. 1989). The use of antibacterial (triclosan) coated polyglactin did not improve SSI rates (Bischofberger et al. 2010). A near-far-far-near suture pattern was associated with increased risk of infection compared to a simple interrupted pattern (Kobluk et al. 1989). The presence of a third, subcutaneous suture layer decreases death space formation but adds more suture material. There is no definite agreement on its positive or negative effect but two-layer closure is considered a safe alternative (Coomer et al. 2007; Smith et al. 2007).

The presence of bacteria or the number of bacterial colony forming units (CFU) at the level of the incision, determined after surgical scrub or at incisional closure, were not predictive for SSI development (Galuppo et al. 1999; Ingle-Fehr et al. 1997). However, the number of CFU measured directly after anesthetic recovery did have an association with SSI (Galuppo et al. 1999). A complicated recovery is also a risk factor for SSI (Freeman et al. 2012). These are indications that the applied surgical aseptic preparation protocols are effective and that true bacterial contamination mainly occurs in the early postoperative period. Protection of the wound during recovery and in the immediate postoperative period seems important. The instillation of an antimicrobial drug in the wound before closure was identified as a protective factor. Covering the wound with an incise drape during anesthetic recovery also protected against SSI development (Galuppo et al. 1999; Ingle-Fehr et al. 1997; Mair and Smith 2005a). Wound protection by a stent, sutured to the skin before recovery and left in place for 3 days postoperatively, was not effective. It even increased the risk of SSI (Mair and Smith 2005a). However, the application of a separate elastic adhesive bandage during the postoperative period did significantly reduce the risk for SSI (Smith et al. 2007).

In addition to the external protection provided by covering the incision, a correct apposition of the skin helps to prevent the entrance of bacteria. In human medicine, the use of intradermal sutures seems favorable in gastrointestinal surgery. Skin

staples promote dead space formation, while transdermal sutures penetrate the dermal barrier, causing more inflammation (Kobayaski et al. 2011). The comparison between staples and sutures has led to various results and at the moment a large randomized controlled trial on the subject is being conducted (Slade Shantz et al. 2012). In horses, reports on the best skin closure techniques to prevent SSI are unfortunately lacking.

ANTIBIOTIC PROPHYLAXIS

The purpose of perioperative antibiotic administration is to obtain bactericidal concentrations at tissue level from the time of first incision until wound closure. For this purpose, administration of the drug within 60 minutes before the incision is recommended for most antibiotics. Only fluoroquinolones and vancomycin have to be administered 120 minutes in advance (Bratzler and Houck 2004). Delayed onset of antibiotic treatment is disadvantageous; postoperative onset even results in the same number of SSI as in patients that did not receive any prophylaxis (Stone et al. 1976). Antibiotic drugs have to be redosed if surgery is still in progress two half-lives after the first dose. The half-life of IV benzylpenicillin (penicillin G) in the horse being only 40-50 min (Horspool et al. 1995; Love et al. 1983) makes repeated dosing often necessary in colic surgery. Since important bacterial contamination probably occurs during anesthetic recovery, it might be appropriate in horses to redose penicillin at the end of surgery, in order to maintain tissue levels for the duration of recovery and even a few hours after.

Prolonged antibiotic therapy after surgery does not help to prevent SSI in human patients and it is associated with the emergence of resistant bacterial strains (Harbarth et al. 2000; McDonald et al. 1998). Guidelines for human medicine therefore recommend restriction of prophylaxis to the first 24h postsurgery, even for abdominal colorectal surgery (Bratzler and Houck 2004). In equine colic surgery, most patients are dosed perioperatively with broad spectrum antibiotics, often IV benzylpenicillin and gentamicin. In a large study performed in the USA, duration

varied from a single dose to 5 days (median 1 day) for colic surgery without intestinal penetration or with bowel decompression. Some surgeons did not administer prophylactic antibiotics at all in these cases. Performing an enterotomy or intestinal resection or the presence of ischemic bowel or peritonitis led to longer duration of prophylaxis, varying from 1-10 days but mostly about 5 days. In specific cases metronidazole was added to the regimen (Traub-Dargatz et al. 2002). There is no evidence-based consensus on long-term antibiotic treatment in surgical colic horses.

TREATMENT

In the face of wound drainage, ultrasonographic evaluation is useful to assess the extension of the infection and to identify internal pockets or abscesses. It is also a reliable method to monitor the progression of healing (Wilson et al. 1989). After evaluation of the wound and sampling for bacterial culture (see below), the basic principles for local wound care apply. It is important to provide a route for exudation by removing a few skin sutures or staples. Wound debridement may be performed to remove devitalized and infected tissue. Any flushing of the wound must be done with caution, because it might propagate the infection along the incision line (Ahern and Richardson 2011). To prevent hernia formation, a specially designed abdominal belt (CM Heal Hernia Belt, CM Equine Products, Norco, CA) may be used. Preliminary results seem favorable (Klohnen 2008).

Bacterial culturing is important for the detection and identification of associated bacteria and for sensitivity testing. Ideally, samples are taken deep in the surgical site after aseptic preparation (Ahern and Richardson 2011). Common isolates from the ventral midline of colic horses in the perioperative period are *Streptococcus* and *Staphylococcus* spp and *Escherichia coli* (Ingle-Fehr et al. 1997). *Streptococci*, *Staphylococci*, Enterobacteriaceae, *Pseudomonas*, *Actinobacter* species and anaerobes have been cultured from -mostly orthopedic- SSI in horses (McDonald et al. 1994;

Schneider et al. 1992; Smith and Ross 2002). Specific information on bacteria involved in ventral midline SSI is lacking.

Even in the presence of a bacterial infection, systemic antibiotic therapy is only indicated in horses that remain febrile after drainage or present with excessive edema or cellulitis (Hardy and Rakestraw 2011). Antibiotic treatment for SSI should always be based upon antibiogram results. Indeed, resistant bacterial strains like methicillin-resistant *Staphylococcus aureus* (MRSA) and β-lactamase producing *Escherichia coli* are an emerging problem in equine medicine. Nasal, fecal and skin carriage is increased after antibiotic treatment or prolonged hospitalization (Johns et al. 2012; Maddox et al. 2012; Van den Eede et al. 2012; Weese et al. 2006). In the face of perioperative antibiotic treatment, wound contamination with resistant nosocomial strains (hospital associated infection) as well as selection of resistant strains already present on the horse (community associated infection) may lead to the involvement of resistant species in SSI. Although clinical MRSA and extended-spectrum β-lactamase (ESBL) infections have been reported in horses (Anderson et al. 2009; Bergström et al. 2012; Dierikx et al. 2012), the relative proportion of resistant bacteria in ventral midline SSI is unknown.

1.3. CONCLUSIONS

Postoperative complications after colic surgery have important economic, welfare and prognostic consequences. This may negatively influence horse owners in their choice for potentially life-saving colic surgery and decrease success rates for the horses that do receive surgical treatment. Two of the most important complications are postoperative ileus and surgical site infections.

Postoperative ileus is a major complication of small intestinal colic surgery. Clinical signs of endotoxemia and the presence of reflux at admission are notorious risk factors for POI development. The performance of a small intestinal resection was

also reported as a risk factor, but last reports date from 2004 (Cohen et al. 2004; French et al. 2002; Roussel et al. 2001).

During the last decade, intra- and/or postoperative prophylaxis with intravenous lidocaine has become increasingly popular. Even though a growing number of studies demonstrate its *in vitro* anti-inflammatory and prokinetic potential, large scale clinical studies in horses are lacking. Its apparent protective effect against endotoxemia (Peiro et al. 2010) also warrants further research.

Advances in surgical techniques and medical care in general, and the administration of IV lidocaine in particular, may have changed POI morbidity and mortality rates and the effects of intestinal resection and other risk factors. This needs to be evaluated in view of evidence based medicine.

However, even with the new treatment possibilities, the multifactorial nature of ileus makes this complication difficult to understand and even harder to tackle. Even though the major pathophysiological mechanisms seem to be unveiled, more research is needed in order to unravel the underlying molecular pathways and develop specific treatments. Many human prokinetic drugs target serotonin receptors. It has been demonstrated in horses that serotonin provokes small intestinal contractions. However, the serotonin receptor is also known for its tachyphylactic features: it desensitizes in the presence of serotonin overload. Changes in serotonin plasma concentrations in colic horses need to be studied to develop pathophysiological insights and to provide a rationale for the use of serotonergic drugs. Serotonin might also be a marker for intestinal compromise. Therefore, a reliable method for free plasma serotonin quantitation in horses needs to be developed.

Another important complication is infection of the surgical incision. Surgical site infections occur frequently, often prolong the hospitalization period and increase the associated cost and may have long-term consequences like hernia and adhesion formation. There are many indications that postoperative wound contamination is a major determinant in SSI development. A number of authors have examined different possibilities for protective covering of the wound, but no information is available on

the results of different skin closure techniques in horses. Knowledge of associated bacteria is also very limited.

Despite the progression already made in surgical and postoperative treatment of equine colic, some of the associated complications still seem hard to avoid. More research is needed to fill in the gaps of current knowledge and improve success rates of colic surgery.

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CHAPTER 2:

SCIENTIFIC AIMS

The general aim of this PhD study was to study two of the most important complications of equine colic surgery: postoperative ileus and surgical site infections. Although they have been extensively studied before, these complications are still highly abundant. Both have important economic, prognostic and welfare consequences. An up to date risk factor analysis and in depth pathophysiological research are needed. A major future goal is to decrease the prevalence of postoperative complications and thereby increase the survival rates of equine colic surgery.

Therefore, the first part of this dissertation focuses on the evaluation of the epidemiology and pathophysiology of postoperative ileus. The **first objective** was to perform a detailed evaluation of the current risk factors for ileus and to determine its morbidity and mortality rates in the Ghent University equine clinic. The **second objective** was to assess the efficacy of the prokinetic drugs lidocaine and metoclopramide in the prophylaxis of postoperative ileus in a clinical situation. The **third objective** was to develop a solid method for the determination of plasma serotonin concentrations and to compare the results with those of a test suitable for clinical purposes. The **fourth objective** was to evaluate plasma serotonin changes in surgical colic horses and to examine the potential of this parameter as a predictor for ileus or non-survival.

In the second part of this PhD study, the epidemiology of surgical site infections occurring after colic surgery in the Ghent University equine clinic was studied. The **fifth objective** was to determine the incidence of surgical site infections in relation to the applied skin closure technique.

CHAPTER 3:

POSTOPERATIVE ILEUS

3.1.

RISK FACTORS FOR EQUINE POSTOPERATIVE ILEUS AND EFFECTIVENESS OF PROPHYLACTIC LIDOCAINE

S. Torfs,[a] C. Delesalle,[a,b] J. Dewulf,[c] L. Devisscher,[d] P. Deprez[a]

[a] Department of Large Animal Internal Medicine, Faculty of Veterinary Medicine, Ghent University, B-9820 Merelbeke, Belgium.

[b] Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, NL-3584 CM Utrecht, The Netherlands.

[c] Department of Reproduction, Obstetrics and Herd Health, Veterinary Epidemiology Unit, Faculty of Veterinary Medicine, Ghent University, B-9820 Merelbeke, Belgium.

[d] Department of Surgery and Anesthesia of Domestic Animals, Faculty of Veterinary Medicine, Ghent University, B-9820 Merelbeke, Belgium

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SUMMARY

Background: Postoperative ileus (POI) is a frequent and often fatal complication of colic surgery. Reliably effective treatments are not available.

Objectives: To determine risk factors and protective factors associated with POI, and to assess the effect of lidocaine IV on short-term survival.

Animals: One hundred and twenty-six horses that underwent small intestinal colic surgery and that survived for at least 24 hours postoperatively.

Methods: Retrospective cross-sectional study. The association of 31 pre-, intra-, and postoperative variables with POI and the association of lidocaine treatment with short-term survival were investigated. Associations were evaluated with univariable logistic regression models, followed by multivariable analysis.

Results: Significant associations of high heart rate (odds ratio [OR] = 1.05, 95% confidence interval [CI] 1.03–1.08), the presence of more than 8L of reflux at admission (OR = 3.02, 95% CI 1.13–8.02) and the performance of a small intestinal resection (OR = 2.46, 95% CI 1.15–5.27) with an increased probability of POI were demonstrated. Prophylactic lidocaine treatment was significantly associated with a reduced incidence of POI (OR = 0.25, 95% CI 0.11–0.56). Lidocaine treatment was also significantly associated with enhanced short-term survival (OR = 0.30, 95% CI 0.09–0.98).

Conclusions and Clinical Importance: The variables associated with an increased risk of POI can be useful in identifying horses at risk of POI and in providing a more accurate prognosis. The results are supportive for lidocaine IV as an effective prokinetic treatment after small intestinal colic surgery.

INTRODUCTION

Postoperative ileus (POI) is a serious and often fatal complication in horses with small intestinal colic. Recent studies indicate that the overall prevalence of POI among horses with colic ranges from 10 to 19%, increasing to 50% when only horses with small intestinal colic are taken into account.[1–7] Risk factors previously associated with the development of POI include an increased PCV and heart rate, the presence of reflux at admission, small intestinal involvement, increased duration of anesthesia, and performance of a small intestinal resection.[2–4,6,8] The associated clinical signs include gastrointestinal reflux, small intestinal distention, hemoconcentration, tachycardia, and abdominal pain.[8,9]

The success rate of treating ileus is rather low and associated financial costs can be high. POI is a risk factor for repeat celiotomy[4,10] and horses that recover from POI are more likely to suffer from recurrent episodes of colic after discharge.[11] The high case-fatality rates associated with equine POI, ranging from 14 to 52%,[1,6,7,12,13] are in sharp contrast with the human situation, where the condition is rarely fatal.[14]

By identifying relevant predisposing or protective factors, more insight can be gained in the pathophysiology of POI in horses, which will help to tackle this troublesome problem and to develop optimal, evidence based treatment protocols. Currently, lidocaine is the prokinetic agent most frequently used for treatment of POI in equine practice,[15] although scientific evidence on its prokinetic and analgesic effectiveness is limited. The evidence on lidocaine as a prokinetic drug is still inconclusive in human medicine.[16]

The purpose of this study was to analyze the association of pre-, intra-, and postoperative factors with development of POI and to assess the influence of postoperative lidocaine treatment on the incidence of POI and on short-term survival.

MATERIALS AND METHODS

STUDIED DATA AND DEFINITIONS

The study population included a total of 157 small intestinal surgical colic cases, presented to the Large Animal Internal Medicine Clinic of Ghent University, Belgium between March 1, 2004 through December 31, 2006. Inclusion criteria were survival for at least 24 hours postoperatively and age ≥ 1 year; pony breeds were excluded from the study.

Recorded variables included sex, age and weight of the horse, duration of signs before admission, sedation at home (no sedation, detomidine, romifidine or an opiate) and sedation at the clinic (no sedation, xylazine or detomidine). The recorded admission parameters were heart rate, mucous membranes (normal, congested or cyanotic), capillary refill time (CRT; < 2 s or ≥ 2 s), skin turgor (normal or decreased), borborygmi on the left side (normal, reduced or tympanic), borborygmi on the right side (normal, reduced or tympanic), plasma packed cell volume (PCV), blood pH, base excess, presence of reflux (> 2 L of reflux upon nasogastric intubation) and quantity of reflux (≤ 8 L or > 8 L). The intraoperative variables included pre-anesthetic sedative (xylazine, detomidine or romifidine), surgeon (S1 through S6), nature of the lesion (strangulating or non-strangulating), small intestinal resection, intestinal emptying (defined as the removal of intestinal contents during or after resection, by jejunal or cecal enterotomy), anesthesia duration, lowest mean arterial pressure (MAP), hypotension (defined as MAP < 70 mm Hg recorded for at least 15 minutes), dobutamine administration, hypoxemia (PaO_2 < 95 mm Hg [12.65 kPa] in at least one arterial blood sample), lowest recorded base excess and lidocaine administration. The postoperative variables included prophylactic lidocaine treatment and prophylactic metoclopramide treatment for the outcome variable POI; for the outcome variable short-term survival, recorded variables were postoperative ileus, curative lidocaine treatment, curative metoclopramide treatment and erythromycin treatment.

Clinical examination at admission encompassed a routine general examination, followed by rectal examination, transabdominal ultrasound (Sonos 100, 2.5 MHz sectorial probe, HP, Diegem, Belgium), and passing of a nasogastric tube for performance of gastric decompression. This tube was left in place until the completion of surgery.

During anesthesia, all but 11 horses received a continuous rate infusion (CRI) with lidocaine (Laocaine, Schering-Plough Animal Health, Segré, France) (loading dose 1.5 mg/kg followed by 0.033 mg/kg/min). A CRI of dobutamine (Dobutamine EG, Eurogenerics, Brussels, Belgium) was started at a dose of 0.5 µg/kg/min in horses with hypotension and was gradually increased to a maximum dose of 1.97 µg/kg/min, aiming to obtain an MAP > 70 mmHg.

Transabdominal ultrasound to check for gastric distention with fluid[17,18] was routinely performed 12 hours after surgery or, according to the clinician's discretion, if a horse had an elevated heart rate or was uncomfortable. If gastric liquid content was observed or if the horse remained uncomfortable, nasogastric intubation was performed. In line with earlier reports,[3,6] the following case definition of POI was adopted: more than 20 L of reflux retrieved within 24 hours, or more than 8 L of reflux retrieved at a single occasion. Short-term survival was defined as survival to discharge from the hospital.

Three different prokinetic drugs were used postoperatively: lidocaine (loading dose 1.3 mg/kg IV followed by a CRI of 0.05 mg/kg/min), metoclopramide (Primperan, Sanofi-Synthelabo, Brussels, Belgium) (0.05 mg/kg IM q6h) and erythromycin (Erythrocine I.V. 1 g, Abbot, Louvain-la-Neuve, Belgium) (2 mg/kg IV in 1 L of physiologic solution q12h). Lidocaine and metoclopramide were used as both prophylactic and curative treatments (see below). Erythromycin was only used as a treatment in horses with prolonged POI, when other prokinetics seemed to have failed. Prophylactic prokinetic treatments were started immediately postoperatively, as soon as the horse was stabled, and discontinued if the horse's clinical parameters, blood PCV and base excess remained within normal limits for 24 hours. For lidocaine, the infusion rate was halved (0.025 mg/kg/min) 24 hours prior to cessation of CRI.

STATISTICAL ANALYSES

Descriptive statistics of continuous variables were presented by median and range. For categorical data, frequencies of occurrence were presented.

The relation between the variables listed above and the occurrence of POI was analyzed using logistic regression (SPSS 15.0, SPSS Inc., IL). First, the relation of each variable to POI was evaluated in a univariable logistic regression model. Odds ratios (OR), including 95% confidence intervals (CI), were reported for all variables with a *P*-value below .20.

For all continuous variables, the shape of the relationship with the outcome variable was assessed by plotting the log odds of the outcome versus the continuous variable.[19] Whenever a non-linear relationship was observed, the variable was categorized using logical cutoff values based both upon the shape of the plot and biological reasoning.

All variables with a univariable $P < .20$ were included in a multivariable logistic regression model, which was constructed in a backward stepwise manner. Pearson's and Spearman's ρ correlations between the parameters were determined, and if two variables were highly correlated ($R^2 > 0.60$), only the variable with the smallest *P*-value was included in the multivariable model. In the final multivariable model, all two-way interactions between significant variables were evaluated. Significance was set at $P < .05$.

Uni- and multivariable models were created in a similar way for short-term survival. The purpose of these models was to evaluate the effect of lidocaine treatment on survival, independent of the presence of POI or other possible confounding factors.

RESULTS

POPULATION CHARACTERISTICS AND CLINICAL FINDINGS AT ADMISSION

The master dataset contained 126 records of horses with small intestinal surgical colic that fulfilled the inclusion criteria. However, certain data could not be retrieved in all horses. The study population included 47 (38%) mares, 45 (36%) geldings and 32 (26%) stallions. The median age of horses was 9 years (range 1 to 26 years). The exact breed of the horses was often (n = 64) not recorded; 39 out of 62 horses of known breed were warmbloods. Median body weight was 548 kg (range 250-720 kg).

The duration of signs at admission ranged from 1 to 72 hours, with a median duration of 8 hours. Given the non-linear relationship between duration of signs at admission and the log odds of POI and short-term survival, it was decided to dichotomize this variable into 2 categories (< 20 hours and ≥ 20 hours). One hundred and five horses arrived within 20 hours; 21 had a longer duration of signs.

Overall, a total of 40 (32%) horses had been sedated; either by the referring veterinarian (26 out of 96 horses; of which 8 horses with detomidine; 8 with romifidine and 10 horses with an opiate) and/or at arrival in the clinic (20 out of 123 horses). For the latter category, in the first year reviewed, mostly detomidine (Domosedan, Pfizer Animal Health, Louvain-la-Neuve, Belgium) (10 µg/kg bwt) was used (n = 12) and later on xylazine (Xyl-M, VMD, Arendonk, Belgium) (0.7 mg/kg bwt) was preferred (n = 8). Both at home and at the clinic, primarily the horses with strangulating lesions had been sedated.

The clinical parameters at admission varied widely. Heart rate varied between 36 and 112 beats/min (median 60 beats/min). Mucous membranes were of normal color in 70 horses (57%), congested in 45 (37%) and cyanotic in 8 (6%). The capillary refill time was prolonged in 50/117 horses (43%) and skin turgor was decreased in 47/118 horses (40%). Borborygmi on the left side were normal in only 7 horses (6%), reduced or absent in 107 (87%) and reduced and tympanic upon

percussion in 9 (7%) cases. On the right side of the abdomen, tympanic sounds were heard more often (*n* = 15; 12%). PCV ranged between 25 and 74% (median 40%). Since the relationship between PCV and the log odds of POI and short-term survival was non-linear and the curve showed a clear bend around 50%, it was decided to dichotomize this variable into 2 categories (< 50% and ≥ 50%). Twenty out of 126 horses (16%) had PCV values ≥ 50%. Blood pH and base excess ranged between 7.21 and 7.55 (median 7.38) and -12 and +11 mEq/L (median +1.9 mEq/L), respectively. Upon nasogastric intubation, 33 horses (26%) had > 2 L of reflux; 20 of them were presented with > 8 L of reflux.

Prior to surgery, horses were sedated using xylazine (*n* = 77; 66%), romifidine (Sedivet, Boehringer Ingelheim, Brussels, Belgium) (80 µg/kg bwt) (*n* = 28; 24%) or detomidine (*n* = 12; 10%), depending on the anesthetist at service. When sedation had already been performed at the internal medicine department, the same sedative was used as anesthetic pre-medication.

INTRAOPERATIVE PARAMETERS

The study population encompassed 84 (67%) strangulating and 42 (33%) non-strangulating small intestinal obstructions. A small intestinal resection was carried out in 55 cases (44%); 50 of these horses had strangulating lesions, and 5 were diagnosed with non-strangulating lesions. An ileal bypass without concurrent resection was performed 5 times. During or after resection, removal of intestinal contents by cecal or jejunal enterotomy was performed in 15/52 (29%) cases, mostly (*n* = 14) strangulating obstructions. In the remaining 66 horses, only decompression, repositioning and/or manual stripping of small intestinal content into the cecum was necessary, followed by an enterotomy of the cecum in 25 horses.

Duration of anesthesia ranged between 30 and 315 minutes (median 130 min). Hypotension (*n* = 102) and/or hypoxemia (*n* = 63) was encountered in many cases. Median lowest MAP was 57 mm Hg (range 18-105 mm Hg) and dobutamine was

administered to 98 horses. The lowest recorded intraoperative base excess ranged from -11 to +8 mEq/L (median -2 mEq/L).

POSTOPERATIVE PERIOD

Prokinetic drugs were administered postoperatively to 109/124 horses (88%). Lidocaine (*n* = 77) and metoclopramide (*n* = 55) treatments were started immediately after surgery for the prevention of ileus (referred to as prophylactic treatment). In 43 of these cases, the combination of lidocaine and metoclopramide was used. Sometimes prokinetic treatment was not given until the horse developed ileus (referred to as curative treatment). In those cases, lidocaine (*n* = 12) was often combined with other prokinetics. Nine horses received lidocaine and erythromycin, 2 received lidocaine, erythromycin and metoclopramide and 1 horse was treated with lidocaine and metoclopramide. A few horses received single treatments with metoclopramide (*n* = 3) or erythromycin (*n* = 5).

Out of 126 horses that survived longer than 24h postoperatively, 41 (33%) developed POI. Frequencies were comparable in the non-strangulating group (14/42; 33%) and the strangulating group (27/84; 32%). In the group of horses that received prophylactic lidocaine treatment, only 16 out of 77 (21%) developed ileus, while in the untreated group, 24 out of 47 horses (51%) developed POI. In the prophylactic metoclopramide group, 15 out of 55 horses (27%) developed POI; out of 69 untreated horses, 25 (36%) were affected.

The onset for need of gastric decompression ranged from less than one day up to 3 days postoperatively (median 1 day), and horses refluxed for 1 up to 15 days (median 6 days). Nine horses needed a repeat celiotomy, 6 of them due to POI.

Overall, 93/126 horses (74%) survived to discharge; survival rates in the non-strangulating group and the strangulating group were similar (31/42 horses; 74%

and 62/84 horses; 74%, respectively). POI had an adverse influence on outcome: only 14/41 POI-affected horses (34%) survived to discharge.

UNIVARIABLE AND MULTIVARIABLE ANALYSIS

The variables that passed univariable screening for association with the development of POI, are listed in Table 1. PCV, turgor and presence of reflux were excluded from the multivariable model because of high correlations with heart rate, CRT and quantity of reflux, respectively. In the multivariable model, the variables heart rate, quantity of reflux (≤ 8 L versus > 8 L), small intestinal resection and prophylactic lidocaine treatment remained significant (Table 2). There were no significant two-way interactions between these variables.

Table 1. Results of univariable analysis for the identification of risk factors for the development of equine postoperative ileus in 126 surgical small intestinal colic cases (2004-2006).

Variable	*n*	*P*-value	OR	95% CI for OR
Age (y)	124	.007	1.11	1.03-1.19
Heart rate (bpm)	123	< .001	1.05	1.03-1.08
CRT	117	.036		
< 2 s (Ref.)	67	-		
≥ 2 s	50	.036	2.31	1.06-5.06
Skin turgor	118	.11		
Normal (Ref.)	71	-		
Decreased	49	.11	1.89	0.87-4.10
PCV	126	.002		
< 50 % (Ref.)	106	-		
≥ 50%	20	.002	4.67	1.72-12.7
Presence of reflux (> 2 L)	125	.17		
No (Ref.)	92	-		
Yes	33	.17	0.56	0.25-1.28
Quantity of reflux	124	.027		
≤ 8 L (Ref.)	104	-		
> 8 L	20	.027	3.02	1.13-8.02
Surgeon	119	.16		
S1 (Ref.)	25	-		
S2	26	.37	2.82	0.29-27.5
S3	22	.40	2.67	0.27-25.9
S4	23	.089	7.20	0.74-70.2
S5	16	.85	1.26	0.12-13.6
S6	7	.29	3.60	0.35-37.6
SI resection	126	.021		
No (Ref.)	71	-		
Yes	55	.021	2.46	1.15-5.27
Lowest MAP (mm Hg)	120	.053	0.97	0.94-1.00
Hypotension	120	.13		
No (Ref.)	18	-		
Yes	102	.13	0.37	0.10-1.35
Prophylactic lidocaine	124	.001		
No (Ref.)	47	-		
Yes	77	.001	0.25	0.11-0.56

OR = odds ratio; CI = confidence interval; Ref. = reference value

Table 2. Results of multivariable analysis for the identification of risk factors for the development of equine postoperative ileus in 120 surgical small intestinal colic cases (2004-2006).

Variable	*n*	*P*-value	OR	95% CI for OR
Heart rate (bpm)	120	.002	1.05	1.02-1.08
Quantity of reflux	120	.032		
≤ 8 L (Ref.)	100	-		
> 8 L	20	.032	4.16	1.13-15.4
SI resection	120	.001		
No (Ref.)	67	-		
Yes	53	.001	6.41	2.23-18.4
Prophylactic lidocaine	120	.013		
No (Ref.)	46	-		
Yes	74	.013	0.31	0.12-0.78

OR = odds ratio; CI = confidence interval; Ref. = reference value

Concerning short-term survival, the parameters that passed univariable screening are shown in Table 3. In the multivariable model, only POI and lidocaine treatment (prophylactic and curative) remained significant (Table 4). No significant interactions were found between these parameters. Lidocaine-treated horses had 3.33-fold higher odds to survive to discharge (95% CI 1.02-11.1, P = .047) than untreated horses, whereas horses suffering from POI were 28.2 times more likely to die (95% CI 9.11-87.2, P < .001).

Table 3. Results of univariable analysis for the identification of risk factors for non-survival in 126 surgical small intestinal colic cases (2004-2006).

Variable	*n*	*P*-value	OR	95% CI for OR
Sex	124	.17		
Mare (Ref.)	47	-		
Gelding	45	.081	2.33	0.90-6.02
Stallion	32	.77	1.18	0.39-3.58
Heart rate (bpm)	123	< .001	1.05	1.02-1.08
CRT	117	.017		
< 2 s (Ref.)	67	-		
≥ 2 s	50	.017	2.81	1.21-6.55

(Table 3. Continued)

Variable	*n*	*P*-value	OR	95% CI for OR
Skin turgor	118	.083		
Normal (Ref.)	71	-		
Decreased	47	.083	2.10	0.91-4.87
PCV	126	.002		
< 50 % (Ref.)	106	-		
≥ 50 %	20	.002	5.17	1.87-14.3
Presence of reflux (> 2 L)	125	.13		
No (Ref.)	92	-		
Yes	33	.13	1.93	0.82-4.57
Quantity of reflux	124	.067		
≤ 8 L (Ref.)	104	-		
> 8 L	20	.047	2.73	1.01-7.36
Surgeon	119	.078		
S1 (Ref.)	25	-		
S2	26	.79	1.20	0.32-4.58
S3	22	.017	4.80	1.32-17.4
S4	23	.82	0.84	0.20-3.62
S5	16	.42	1.82	0.43-7.69
S6	7	.73	0.67	0.07-6.87
SI resection	126	.063		
No (Ref.)	71	-		
Yes	55	.063	2.15	0.96-4.81
Intestinal emptying	52	.077		
No (Ref.)	37	-		
Yes	15	.077	3.09	0.89-10.7
Anesth. duration (10 min)	120	.14	1.05	0.98-1.13
Lowest MAP (mm Hg)	120	.10	0.98	0.95-1.01
Lidocaine in anesthesia	124	.037		
No (Ref.)	11	-		
Yes	113	.037	0.03	0.07-0.93
Lidocaine (proph. + cur.)	124	.074		
No (Ref.)	35	-		
Yes	89	.074	0.46	0.20-1.08
Erythromycin (curative)	124	< .001		
No (Ref.)	108	-		
Yes	16	< .001	9.11	2.86-29.1
Postoperative ileus	126	< .001		
No (Ref.)	85	-		
Yes	41	< .001	25.4	8.87-72.7

OR = odds ratio; CI = confidence interval; Ref. = reference value

Table 4. Results of multivariable analysis for the identification of risk factors for non-survival in 124 surgical small intestinal colic cases (2004-2006).

Variable	*n*	*P*-value	OR	95% CI for OR
Lidocaine (proph. + cur.)	124	.047		
No (Ref.)	35	-		
Yes	89	.047	0.30	0.09-0.98
Postoperative ileus	124	< .001		
No (Ref.)	84	-		
Yes	40	< .001	28.2	9.11-87.2

OR = odds ratio; CI = confidence interval; Ref. = reference value

DISCUSSION

POSTOPERATIVE ILEUS MORBIDITY, MORTALITY AND RISK FACTORS

Postoperative ileus in horses in this study was associated with highly increased odds of death. This underlines once again the need for reliable identification of horses at risk and for the development of effective prophylactic and curative treatment protocols for this condition. A high heart rate, the presence of more than 8 liters of reflux at admission and the performance of a small intestinal resection were identified as risk factors associated with development of POI.

Several studies have shown that an impaired cardiovascular state, particularly an elevated PCV, is related to a higher incidence of equine POI.[3,4,6,8] This is again confirmed in the present study, in which the variables PCV, CRT and heart rate all passed univariable screening. Heart rate and PCV were highly correlated (R^2 = 0.61), and in the final multivariable model only heart rate, the main associated factor, was retained.

The association between the presence of reflux at admission and the occurrence of POI has been demonstrated previously.[2] Furthermore, the findings of this study are suggestive for an increased risk of POI in horses presented with more than 8 L of reflux.

Although use of α_2-agonists has been reported to suppress duodenal motility,[20] in this study no significant association to POI of sedation or type of sedative used either at home, at admission or pre-anesthetically could be demonstrated.

In line with expectations, the performance of a small intestinal resection was strongly associated with an increased risk for development of POI. This highlights, in accordance with earlier findings,[1] the importance of timely referral and prompt surgical intervention. In cases where a resection was performed, the removal of intestinal contents via the site of resection or by cecotomy did not have a significant influence on the prevalence of POI. When interpreting these results, one should take into account the limited number of occasions during which this procedure was carried out in the studied population (n = 15). Further research is necessary to gain a clearer view on that matter.

PROPHYLAXIS AND TREATMENT OF POSTOPERATIVE ILEUS

For the medical treatment of POI, three types of drugs were used during the study. Erythromycin was only used in very persistent cases and was not significantly associated with outcome in the multivariable model. Metoclopramide had no significant influence on occurrence of POI, in contrast to a previous study.[21] However, due to the occasional occurrence of extrapyramidal side effects associated with its intravenous use, metoclopramide was administered intramuscularly and at a much lower dose in this study. This could explain the observed lack of effect.

The current findings are supportive for a significantly reduced risk of POI in horses that received prophylactic lidocaine treatment. The results also suggest a beneficial effect of lidocaine treatment on survival rates. Interestingly, the positive effect of lidocaine on survival was not related just to its protective effect in preventing POI, for the effect on survival was significant in a multivariable model, independent of POI. Various other properties of lidocaine, such as anti-inflammatory,

analgesic and/or anti-endotoxin effects, likely mediated this effect of lidocaine on short-term survival.

Results of studies on lidocaine treatment have heretofore been variable. *In vitro*, the prokinetic effect of lidocaine was restricted to proximal duodenal smooth muscle strips, with no effect on jejunal or pyloric antral strips.[22] No evidence was found for any prokinetic effect of lidocaine treatment on postoperative jejunal motility in healthy horses.[23] Prophylactic lidocaine treatment did have some positive effects on intestinal contractility after colic surgery, but there was no effect on the development of gastric reflux or on survival.[24] This lack of effect on survival was in accordance with a later study,[25] in which POI-affected horses were nevertheless found to exhibit an earlier inhibition of reflux and an earlier discharge after treatment with lidocaine.

The lack of prokinetic effects of lidocaine, encountered in both *in vitro* studies and in healthy horses, could indicate that the drug exerts an indirect effect on motility by decreasing pain or intestinal inflammation. Lidocaine has been demonstrated to inhibit neutrophil activity[26] and attenuate cytokine response, as well as accelerate recovery of bowel function, in humans.[27] After colic surgery, significant neutrophilic inflammation occurs in the equine jejunum,[28] which supports the hypothesis that intestinal inflammation plays a role in the pathogenesis of POI. The anti-inflammatory properties of lidocaine could mediate the effects on POI and short-term survival that were observed in this study. It has also been demonstrated that systemic lidocaine treatment can protect the equine jejunum against the negative effects of flunixine meglumine,[29] which is reported to delay jejunal mucosal recovery after ischemic injury.[30] Before induction of anesthesia, as well as postoperatively, all the horses in the present study received flunixin meglumine as an analgesic treatment. It would be interesting to evaluate the effect of lidocaine in the absence of nonselective COX-inhibitors.

In 10 out of 47 horses in this study that did not receive prophylactic postoperative lidocaine treatment, lidocaine was not administered during surgery either. Therefore it cannot be ruled out that the demonstrated effects of lidocaine can be partially attributed to intraoperative administration. A trend (P = .084) towards

reduced odds of POI has been reported in horses that had been treated intraoperatively with lidocaine.[6] More research is necessary for a final conclusion.

CONCLUSIONS

Review of the literature reveals substantial variation between studies, with regard to the prevalence and outcome of POI. This can be explained by differences in the studied populations or by different definitions of POI, as well as by variations in treatment protocols. The variables heart rate, quantity of reflux at admission and small intestinal resection can be a useful aid in identifying horses at risk for POI and in providing a more accurate prognosis. The results of this study are suggesting a protective effect of intravenous lidocaine treatment against the development of POI and a positive effect on short-term survival. Further research is needed to get more insight into the prokinetic properties and mechanism of action of lidocaine.

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3.2.

COMPARATIVE ANALYSIS OF SEROTONIN IN EQUINE PLASMA WITH LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY AND ENZYME-LINKED IMMUNOSORBENT ASSAY

S. Torfs,[a] A. Maes,[b] C. Delesalle,[a,c] P. Deprez,[a] S. Croubels[b]

[a] Department of Large Animal Internal Medicine, Faculty of Veterinary Medicine, Ghent University, B-9820 Merelbeke, Belgium.

[b] Department of Pharmacology, Toxicology, and Biochemistry, Faculty of Veterinary Medicine, Ghent University, B-9820 Merelbeke, Belgium.

[c] Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, NL-3584 CM Utrecht, The Netherlands.

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SUMMARY

Serotonin is regularly measured in equine platelet poor plasma in research settings. However, reported reference values vary between studies, partially because plasma serotonin concentrations are very low and a reliable and affordable detection method is lacking. A simple, rapid, and sensitive method for serotonin determination in equine platelet poor plasma using liquid chromatography–tandem mass spectrometry (LC-MS/MS) was developed and validated. Results of a commercially available enzyme-linked immunosorbent assay (ELISA) were compared to the LC-MS/MS results, in order to validate a test more suitable for use in a clinical situation.

For LC-MS/MS, 500 µl of plasma was required, and deuterated serotonin was used as an internal standard. The sample preparation was based upon a simple liquid extraction into ethyl acetate. Chromatographic separation was performed with an acetic acid–acetonitrile mobile phase gradient elution. Linearity was demonstrated between 3 ng/ml and 100 ng/ml. A limit of quantification of 3 ng/ml was achieved, corresponding to a limit of detection of 0.10 ng/ml.

Comparison of LC-MS/MS and ELISA with Passing–Bablok regression and Bland–Altman plotting showed a poor agreement between the 2 methods, with an increasing difference within the higher range of measurements. Caution is needed when extrapolating results from sources using different analytical techniques.

INTRODUCTION

Serotonin (5-hydroxytryptamine [5-HT]) is an important signaling molecule in the body (Fig. 1 for chemical structure), exerting several neurotransmitter functions inside and outside the brain and playing a key role in regulating gastrointestinal motility. In the circulatory system, serotonin has a hormonal function modulating the vascular tone and also plays a role in platelet activation.[8,16] Most of the body's serotonin is produced by the enterochromaffin cells located in the intestinal mucosa. Overproduction of serotonin by enterochromaffin cells causes a 5-HT overflow towards the intestinal lumen and the portal circulation.[16] Plasma serotonin is quickly transported into the platelets via specialized transporters on the platelet membrane. Upon activation, platelets release significant amounts of serotonin, causing a rise in plasma 5-HT.[10,16] Therefore the concentration of free serotonin is typically measured in platelet poor plasma (PPP), produced by prolonged or high-speed centrifugation of plasma and containing < 10,000 platelets/µl.[27]

Increased plasma concentrations of 5-HT have been linked to multiple conditions in human medicine.[8] In horses, elevated 5-HT levels have been reported following endotoxemia.[5,10,21] An association has been found between increased plasma 5-HT concentrations and digital hypoperfusion causing laminitis.[4-6,21,22] Other research groups have described serotonin changes in surgical colic cases,[3,13] recurrent airway obstruction,[17] Cushingoid horses,[18] and other acute or chronic disease states.[3]

Serotonin plasma levels are also subject to physiological variations.[1,2,7,18,31] Even in healthy horses, reported reference values for serotonin are not consistent, which hampers further research into the role of serotonin in equine diseases. Reported PPP serotonin values in healthy horses range from 2.5 ng/ml to 90 ng/ml with a majority varying between 3 ng/ml and 30 ng/ml.[1,2,4,6,7,13,17-19,21] One possible explanation for this inconsistency is the use of different analytical techniques. Therefore, it is essential to develop fast and reliable detection methods, which are well validated for use on equine plasma samples.

Different methods have been described for serotonin measurement; however, most were developed for human plasma analysis. High-performance liquid chromatography (HPLC) with amperometric detection,[25,32] electrochemical detection,[1,6,30,33,35] coulometric detection,[20,34] and fluorescence detection[13] have been reported. It must be mentioned that none of these methods make use of a more conventional ultraviolet detector, due to the insufficient ultraviolet-absorbing capacities of serotonin. Therefore, all of these methods require specific detectors or inclusion of extra derivatization steps of the analyte.

Some liquid chromatography–tandem mass spectrometry (LC-MS/MS) methods have been developed for serotonin determination in human whole blood,[11] platelet rich plasma,[29] and PPP.[12,24] Most of these methods are indeed very specific and fast, the latter[12,24] based on the use of on-line sample preparation technologies that require extra hardware investments. Others used off-line solid-phase extraction as sample clean-up, which is a time-consuming and expensive method in a routine analytical environment. Both the complexity of the matrix to be assayed and the expected serotonin concentrations have to be considered when selecting a sample preparation technique.[25] It was, therefore, the aim of the current study to develop a simple, rapid, and sensitive method to quantify serotonin in equine PPP with LC-MS/MS detection.

Besides chromatographic methods, commercially available enzyme-linked immunosorbent assays (ELISAs)[3,23] or radioimmunoassays (RIAs)[18] have occasionally been used for rapid determination of serotonin in horse plasma or serum. A second aim of the present study was to compare the results obtained with the developed LC-MS/MS method with those obtained using a commercially available human serotonin ELISA kit. The use of such kits would greatly facilitate serotonin analysis in equine medicine, especially in a clinical situation.

MATERIALS AND METHODS

SAMPLES

Samples were obtained from healthy horses (*n* = 24; 41 samples) and horses undergoing laparotomy for small intestinal colic (*n* = 34; 123 samples). Of these samples, 152 platelet poor ethylenediamine tetra-acetic acid (EDTA) plasma samples were prepared according to protocols earlier described.[7,13] Further, 6 activated platelet samples, prepared by adding collagen (Equine collagen type 1, American Biochemical and Pharmaceutical Ltd., Epsom, UK) (15 µg/ml) to platelet rich EDTA plasma, and 6 serum samples were collected to obtain a wide range of serotonin concentrations.

LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY

STANDARD SOLUTIONS

For LC-MS/MS analysis, deuterated serotonin (5-HT-d4) (CDN Isotopes, Nieuwegein, The Netherlands) was used as an internal standard. Separate stock solutions of serotonin (Sigma-Aldrich NV/SA, Bornem, Belgium) and 5-HT-d4 of 1,000 µg/ml were prepared in 10% acetic acid (VWR International, Leuven, Belgium) in HPLC water (VWR International, Leuven, Belgium) and stored at +4°C. Stock solutions were determined to be stable for at least 182 days under these conditions. The stock solution was further diluted with HPLC water to obtain working solutions ranging from 30 ng/ml to 1,000 ng/ml for spiking PPP samples. The stock solution of the internal standard was also diluted with HPLC water to a final concentration of 1,000 ng/ml. Tuning solutions of 1,000 ng/ml serotonin and internal standard were made by diluting the stock solution with HPLC water.

SAMPLE PREPARATION

The LC-MS/MS method for determination of serotonin in equine PPP is based on a simple liquid extraction. To 500 µl of plasma, 50 µl of the internal standard solution (1,000 ng/ml) was added. The samples were vortex mixed for 10 sec; thereafter, 100 µl of 1 M sodium hydroxide (VWR International, Leuven, Belgium) was added, and the samples were vortex mixed again. Next, 4 ml of ethyl acetate (VWR International, Leuven, Belgium) was added, and the samples were put on a rolling device for 20 min. After centrifugation for 10 min at 3,200 × g, the supernatant was transferred to another tube and evaporated to dryness under a gentle stream of nitrogen at 40°C. The residue was reconstituted in 250 µl of 0.1% acetic acid in HPLC water, and an aliquot of 20 µl was injected into the LC-MS/MS system.

LC-MS/MS METHOD

The LC-MS/MS analyses were performed using a commercial system (Acquity 2695 LC system Quattro Ultima, Waters, Zellik, Belgium) consisting of a vacuum degasser, a quaternary MS pump, an autosampler with cooling device, and a triple quadrupole mass spectrometer (Acquity 2695 LC system Quattro Ultima, Waters, Zellik, Belgium) equipped with an electrospray ionization source operating in positive ion mode, run by Masslynx software (Acquity 2695 LC system Quattro Ultima, Waters, Zellik, Belgium).

For chromatographic separation, a 3.5 µm column (Eclipse Plus C18 3.5 µm column, Agilent Technologies Belgium NV/SA, Diegem, Belgium) (100 × 3.0 mm) with a guard column of the same type was used. A gradient elution, at a flow rate of 0.2 ml/min, was performed with mobile phase A consisting of 0.1% acetic acid in HPLC water, and mobile phase B of acetonitrile (VWR International, Leuven, Belgium). The following linear gradient was used: 0–3 min 99% A to 1% B, 5–6 min 60% A to 40% B, and 6.5–16 min 99% A to 1% B.

Operating conditions for the electrospray ionization source were optimized by infusing a mixture of serotonin and 5-HT-d4 each at a concentration of 10 µg/ml via a syringe pump. The following tune parameters were obtained for optimal serotonin detection: capillary voltage, 3.0 kV; cone, 20 V; cone gas flow-rate, 50 l/hr; desolvation gas flow rate, 750 l/hr; source temperature, 120°C; desolvation temperature, 200°C. These tune parameters were also suitable for the detection of 5-HT-d4, given the structural similarity between these components. The optimal collision energy in the MS/MS mode, corresponding to nearly 100% fragmentation of the protonated molecular ions of serotonin (m/z 176.9 for a relative molecular mass [M_r] of 176.21), was found to be 10 V. For 5-HT-d4 (m/z 180.9 for M_r of 180.23), the optimal collision energy was also determined at 10 V. Under these conditions, the most abundant product ion was at m/z 160 and 164 for serotonin and 5-HT-d4, respectively. Quantification was performed with the Masslynx software (Acquity 2695 LC system Quattro Ultima, Waters, Zellik, Belgium), using the above-mentioned product ions.

LC-MS/MS METHOD VALIDATION

The proposed method was validated by a set of parameters that are in compliance with the recommendations as defined by the European Commission.[14,15] The method was validated in pooled PPP obtained from 3 healthy horses. The following parameters were determined:

1. Linearity: determined on calibration curves using spiked samples at concentrations of 3, 5, 10, 20, 50, and 100 ng/ml. Peak area ratios between serotonin and 5-HT-d4 were plotted against the concentration of serotonin, and a linear regression was performed. The acceptance criterion was a correlation coefficient $R \geq 0.99$ and a goodness-of-fit coefficient $g \leq 10\%$.

2. Accuracy: determined by analyzing 6 independently spiked samples at the same spike level (2 levels evaluated: 5 ng/ml and 20 ng/ml). The accuracy (%),

expressed as the difference between the mean found concentration and the spiked concentration, should be in the range of –30% to +10% for levels < 10 ng/ml and –20% to + 10% for levels ≥ 10 ng/ml.

3. Precision: expressed as the relative standard deviation (RSD, %). For the within-day precision, the RSD should be lower than RSD_{max}, which is determined as two-thirds of the values calculated according to the Horwitz equation (i.e., $RSD_{max} = 2/3 \times 2^{(1-0.5 \log C)}$), with C being the concentration (g/ml) at which the sample is fortified. Precision was determined using the same samples as for the accuracy. The between-day precision was evaluated on quality control samples spiked at 10 ng/ml and 20 ng/ml. The samples were prepared and analyzed on different days. The RSD should be lower than the $RSD_{max} = 2^{(1-0.5 \log C)}$

4. Limit of quantification (LOQ): determined as the lowest concentration for which the method is validated with an accuracy and precision that fall within the ranges recommended by the European Union.

5. Limit of detection (LOD): determined as the lowest measured content from which it is possible to deduce the presence of the analyte with reasonable statistical certainty, using the criterion of a signal-to-noise (S/N) ratio equal to 3.

ENZYME-LINKED IMMUNOSORBENT ASSAY

Results obtained from a commercial human serotonin ELISA kit (Genway Biotech, San Diego, CA) were compared with the LC-MS/MS results. The ELISA was performed following the manufacturer's instructions. The assay principle is a competitive ELISA, with colorimetric detection performed by an ELISA microplate reader (Multiskan FC, Thermo Scientific, Erembodegem, Belgium) at 405 nm. The sample preparation step includes derivatization of serotonin to N-acylserotonin, which leads to a 23.5-fold dilution of plasma samples. The calibration curve was prepared with acylated serotonin standard delivered with the kit.

The recovery of serotonin from equine plasma was assessed by spiking PPP with equal amounts of acylated serotonin standard (Genway Biotech, San Diego, CA) at different concentrations (0.00, 0.08, 0.24, 0.73, 2.2, 6.6, and 19.8 ng/ml). Also, a dilution series was prepared by diluting acylated serotonin standard (19.8 ng/ml) with equine PPP (1:1; 1:2; 1:4; 1:8). Platelet poor plasma was used to obtain the lowest possible background serotonin levels. All analyses were performed in duplicate. Recovery was calculated as a percentage using the following formula: (measured 5-HT level/calculated 5-HT level) × 100. The recovery was assessed for sample serotonin concentrations ranging from 3.9 ng/ml to 236 ng/ml.

DATA ANALYSIS

Agreement between LC-MS/MS and ELISA results was assessed by Passing–Bablok regression analysis and Bland–Altman plotting. Passing–Bablok regression[28] is a nonparametric linear regression procedure. After plotting the concentrations determined by the 2 methods, the regression curve should not significantly deviate from the equation line (y = x). A slope significantly different from 1 indicates the presence of a proportional error while an intercept different from 0 can be related to a systematic error. Bland–Altman plotting[9] assesses the presence of a proportional error by evaluating the relationship between the differences between 2 observations (ELISA and LC-MS/MS serotonin concentration) and their average. The method is also suitable to reveal systematic bias and to identify outliers. All analyses were performed using MedCalc statistical software (MedCalc Software BVBA, Mariakerke, Belgium), and P values of < 0.05 were considered significant.

RESULTS

METHOD DEVELOPMENT AND VALIDATION: LC-MS/MS

The chemical structure of serotonin is shown in Figure 1. In the MS mode, the most prominent ion is the protonated molecular ion $[M+H]^+$ at m/z 176.9 for serotonin and at m/z 180.9 for 5-HT-d4. In the MS/MS mode, the most abundant ion is at m/z 160 for serotonin and at m/z 164 for 5-HT-d4. Figure 1 also shows an extracted-ion chromatogram of serotonin and 5-HT-d4 for a PPP sample spiked at the LOQ level of 3 ng/ml (Fig. 1A) and of an unspiked PPP sample (Fig. 1B). At the trace of 5-HT-d4, no peak could be detected in the PPP sample used as blank sample. For serotonin, there was a peak detected due to the endogenous serotonin level, but the intensity was almost 10 times lower than the spiked plasma at the LOQ level.

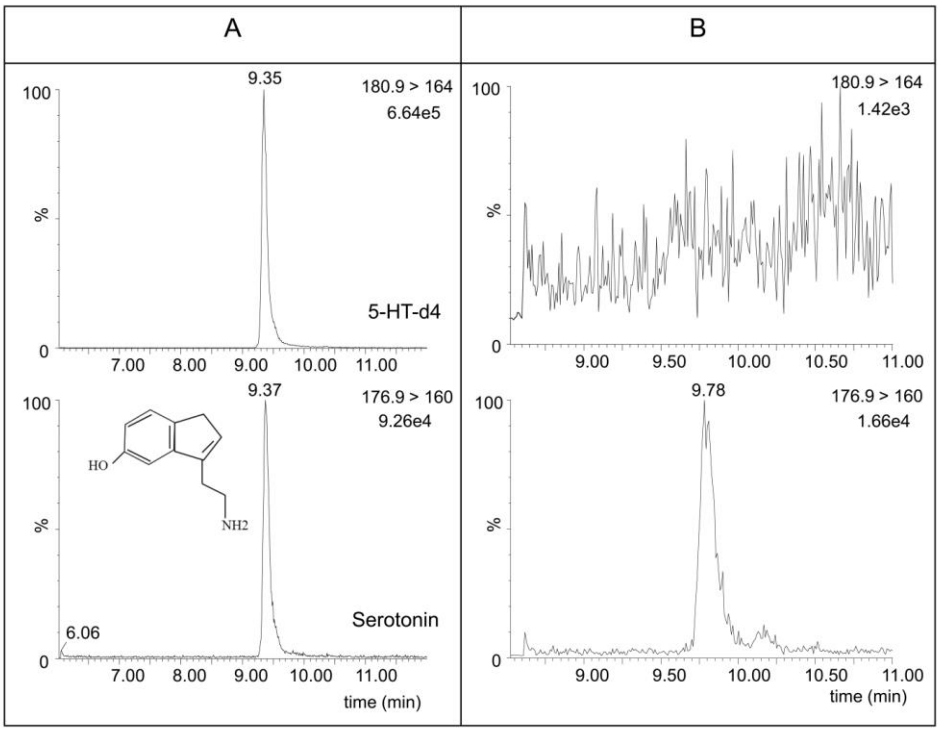

Fig. 1. Structure of serotonin and an extracted-ion chromatogram of serotonin and deuterated serotonin (5-HT-d4) for an equine platelet poor plasma sample spiked at 3 ng/ml (**A**) and for a unspiked equine platelet poor plasma sample (**B**).

In Figure 2, the calibration curve, ranging from 3 ng/ml to 100 ng/ml, obtained with the LC-MS/MS method is presented as the mean of 8 calibration curves. The 8 curves were made over a period of 259 days, and each curve originated from a new set of extractions. The goodness-of-fit coefficients (g) of the individual calibration curves were all < 10%, and the correlation coefficients (R) were all > 0.99.

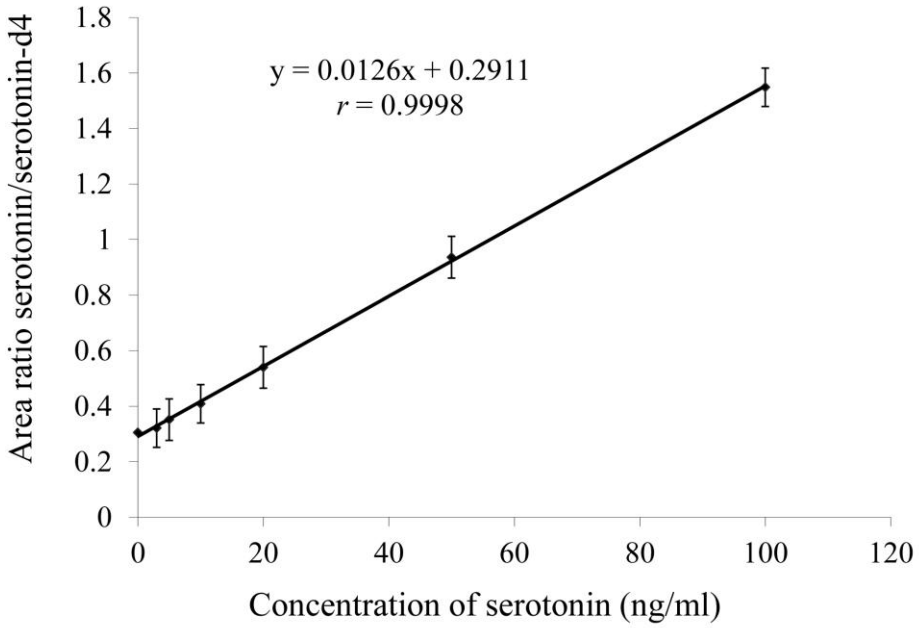

Fig. 2. Calibration curve of serotonin in horse plasma (platelet poor plasma) represented as the mean of 8 calibration curves obtained over a period of 259 days.

The results of the accuracy, within-day, and between-day precision evaluation are summarized in Table 1. The accuracy and precision fell within the specified ranges, proving a good repeatability and reproducibility of the method.

Table 1. Within- and between-day accuracy and precision of the liquid chromatography–tandem mass spectrometry method for quantification of serotonin in horse plasma (platelet poor plasma).

	Mean (ng/ml)	Accuracy (%)	RSD* (%)	RSD* max (%)
Within-day at:				
3 ng/ml† (*n* = 6)	3.0	–0.3	8.9	25.6
5 ng/ml (*n* = 6)	4.4	–12.7	5.3	23.7
20 ng/ml (*n* = 6)	18.9	–5.3	4.4	19.2
Between-day at:				
10 ng/ml (*n* = 18)	9.9	–1.2	8.1	32.0
20 ng/ml (*n* = 7)	21.7	8.5	8.2	28.8

* RSD = relative standard deviation.
† Limit of quantification.

Because a spike level of 3 ng/ml could be quantified fulfilling the criteria for accuracy and precision, it was set as the LOQ. The results are also summarized in Table 1. With an LOQ below the normal endogenous concentrations of serotonin, the sensitivity was considered suitable for the determination of serotonin levels in horse PPP.

For the determination of the LOD, the S/N ratio of the serotonin peak in the LOQ samples was used. The mean S/N ratio for the 6 LOQ samples at 3 ng/ml was 92.4, corresponding to an LOD of 0.10 ng/ml.

METHOD VALIDATION: ELISA SEROTONIN RECOVERY FROM EQUINE PLASMA

Recovery was calculated from equine PPP spiked with 7 different concentrations of 5-HT and from a dilution series of serotonin standard with 4 different amounts of equine PPP. Mean recovery was 105% (range: 83–127%; Table 2).

Table 2. Recovery of spiked serotonin from equine platelet poor plasma with a commercial enzyme-linked immunosorbent assay kit.*

Sample	Expected serotonin (ng/ml)	Mean serotonin (ng/ml)	Recovery (%)
Spiked			
0.00 ng/ml	3.9	4.2	108
0.08 ng/ml	4.8	4.0	83
0.24 ng/ml	6.7	6.0	90
0.73 ng/ml	12.5	12.1	97
2.2 ng/ml	29.7	31	104
6.6 ng/ml	81	71	88
19.8 ng/ml	236	294	125
Diluted			
1:1	236	300	127
1:2	122	147	120
1:4	65	68	105
1:8	36	40	111

* Expected serotonin values were calculated from the concentration of serotonin in the acylated platelet poor plasma sample and the acylated serotonin standard, multiplied by the dilution factor of 23.5 that was obtained in the acylation step.

METHOD COMPARISON

Results obtained from equine PPP, activated PRP, and serum with ELISA and LC-MS/MS were statistically compared. Regression analysis showed that the 2 methods were highly correlated (Pearson R^2 = 0.94, P < 0.001, n = 164). However, Passing–Bablok analysis gave a regression equation of ELISA = 1.62(LC-MS/MS) + 0.53. The 95% confidence interval (CI) for the slope did not include 1 (95% CI: 1.50–1.81; P < 0.001), indicating the presence of a proportional error (Fig. 3).

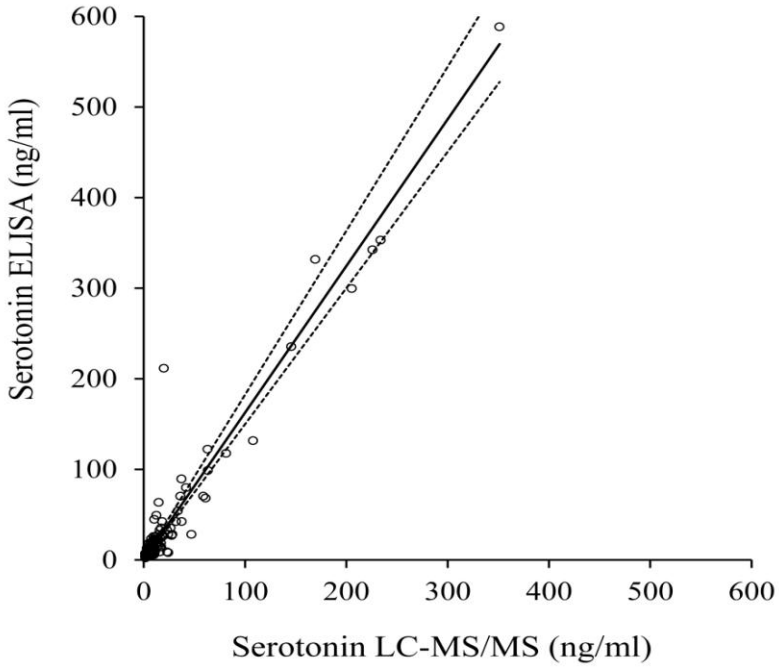

Fig. 3. Passing–Bablok analysis between plasma and serum analyzed by enzyme-linked immunosorbent assay (ELISA) or liquid chromatography–tandem mass spectrometry (LC-MS/MS) gave the correlation ELISA = 1.62(LC-MS/MS) + 0.53. The 95% confidence interval (CI) of the slope was 1.50–1.81; R^2 = 0.94; P < 0.001; n = 164. The Passing–Bablok regression line is represented by the solid line, with its 95% CI represented by the dashed line.

Bland–Altman plotting also demonstrated poor agreement between the methods, with an increasing difference within the higher range of measurements (Fig. 4). However, a second analysis only focusing on 5-HT values in the lower range of < 100 ng/ml did not demonstrate a good agreement, either.

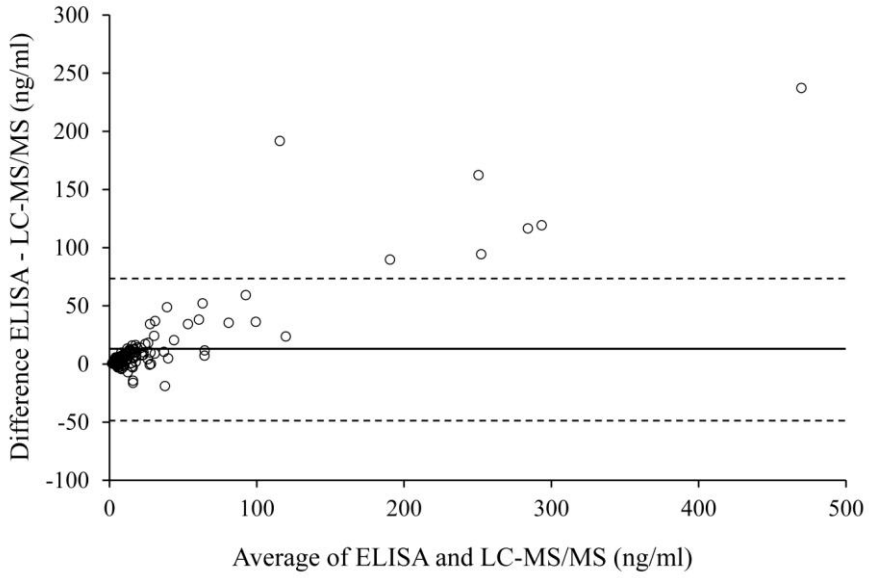

Fig. 4. Bland–Altman plot showing poor agreement between the enzyme-linked immunosorbent assay (ELISA) and liquid chromatography–tandem mass spectrometry (LC-MS/MS) method for determination of serotonin (5-HT) in equine plasma and serum, with a mean bias (solid line) of 12.5 ng/ml (ELISA–LC-MS/MS) and 95% limits of agreement (dashed lines) of –49.2 ng/ml to 74.3 ng/ml.

DISCUSSION

Multiple research groups have studied serotonin physiology and pathology in horses. However, a gold standard method for serotonin analysis is not available or published. Most commonly applied are HPLC and, to a lesser extent, ELISA methods. In the current study, a LC-MS/MS method was developed and fully validated for use in equine PPP. The method is rapid and sensitive, and especially for target analysis, LC-MS/MS has a higher specificity than the more frequently used HPLC methods.[26] The

LC-MS/MS method was successfully applied for the measurement of serotonin in healthy horses and horses suffering from acute small intestinal colic. With a validated linearity in the range of 3–100 ng/ml, the developed LC-MS/MS method could be extended to research into other conditions like endotoxemia and laminitis, in which 3- to 4-fold increases in plasma serotonin values have been claimed.[4,21] Because of its high sensitivity (LOD 0.10 ng/ml), the method can also be applied in studying normal serotonin physiology in horses. Because serum serotonin values are in a much higher range, varying from over 200 ng/ml in the current study to 616 ng/ml[3] and 976 ng/ml[23] in previous studies, extended validation of this LC-MS/MS method is needed before its implementation in the quantitation of serum serotonin concentrations.

A 3.5-µm HPLC column[f] was used for chromatographic separation because it can be applied for a wide range of compounds and covers a wide pH range that accommodates most mobile phases. In this case, acetonitrile and acetic acid in HPLC water were used as mobile phases. Small peaks of 0.5 min were achieved with a good symmetrical peak shape. This simple chromatographic method can be implemented in any laboratory.

During the LC-MS/MS method development, a simple precipitation of plasma proteins was tested first as sample clean-up. Protein precipitation with a strong acid, however, resulted in a significant loss in detector sensitivity. Protein precipitation using acidified acetonitrile on the other hand gave rise to a turbid supernatant after centrifugation, which could not be injected on the LC-MS instrument. A more intensive sample clean-up was tested further, using solid-phase extraction with a weak cation exchanger. A poor extraction recovery was obtained despite the wide range of wash and elution solvents tested. The final extraction methodology, as described in the Materials and Methods section, makes use of a liquid extraction of the plasma samples after adjusting the pH with sodium hydroxide. This methodology resulted in acceptable serotonin recovery rates.

Serotonin is regularly determined in horse plasma in research settings. Although several ELISA kits are commercially available for serotonin determination in human

plasma, such kits should not be used in horse plasma without extensive validation. In PPP, serotonin quantities are relatively low, making accurate and precise measurement quite difficult.[25] The ELISA kit evaluated in the current study had acceptable serotonin recovery rates in PPP, enabling use of the kit in a clinical situation. However, comparison of LC-MS/MS and ELISA showed a limited agreement between the 2 methods. Therefore, caution is warranted when extrapolating results from different analytical techniques.

A limitation of the current study is the nonexistence of totally serotonin-free horse plasma. Therefore, the samples used as "blanks" for serotonin recovery studies still contained a low serotonin concentration. The use of PPP samples of healthy horses kept this endogenous serotonin as low as possible. A chromatogram of unspiked PPP (Fig. 1B) demonstrates the very low endogenous serotonin concentrations, in comparison to the spiked serotonin (Fig. 1A).

Interlaboratory variation and the demonstrated lack of agreement between different measurement methods may partially explain the wide range of reported normal values for plasma serotonin. Other possible influences are different protocols used for blood sample stabilization, preparation, and centrifugation. Inadequate sample handling may lead to *in vitro* platelet activation and aggregation with consecutive serotonin release, causing a false increase in plasma serotonin. The use of different centrifugal speeds in the preparation of PPP causes variation in the plasma platelet content and, consequently, in the serotonin concentration.[6]

CONCLUSIONS

It can be concluded that reliable measurement of equine plasma serotonin is possible, but there are a number of restrictions. For an accurate determination of plasma levels, it is essential to use PPP. In horses, this requires relatively high centrifugal speeds,[6] which limit the feasibility in a clinical situation. Furthermore, it is not advisable to make comparisons between absolute serotonin values determined

in different circumstances or analyzed with different methods. Therefore, the establishment of method-specific serotonin plasma values is required. For chromatographic methods, the extraction step is a pitfall, and inadequate extraction may lead to underestimation of serotonin values. The newly developed LC-MS/MS method described herein is suitable for determining serotonin plasma reference values and for analyzing serotonin changes associated with pathological conditions.

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The authors Sara C. Torfs and An A. Maes contributed equally to this work.

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3.3.

FOLLOW UP OF PLASMA SEROTONIN IN HORSES OPERATED FOR SMALL INTESTINAL COLIC

S. Torfs,[a] A. Maes,[b] C. Delesalle,[a,c] S. Croubels,[b] P. Deprez[a]

[a] Department of Large Animal Internal Medicine, Faculty of Veterinary Medicine, Ghent University, B-9820 Merelbeke, Belgium.

[b] Department of Pharmacology, Toxicology, and Biochemistry, Faculty of Veterinary Medicine, Ghent University, B-9820 Merelbeke, Belgium.

[c] Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, NL-3584 CM Utrecht, The Netherlands.

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SUMMARY

Objectives: To compare plasma serotonin concentrations in healthy horses and horses presented for surgical small intestinal (SI) colic, and to evaluate their association with postoperative ileus and non-survival.

Sample population: EDTA plasma samples of 34 horses with a surgical SI problem. Plasma and serum samples of 24 healthy controls.

Procedures: Samples from the colic group were collected at several pre- and postoperative time points. Serotonin concentrations in platelet poor plasma were determined with both liquid-chromatography tandem mass spectrometry and ELISA and compared with healthy controls. The possibility of inadvertent serotonin release during sample handling and pretreatment was assessed by analyzing platelet rich plasma samples that were activated by either freeze-thawing, sonication or the addition of collagen. The platelet activation parameters β-thromboglobulin and platelet factor 4 were measured in all samples.

Results: Platelet poor plasma serotonin concentrations were significantly lower in SI surgical colic horses compared to controls, in both pre- and postoperative samples. No association with ileus or non-survival could be demonstrated. Serotonin concentrations in platelet rich plasma were affected by sonication or addition of collagen, but not by freeze-thawing. The concentrations of β-thromboglobulin and platelet factor 4 were not affected by platelet activation.

Conclusions and clinical relevance: Plasma serotonin concentrations is not suitable as a prognostic factor in horses with SI surgical colic. The cause and clinical relevance of the observed decrease in free plasma serotonin have to be investigated further.

INTRODUCTION

Serotonin (5-hydroxytryptamine, 5-HT) is an important neurocrine messenger in the intestinal tract. Intestinal serotonin is produced by enterochromaffin (EC) cells and to a lesser extent by serotonergic neurons, and released upon mucosal stimulation. It interacts with serotonin receptors on afferent neurons, initiating peristaltic and secretory reflexes. Serotonin is eliminated from the interstitium by serotonin transporters on enterocytes and neurons; serotonin overflow from the gut reaches the intestinal lumen and portal circulation. In the circulation, it is quickly removed from the plasma by uptake into the platelets and by hepatic metabolism. The platelets release serotonin again upon activation. Free plasma serotonin exerts important systemic functions: it modulates platelet aggregation and is involved in vasomotor function.[1-4]

Several studies have demonstrated an augmented serotonin release after experimental intestinal ischemia and reperfusion. In intestinal transplantation, a serotonin decrease in the intestinal wall and increase in the lumen and preservation fluid is used as a marker for ischemia-reperfusion injury.[5-7] Increased serotonin concentrations after ischemia-reperfusion have been found in the intestinal lumen[6] and peritoneal fluid[8] but also in the mesenteric,[9] portal and hepatic veins.[10] In rats, intestinal ischemia-reperfusion caused a short-lived serotonin increase in peripheral plasma.[11] Increased plasma serotonin concentrations were also reported in strangulating small intestinal (SI) colic horses.[8] On the other hand, another study showed a decreased serum serotonin concentration in horses suffering from acute abdominal pain.[12]

Despite continuing improvements in the surgical and postoperative care for colic horses, postoperative complications like ileus, but also endotoxemia and laminitis, are still a substantial problem. Recent publications report up to 33% of horses developing ileus after SI colic surgery.[13-15] Estimating the risk of developing ileus for an individual horse is often a challenge. Both the local lesions at the level of the intestinal wall and the associated systemic inflammatory and endotoxemic reactions

contribute to the development of ileus. Plasma serotonin concentrations could reflect intestinal integrity as well as the circulatory effects associated with inflammation or endotoxemia. Therefore serotonin quantitation could be an aid in prognosticating the outcome in horses with SI colic. Knowledge of plasma serotonin changes in colic horses is also important in the quest for an effective treatment for ileus. Indeed, certain classes of prokinetic drugs target serotonin receptors.[1,16] There could be a risk of receptor desensitization[17] when these drugs are used in patients with already elevated serotonin levels.

The purpose of the current study was to investigate changes in plasma serotonin concentrations in horses presented for surgical SI colic. Serotonin concentrations were determined at several time points during the perioperative period and compared with healthy controls. The possible associations of plasma serotonin levels with the development of ileus and with non-survival were evaluated. We also assessed the possibility of inadvertent serotonin release by platelet activation during sample handling and pretreatment.

MATERIALS AND METHODS

SAMPLE POPULATION OF SI SURGICAL COLIC HORSES AND HEALTHY CONTROLS

The colic group consisted of 34 horses that underwent small intestinal colic surgery at the Faculty of Veterinary Medicine, Ghent University, Belgium between July 2009 and September 2010. The horses (15 mares, 16 geldings, 3 stallions) were mainly (*n* = 24) warmbloods, with an age range from 3 months to 20 years (mean age 11 years). Strangulating lesions were found in 23 horses, 11 had a non-strangulating obstruction. Seven horses developed postoperative ileus (defined as > 20 liters of reflux within 24 h or > 8 liters in a single occasion). A total of 12 horses did not survive to discharge, 4 of them were euthanized during surgery.

The control group consisted of 24 healthy warmblood horses (*n* = 19) and trotters (*n* = 5) (13 mares, 9 geldings and 2 stallions; aged 6-20 years; mean age 12 years).

COLLECTION AND PREPARATION OF SAMPLES

Blood samples of the colic horses were collected at admission and immediately postoperatively. This was followed by daily sampling until the intestinal transit resumed, or until death or euthanasia of the horse. The daily samples were always collected in the morning; the control group was also sampled in the morning to avoid possible circadian variations.

In accordance with previously published studies, samples were collected into EDTA tubes and immediately put on ice. Directly after collection, clomipramine (Clomipramine hydrochloride, Sigma-Aldrich, Bornem, Belgium) (final concentration 1µM), phenelzine (Phenelzine sulphate, Sigma-Aldrich, Bornem, Belgium) (10 µM) and acetyl salicylate (Aspégic Injectable, Sanofi-Aventis, Diegem, Belgium) (1 mM) were added to inhibit platelet serotonin uptake, metabolism and release, respectively.[8,18] Samples were centrifuged at 300 × g for 10 minutes at 4°C, followed by 5000 × g for 20 minutes at 4°C for the supernatant, to obtain platelet poor plasma (PPP). Platelet rich plasma (PRP) for the activation protocol (see "Assessment of platelet activation") was produced by adding only clomipramine (1 µM) and phenelzine (10 µM) to EDTA plasma and centrifuging at 300 × g for 10 minutes at 4°C. All samples were stored at -80°C until assayed.

MEASUREMENT OF PLASMA SEROTONIN CONCENTRATIONS

Plasma serotonin concentrations were analyzed by a liquid-chromatography tandem mass spectrometry (LC-MS/MS) method previously described.[19] In brief, to an aliquot of 500 µL PPP, a solution of deuterated serotonin (5-HT-d4, CDN Isotopes,

Nieuwegein, The Netherlands) was added as an internal standard. A liquid extraction into ethyl acetate was performed, followed by chromatographic separation on an C18 column (Eclipse Plus C18 column, Agilent, Diegem, Belgium) with an acetic acid-acetonitrile mobile phase gradient elution. The linearity of the LC-MS/MS method has been assessed between 3 and 100 ng/ml, the method had a limit of quantification of 3 ng/ml and its detection limit was 0.1 ng/ml.[19]

Additionally, all samples were also analyzed using a commercially available serotonin ELISA kit (Serotonin ELISA, Genway Biotech, San Diego, CA), formerly validated for use in equine PPP.[19]

ASSESSMENT OF *IN VITRO* PLATELET ACTIVATION

Platelets are able to store serotonin in their dense granules and release it upon activation.[4] Inadvertent platelet activation during sample collection and preparation might therefore increase plasma serotonin concentrations. To assess the sensitivity of equine platelets for *in vitro* platelet activation and to obtain positive control samples, serotonin concentrations were assayed after the application of several activation protocols on PRP obtained from healthy control horses. In activation group 1, PRP samples of 12 horses were subjected to 2 freeze-thaw cycles. In activation group 2, 6 samples underwent sonication (Vibra-Cell, Sonics & Materials, Inc., Newtown, CT) during 30 seconds and for activation group 3, platelets were activated by adding collagen (Equine collagen type 1, American Biochemical and Pharmaceutical Ltd., Epsom, UK) (15 µg/ml) to 6 PRP samples. Finally, a group of 6 serum samples, allowed to cloth completely during 1 h at 37°C and centrifuged for 10 minutes at 1500 × g, served as positive controls with maximum platelet activation.

As an additional test for platelet activation, β-thromboglobulin (β-TG) and platelet factor 4 (PF4) were determined for all samples from the colic group as well as the negative and positive controls. These chemokines are released into the plasma by activated platelets, together with serotonin. In human platelets, it has been

demonstrated that β-TG and PF4 are more easily released than serotonin by *in vitro* procedures.[20] In case of *in vivo* activation, β-TG immediately binds to the endothelium, causing the ratio β-TG/PF4 to decrease. Therefore, augmented β-TG and PF4 concentrations together with an increased β-TG/PF4 ratio are an indication of *in vitro* platelet activation.[21] The analyses of β-TG and PF4 were performed according to a sandwich ELISA protocol that was previously described and validated.[8] The β-TG/PF4 ratios were determined by comparing optical density (OD) values.

STATISTICAL ANALYSIS

The results were analyzed using computer software packages (IBM SPSS Statistics 20, IBM, Brussels, Belgium). For the serotonin plasma data, a log transformation to a common logarithm was necessary to obtain a normal distribution. The effect of group (colic or control) on plasma serotonin concentrations was evaluated using linear regression analysis. Linear regression was also applied for examining the association between serotonin concentrations and development of postoperative ileus and non-survival. One-way ANOVA and Dunnett post hoc tests were used for comparison of serotonin concentrations in the different activated samples with negative controls. Results were expressed as mean (SEM) or median (SD); significance was set at a value of $P < 0.05$.

RESULTS

PLASMA 5-HT IN HORSES SUFFERING FROM SI SURGICAL COLIC

Serotonin values measured by LC-MS/MS in PPP of healthy horses and SI colic horses at admission, postoperatively and the next morning (morning 1) are summarized in Table 1. All three colic groups had significantly lower serotonin concentrations than the control group. For the admission samples, linear regression analysis gave the

regression equation: log(5-HT) = -0.403 × group + 1.225 ($P < 0.01$; $R^2 = 0.40$). For the postoperative samples, the equation was: log(5-HT) = -0.407 × group + 1.225 ($P < 0.01$; $R^2 = 0.29$) and for the first morning sample: log(5-HT) = -0.473 × group + 1.225 ($P < 0.01$; $R^2 = 0.43$).

Table 1. Mean (SEM) and median (SD) plasma serotonin concentrations measured by LC-MS/MS in healthy control horses; in surgical small intestinal colic horses at admission, directly postoperatively and the next morning; in activated samples. A log transformation was performed before statistical analysis.

	Mean (SEM) 5-HT (ng/ml)	**Median (SD) 5-HT (ng/ml)**
Platelet poor plasma samples		
Healthy control (*n* = 24)	19.5 (2.4)	17.8 (12.0)
Colic admission (*n* = 31)	8.0 (1.0)	6.3 (5.7)
Colic postoperative (*n* = 31)	10.9 (3.5)	6.2 (19.2)
Colic morning 1 (*n* = 28)	7.3 (1.3)	6.5 (6.8)
Activated samples		
Freeze-thawed PRP (*n* = 11)	14.7 (2.0)	13.5 (6.5)
Sonicated PRP (*n* = 6)	59.0 (5.2)	59.5 (12.6)
Collagen PRP (*n* = 6)	57.2 (6.5)	59.9 (15.9)
Serum (*n* = 6)	221.9 (29.3)	215.6 (71.7)

All serotonin samples were also analyzed with a commercial serotonin ELISA kit. The ELISA results were equivalent to LC-MS/MS results, with significantly lower serotonin concentrations in all colic groups compared to controls (admission: $n = 56$, $P < 0.01$, $R^2 = 0.36$; postoperative: $n = 54$, $P < 0.01$, $R^2 = 0.29$; morning 1: $n = 51$, $P < 0.01$, $R^2 = 0.40$).

ASSOCIATION OF PLASMA 5-HT WITH DEVELOPMENT OF ILEUS AND NON-SURVIVAL

Linear regression after log transformation of data did not demonstrate any association between PPP serotonin concentrations and either development of ileus or non-survival. Associations were evaluated at admission (ileus: $n = 27$, $P = 0.86$; non-survival: $n = 26$, $P = 0.58$), postoperatively (ileus: $n = 29$, $P = 0.56$; non-survival: $n = 26$, $P = 0.92$) as well as the next morning (ileus: $n = 28$, $P = 0.58$; non-survival: $n = 26$, $P = 0.54$).

ASSESSMENT OF *IN VITRO* PLATELET ACTIVATION

The activation of PRP by freeze-thaw cycles did not cause increased serotonin concentrations compared to controls ($P = 0.57$). Moderate increases in free serotonin (Table 1) were observed after both sonication ($P < 0.01$) and addition of collagen ($P < 0.01$). In serum, markedly increased serotonin concentrations (Table 1) were observed ($P < 0.01$) compared to PPP.

The OD values for the platelet activation parameters β-TG and PF4 were very low for most horses. For β-TG, mean OD was 0.06 (SD 0.11) and for PF4, mean OD was 0.07 (SD 0.16). There was no effect of any applied activation protocol (activation groups 1-4) on either β-TG or PF4. However, a number of individual horses (3 healthy controls and 2 colic horses) had consistently higher OD values ranging from 0.40 to 0.72 for β-TG and from 0.45 to 1.06 for PF4. These values remained constant in repeated sampling and were not influenced by applying activation protocols. The β-TG/PF4 ratio remained unchanged in all of these 5 horses, indicating that the elevated levels reflected an *in vivo* situation and not *in vitro* platelet activation.[21]

DISCUSSION

The main finding of this study was that PPP serotonin concentrations were significantly lower in SI surgical colic horses than in healthy controls. The serotonin concentrations remained low at least until the first morning postoperatively. Decreased serotonin concentrations in colic horses were also observed in a previous study[12] comparing serum serotonin concentrations in healthy horses and horses suffering from various conditions. Significantly lower serotonin values were found in horses with acute colic. However, the results are in contrast to those of another study,[8] that demonstrated higher plasma serotonin concentrations in horses that underwent SI colic surgery.

Platelet poor plasma serotonin concentrations are thought to reflect the amount of recently synthesized and secreted 5-HT in EC cells. However, this association is confounded by many variables. Enhanced platelet fragility may increase PPP serotonin concentrations. Serotonin clearance by uptake into platelets, enterocytes and hepatic cells is affected by serotonin transporter (SERT) expression, activation and desensitization.[2] Besides SERT, backup transport mechanisms with a low affinity but high capacity play a role.[17] After uptake into the cells, serotonin can be inactivated primarily through monoamine oxidase mediated breakdown to 5-hydroxyindoleacetic acid. The latter is excreted predominantly in the urine.[3]

Platelet stabilization by previous administration of non-steroidal anti-inflammatory drugs (NSAIDs) could possibly account for the lower serotonin concentrations found in the colic group. NSAIDs were administered to 22 out of 34 colic horses before admission to the clinic, and to all horses in the peri-operative period. Serum glucocorticoid levels are also elevated in colic horses[12,22] and could be associated with platelet stabilization[23] and decreased serotonin concentrations. Indeed, lower plasma serotonin values have been observed in horses with Cushing's disease compared to controls.[24] However, it is questionable if the platelet effects of NSAIDs or endogenous glucocorticoid upregulation would outweigh endotoxemic

events leading to increased platelet activation in surgical colic. Unfortunately it was not possible to evaluate the degree of platelet activation by assaying β-TG and PF4.

Intestinal ischemia and reperfusion has been associated with a massive serotonin release towards the intestinal lumen and with a decreased number of EC cells in the mucosa.[5,6,25] In our study population, the small intestinal content was removed during colic surgery through enterotomy or by emptying the intestine towards the cecum. A temporary depletion of intestinal serotonin stores could therefore account for the prolonged decrease of PPP serotonin concentrations. Evaluating the duration of the 5-HT deficit, we found that PPP serotonin values were significantly lower until at least the morning after surgery. A limited number of horses were also sampled the 2nd (*n* = 17) and 3rd (*n* = 10) morning postoperatively. Serotonin concentrations appeared to stay low, but unfortunately the group was too small for statistical confirmation.

The plasma concentration of serotonin is also influenced by the diet. Lower concentrations are measured in fasted horses compared to fed horses.[26] An increased serotonin release from EC cells after food intake, with increased plasma levels persisting for several hours, is also observed in humans.[27] In the current study, horses in the colic group received little, if any, food in the direct postoperative period, which could account for at least part of the observed decrease in PPP serotonin concentrations.

Blood sample handling and preparation might result in *in vitro* platelet activation. Several tests were performed to assess the possibility of inadvertent changes to PPP serotonin levels in either the colic or control group. Unfortunately, β-TG and PF4 were found to be unsuitable parameters for platelet activation in the horse. In contrast with human platelets,[20] horse platelets released serotonin but no β-TG or PF4 during *in vitro* activation. However, the lysis of platelets by sonication or their activation by the addition of collagen was required for obtaining a serotonin release. Implementation of a freeze-thaw protocol was not sufficient to augment free serotonin levels. Therefore it may be concluded that the risk of provoking inadvertent serotonin changes by *in vitro* platelet activation is low in horses. This

risk was reduced even further in the current study by immediate cooling of samples, adding stabilizing agents and cooled centrifugation. The latter can however lead to somewhat higher serotonin values compared to centrifugation at room temperature, probably because the reuptake of serotonin into platelets is suppressed.[20] This and other differences in sample pretreatment protocols, together with technical difficulties in serotonin analysis, are reflected by the broad range (2.5 – 90 ng/ml) of serotonin reference values reported for healthy horses.[8,18,24,26,28] A strength of the current study is the PPP serotonin determination with 2 different methods, yielding equivalent results for LC-MS/MS and ELISA assays.

Changes in serotonin levels might not only be the result, but also the cause of disturbed intestinal functioning. In human medicine, an artificial increase in plasma serotonin by the administration of selective serotonin reuptake inhibitors may lead to both increased and decreased intestinal transit times.[29] The final effects on the intestine will depend on serotonin concentrations, but also on the balance between receptor activation and desensitization.

CONCLUSIONS

In the current study no associations could be demonstrated between PPP serotonin concentrations and the occurrence of postoperative ileus or non-survival. Since serotonin is both an important mediator of intestinal function and an indicator of intestinal injury, it would be interesting to assess local serotonin concentrations at the level of the intestinal mucosa and evaluate their association with postoperative intestinal recovery. In view of the results of this study, PPP serotonin concentrations do not seem suitable as a prognostic factor in SI colic surgery. However these results need to be confirmed in a larger group of horses.

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CHAPTER 4:

SURGICAL SITE INFECTIONS

RISK FACTORS FOR INCISIONAL COMPLICATIONS AFTER EXPLORATORY CELIOTOMY IN HORSES: DO SKIN STAPLES INCREASE THE RISK?

S. Torfs,[a] T. Levet,[b] C. Delesalle,[a,c] J. Dewulf,[d] L. Vlaminck,[b]

F. Pille,[b] L. Lefere,[a] A. Martens[b]

[a] Department of Large Animal Internal Medicine, Faculty of Veterinary Medicine, Ghent University, B-9820 Merelbeke, Belgium.

[b] Department of Surgery and Anesthesia of Domestic Animals, Faculty of Veterinary Medicine, Ghent University, B-9820 Merelbeke, Belgium

[c] Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, NL-3584 CM Utrecht, The Netherlands.

[d] Department of Reproduction, Obstetrics and Herd Health, Veterinary Epidemiology Unit, Faculty of Veterinary Medicine, Ghent University, B-9820 Merelbeke, Belgium.

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ABSTRACT

Objective: To assess risk factors for celiotomy incisional infection in horses, especially the use of staples for skin closure.

Study Design: Case series.

Animals: Horses (n = 356) that had 1 exploratory celiotomy for colic and survived ≥ 2 weeks after surgery between March 1, 2004 and December 31, 2007.

Methods: Incisions were classified as "normal" (no complication, only edema, serous drainage lasting < 24 hours) or as "surgical site infection (SSI)" (persistent serosanguinous drainage or purulent drainage with or without positive bacterial culture). All possible risk factors, including method of skin closure (monofilament sutures or staples), were statistically analyzed using univariable and multivariable logistic regression.

Results: Of 356 horses, 303 (85%) had normal wound healing and 53 (15%) developed a SSI (purulent: 48 [14%]; persistent serosanguinous: 5 [1%]). Bacterial cultures were positive in 33 of 40 cases. Factors significantly associated with SSI in the multivariable analysis were: use of staples for skin closure (OR 3.85, P < .001) and surgical site closure by a 1st or 2nd year resident (OR 2.20, P = .016). Lavage of the linea alba with sterile saline solution after closure was a protective factor (OR 0.38, P = .004).

Conclusion: Use of staples for skin closure and less experienced surgeons closing the abdomen are risk factors for incisional infection. Incisional lavage after linea alba closure was a protective factor.

Clinical Relevance: Despite their ease and speed of application, skin staples can lead to an increase in celiotomy wound complications in horses.

INTRODUCTION

Although survival after exploratory celiotomy has improved, incisional complications still represent a challenge for equine surgeons because of their impact on the duration of recovery and the associated cost for owners. Incisional drainage and infection have been strongly associated with hernia formation,[1,2] with up to 62.5 times more chance of herniation in horses that have incisional drainage.[3]

Reported wound infection rates vary from 7.4%[4] - 37%[5] reflecting the combined effect of intrinsic (patient related) and extrinsic (management and care related) risk factors predisposing horses to wound complications, as well as variations in inclusion criteria and in definitions of incisional complications. Recently, incisional complications have been described using the general term "surgical site infection" (SSI) for equine abdominal surgery.[6]

Many risk factors for wound infection have been identified including use of polyglactin 910 to close the linea alba;[7] trauma to the incisional edges;[5,7,8] and endotoxemia on admission.[9] Some studies identify contamination with intestinal content during surgery as a risk factor,[5,7,10] whereas others do not.[3,11,12-14]

Assessing bacterial contamination of the incision during surgery seemingly does not predict wound complications or which organisms will be involved in SSI.[3,14] There is however a correlation between a high number of bacterial colony-forming units (CFU) obtained from the incision site immediately after recovery from anesthesia and the development of SSI.[14] These data suggest that true contamination mainly occurs in the early postoperative period. Bacterial entrance can be avoided by protecting the wound, e.g. with abdominal bandages,[9] and by using a closure technique (staples or sutures) that provides the best skin apposition.

Our aim was to determine intrinsic and extrinsic risk factors associated with surgical site infection after celiotomy, based upon a large-scale retrospective study.

MATERIALS AND METHODS

INCLUSION CRITERIA

Surgical records (March 2004 - December 2007) of the Veterinary Teaching Hospital, Ghent University were reviewed. Horses were included if they 1) had exploratory celiotomy for investigation and treatment of abdominal pain; 2) had recovered successfully from anesthesia; and 3) had survived ≥ 2 weeks after surgery. Horses that had repeat celiotomy before discharge from the hospital were excluded from the study.

DEFINITIONS

Wounds were classified as "normal" when there was either no wound complication or only edema at the surgical site. Wounds with serous drainage for < 24 hours after surgery were also classified as normal. Wounds were classified as surgical site infection (SSI) when there was either persistent serosanguinous drainage starting > 24 hours after surgery, or purulent drainage (suppuration), with or without positive bacterial culture.

RISK FACTORS EVALUATED

Intrinsic preoperative risk factors evaluated were: age, sex and weight of the horse; duration of colic before admission; heart rate, packed cell volume (PCV), skin turgor and mucous membrane color; presence of clinical signs of endotoxemia on admission (defined as any 2 of: congested mucous membranes with prolonged capillary refill time, heart rate > 50/min, and PCV > 50%).[9]

Intrinsic intraoperative risk factors included type of primary lesion (non-strangulating or strangulating), anatomic location of the primary lesion (small or

large intestine), increased risk for abdominal contamination during surgery (performing an enterotomy or enterectomy), hypoxemia (PaO_2 < 65 mmHg), highest mean arterial blood pressure (MAP) during anesthesia, hypotension (MAP < 60 mm Hg during at least 15 minutes) and recovery score. Recovery was mainly unassisted and scored as "uneventful" if the horse got up in a calm way with few attempts and was only moderately ataxic, and as "complicated" if the horse needed several attempts and showed excitation or severe ataxia. Horses were lightly sedated with xylazine (0.2 mg/kg) before recovery, to prevent excitation.

Extrinsic intraoperative factors included duration of general anesthesia, the experience of the surgeon closing the abdomen (postgraduate year (PGY) 3 resident or senior surgeon versus PGY 1 or 2 resident), administration of procaine benzylpenicillin into the abdomen before closure, closure of the peritoneum, dissection of the subcutaneous tissues to expose the linea alba before closure, lavage of the linea alba with sterile saline solution, and local administration of procaine benzylpenicillin before closure of the subcutaneous tissues. Method of skin closure (conventional skin sutures in a continuous pattern or staples) was also recorded, as well as the type of protection used for the recovery (a stent sutured with 0 polypropylene or a stent maintained by an adhesive sheet [Fixomull, Smith and Nephew, Brussels, Belgium]).

In all horses, the linea alba was closed with a simple continuous pattern of 6 polyglactin 910 and the subcutaneous tissues were closed with a continuous pattern of 1 polyglactin 910. Sutures used for skin closure were only monofilament sutures in a continuous pattern and mainly nonabsorbable suture material (0 polypropylene). Skin staples were nonabsorbable, stainless steel, 6.9 mm × 3.9 mm (Leukoclip SD, Smith and Nephew, Brussels, Belgium).

Intrinsic postoperative factors included the development of postoperative ileus (POI), diarrhea or laminitis. Wound protection in the postoperative period (no further protection, protection by a stent or protection by an abdominal bandage) was an extrinsic postoperative factor.

Postoperatively, horses were generally bedded on straw and they were tied to prevent them from lying down. Antibiotic administration was sodium benzylpenicillin (20,000 U/kg IV every 8 hours) and gentamicin (6.6 mg/kg IV once daily) immediately preoperatively and for 3 - 5 days postoperatively depending on the type of surgery. During hospitalization, the surgical site was monitored daily or at bandage change and evaluated for possible complications. Abdominal bandages (elastic adhesives) or stents, if applicable, were placed directly after the recovery, changed when necessary (depending on the wound and at least every 3rd day) and removed after 3 - 7 days upon clinician's discretion.

STATISTICAL ANALYSIS

Descriptive results of continuous variables are expressed as median and range. For categorical data, frequencies of occurrence are reported. The relation between potential intrinsic and extrinsic risk factors and the outcome variable SSI, was analyzed using logistic regression (SPSS 15.0 for Windows, SPSS Inc., Chicago, IL). Univariable analysis was used to screen all variables for association with the outcome variables. Variables with a univariable $P < 0.20$ were included in a multivariable logistic regression model, which was constructed in a stepwise backward manner. Spearman's ρ correlations between the selected variables were determined, and if 2 variables were highly correlated ($R^2 > 0.60$), only the variable with the smallest *P*-value was included in the multivariable model.

In the final multivariable model, all 2 way interactions between significant variables were evaluated. Significance was set at $P < .05$. Odds ratios (OR), including 95% confidence intervals (CI), are reported for all significant variables.

RESULTS

Three hundred fifty-six horses met the inclusion criteria and 85% (303) had normal wound healing and 15% (53) had SSI. Most (14%, 48) developed incisional suppuration whereas 1% (5) had a serosanguinous drainage. Thirty-three of 40 microbial cultures were positive. *Staphylococcus aureus* (*n* = 12, including 4 Methicillin-resistant *S. aureus* [MRSA]), *E. coli* (8) and other enterobacteriaceae (3), DNAse negative *Staphylococcus* spp (6), *Enterococcus fecalis* (4), Gram negative anaerobic pathogens (3), *Pseudomonas aeruginosa* (2), *Streptococcus equi* (1), *Proteus mirabilis* (1) and *Candida* sp (1) were isolated. Nine wounds (27%) had a mixed bacterial culture.

In the univariable analysis, significant risk factors for SSI were all intraoperative variables (Table 1). Twelve percent (32/267) of horses with surgical sites closed by an experienced surgeon had SSI compared with 23% (21/89) when a PGY 1 or 2 closed the incision. If the peritoneum was sutured, 12% (35/287) of horses developed SSI whereas without closure the prevalence increased to 26% (18/69). In horses that had subcutaneous dissection before linea alba closure, 7% (10/129) SSI occurred compared with 20% (43/217) when the subcutaneous tissue was not dissected. Nineteen percent (46/239) of horses not administered benzylpenicillin after linea alba closure developed SSI compared with 6% (7/110) for horses administered benzyl penicillin. If a wound lavage was performed after linea alba closure, 13% (30/242) had SSI compared with 20% (23/114) in horse where the incision was not lavaged. Skin closure with a continuous suture pattern was associated with 10% (23/242) SSI whereas use of staples had a prevalence of SSI of 27% (30/114). If a sutured stent was used during anesthesia recovery, 9% (12/133) developed SSI compared with 19% (41/221) when the stent was maintained by an adhesive sheet.

Table 1. Results of univariable analysis for the identification of risk factors for development of abdominal incision SSI in 356 surgical colic cases (2004-2007). Only significant factors are listed.

Variable	*n*	*P*-value	OR	95% CI for OR
Experience surgeon	356	0.009		
PGY 3 resident / Senior (Ref.)	267	-		
PGY 1 / 2 resident	89	0.009	2.26	1.22-4.18
Peritoneum closure	356	0.004		
No (Ref.)	69	-		
Yes	287	0.004	0.39	0.20-0.75
Subcutaneous dissection	356	0.002		
No (Ref.)	217	-		
Yes	139	0.002	0.39	0.15-0.64
Penicillin after linea alba closure	356	0.002		
No (Ref.)	239	-		
Yes	117	0.002	0.26	0.11-0.61
Linea alba lavage*	356	0.057		
No (Ref.)	114	-		
Yes	242	0.057	0.56	0.30-1.02
Skin closure	356	< 0.001		
Monofilament sutures (Ref.)	242	-		
Staples	114	< 0.001	3.44	2.00-6.22
Type of stent for recovery	354	0.017		
Sutured stent (Ref.)	133	-		
Stent + adhesive sheet	221	0.017	2.29	1.16-5.54

OR = odds ratio; CI = confidence interval; Ref. = reference value; SSI = surgical site infection; PGY = post graduate year.
*Included in the table regardless its *P*-value > 0.05, because of its significant result in multivariable analysis.

A significant correlation (R^2 = 0.62) was found between the variables "subcutaneous dissection" and "penicillin after linea alba closure". Because they were both highly significant in the univariable screening model, both were tested in alternative multivariable models, but neither of them remained significant.

Table 2. Results of multivariable analysis for the identification of risk factors for development of abdominal incision SSI in 356 surgical colic cases (2004-2007).

Variable	*n*	*P*-value	OR	95% CI for OR
Experience of surgeon	356	0.016		
PGY 3 resident / Senior (Ref.)	267			
PGY 1 / 2 resident	89	0.016	2.20	1.16-4.18
Linea alba lavage	356	0.004		
No (Ref.)	114			
Yes	242	0.004	0.38	0.20-0.74
Skin closure	356	< 0.001		
Monofilament sutures (Ref.)	242			
Staples	114	< 0.001	3.85	2.04-7.29

OR = odds ratio; CI = confidence interval; Ref. = reference value; SSI = surgical site infection; PGY = post graduate year.

In the final multivariable model (Table 2), significant risk factors for SSI were the use of staples for skin closure compared with a continuous suture pattern, and closure of the surgical site by a PGY 1 or 2 resident versus closure by more experienced surgeons. Wound lavage after linea alba closure with sterile saline solution was a protective factor. There were no significant 2 way interactions between the variables in the model.

DISCUSSION

Our rate of celiotomy wound suppuration was 14%, which is relatively low compared with recently published rates of 21.4%[11] and 15.3%[9] in studies where horses undergoing repeat celiotomy were also excluded. Horses undergoing repeat celiotomy have far higher rates of infection (87.5%).[12]

Interestingly, skin incisions closed with staples had a 3.85-fold higher risk of developing SSI compared with those closed with a continuous suture pattern. Ingle-Fehr et al.[3] and Galuppo et al.[14] already noted the importance of early postoperative contamination, especially during recovery. Their findings emphasize the importance

of optimal wound sealing. Indeed, even during uneventful recoveries, protective stents or adhesive sheet are often displaced or dislodged, exposing the wound to contamination.

To our knowledge, this is the first time that the use of staples has been identified as a risk factor for wound infection in horses that had exploratory celiotomy for colic. The use of staples has been investigated in 2 studies[7,12] of incisional complications in horses. In both reports, there was a tendency toward a higher infection rate for staples versus sutures. In human medicine, limited data are available on the effects of staples on SSI; however, the use of staples compared with intradermal sutures was demonstrated to entail an increased risk of SSI after caesarean section,[15,16] and to lead to more wound complications (erythema, wound drainage, wound dehiscence and necrosis) after cardiac operations.[17]

Possibly, the apposition of skin edges and sealing of the wound is less effective with staples, compared with sutures, allowing early postoperative contamination. Clinically, the choice of skin closure material varies among surgeons according to their experience and the condition of the wound (contamination, tension, etc.). Earlier literature comparing sutures with staples mainly focused upon speed of insertion;[18] however, the difference in time of application between staples and a conventional continuous pattern decreases with surgical experience and may be an irrelevant factor considering total surgical time and the increased infection risk.

In general, monofilament sutures for skin closure appear to be associated with a decreased risk for SSI compared with other suture material.[19,20] In our study, only monofilament sutures (mainly nonabsorbable polypropylene) were used and had a major advantage over staples. Intradermal patterns are chosen in people for esthetic reasons with good results with respect to SSI.[15,17,21] In dogs and cats, use of intradermal sutures seemingly minimizes licking and scratching at the incision.[22] However, esthetics and self-mutilation are not a concern in equine celiotomy incisions and therefore we use a full-thickness continuous pattern because of its ease and speed of application. Whether an intradermal, full-thickness continuous or an interrupted pattern is the best skin suture pattern to prevent SSI in associated with

closure of ventral median celiotomy in horses is not apparent and further investigation is warranted.

Another significant factor for the development of SSI was the experience of the surgeon closing the abdomen. In the present study, PGY 3 residents have been classified as experienced surgeons because of the high log of abdominal surgeries in our clinic, allowing residents to gain a lot of experience during the first 2 years. Comparable results concerning abdominal wound dehiscence after laparotomy (which is closely related to SSI) have been reported in human surgery.[23] It could be hypothesized that when PGY 1 or 2 residents are closing the surgical site, they increase anesthesia and surgery time, which could lead to more wound complications. However, no association was found between SSI and anesthesia duration in our study population, suggesting that a difference in surgical skill for wound closure is the causative factor. It is generally accepted that inappropriate bites (overly large) or excessive tightening of the sutures can lead to ischemia and necrosis of the linea alba,[24] predisposing to infection and dehiscence. On the contrary, less than secure closure may allow peritoneal fluid to pass through the linea alba and accumulate subcutaneously, predisposing to infection. Sufficient experience and strict compliance with Halsted's principles are required to obtain a secure closure of the abdominal wall.

Based on our results, lavage of the surgical site with saline solution can be recommended to prevent SSI. This is most likely beneficial because of physical removal of blood clots and gross contamination. Surgeons could consider including this procedure as a standard step in celiotomy wound closure.

Dissection of the subcutaneous tissue was carried out by some surgeons to facilitate suturing of the linea alba. Subcutaneous dissection was associated with a lower risk of SSI in the univariable analysis. This finding might be surprising because subcutaneous dissection increases dead space and enhances bleeding at the surgical site. Nevertheless, this risk factor was no longer significant in the multivariable analysis, showing that it might not be a determinant factor for SSI.

Despite being a significant protective factor in the univariable analysis (OR = 0.26; *P* = .002), local application of procaine benzylpenicillin after linea alba closure was not retained in the multivariable model. Similar results have been reported[10] where the administration of antibiotics in the wound was a protective factor (OR = 0.52); nevertheless, no multivariable analysis was performed. Few pathogens isolated from draining wounds were susceptible to benzylpenicillin (*Streptococcus* spp, some *staphylococci*), suggesting that intra- or postoperative penicillin administration may have prevented SSI with susceptible bacteria. On the other hand, the fact that most pathogens we isolated were resistant to this antibiotic (Enterobacteriacea, *Enterococcus fecalis*, *S. aureus* including MRSA) makes this recommendation controversial. Unnecessary use of antibiotics may kill susceptible commensal organisms and favor resistant commensal and opportunistic bacteria. Moreover, it could exacerbate resistance development, which should be avoided especially in hospital environment where multiresistant nosocomial bacteria (MRSA, *E. coli*) are emerging.[21] One horse developed a severe SSI involving not only *S. intermedius* and *Ps. aeruginosa*, but also a *Candida* sp. Since skin *Candida* infections are rare in the horse, this may reflect either immunocompromise, accidental contamination, or the impact of excessive use of broad-spectrum antimicrobial agents.[25]

Unlike some studies,[5,7,9,10] no association was found between SSI and either endotoxemia on admission, prolonged duration of general anesthesia (> 120 min), or exposure to contamination (performing an enterotomy and/or intestinal resection). Unlike the results of Smith et al.,[9] we found no protective effect of applying an abdominal bandage postoperatively. This was a surprising result, although it should be stated that throughout the 4 years of the study, abdominal bandages were not used in a standardized way or during a standardized period, which might have influenced the results.

CONCLUSIONS

We identified new risk factors for surgical site infection after celiotomy in horses. The use of staples for skin closure proved to be an important risk factor for the development of abdominal incision SSI. A less experienced surgeon closing the surgical site was also a risk factor, whereas wound lavage after linea alba closure had a protective effect.

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CHAPTER 5.

GENERAL DISCUSSION

The main objective of this PhD study was the study of the epidemiology and pathophysiology of postoperative ileus and surgical site infections, with the ultimate goal of reducing their prevalence and improving survival rates. In this general discussion section, the results of this PhD thesis will be commented on in view of the existing literature. Limitations of the research and future perspectives will also be discussed.

POSTOPERATIVE ILEUS

EPIDEMIOLOGY

The first objective of this thesis was to evaluate the risk factors, morbidity and mortality rates for ileus in a current clinical situation. It was decided to study only small intestinal surgical cases for this purpose, because literature research demonstrated that surgery for large intestinal colic was associated with a very low risk of developing POI. A preliminary study confirmed that over 80% (62/77) of POI cases were either operated for small intestinal pathology or for general small and large intestinal fermentation colic. Small intestinal pathology was associated with a 5.8 times increased odds for POI development (unpublished data).

Risk factors for POI, as identified in this thesis, were increased heart rate and high quantity of reflux (> 8 liter) at admission and small intestinal resection (Chapter 3.1). In order to use heart rate at admission as a dichotomized predictive parameter for POI development, it would have been useful to create a ROC curve and determine the best cut-off value. The association with preoperative tachycardia was in line with earlier studies. An impaired cardiovascular state at admission had already been linked with POI years ago. Blikslager et al. (1994) found an association of POI with both admission PCV and heart rate. A univariable association of POI with heart rate was demonstrated by Mair and Smith (2005a), who did not analyze PCV. However, most other authors (Cohen et al. 2004; French et al. 2002; Holcombe et al. 2009; Roussel et al. 2001) report PCV as the main associated factor. In our study heart rate

and PCV were strongly correlated (R^2 = 0.61) and PCV was associated with POI development in a univariable model. High heart rate and PCV are both present in endotoxemia and hypovolemic shock. However, the observed statistical associations do not imply a causal relationship between endotoxemia or hypovolemia and POI. Fluid sequestration in the GI tract may be a confounding factor, causing dehydration and GI dilation. This may lead to both increased PCV and tachycardia and predispose to ileus. The stronger association in our study of heart rate with POI might reflect underlying effects of pain caused by gastric and small intestinal distention. The association of POI with preoperative reflux also indicates that preoperative GI distention may be an important underlying factor. Other findings that suggest an important role for intestinal dilation are the early morphological changes occurring in the intestinal wall proximal to a strangulated intestine (De Ceulaer et al. 2011a; De Ceulaer et al. 2011b). For further assessment of the effects of dilation, it would be interesting to evaluate if the same changes occur aboral from a strangulated region, where there is proximity of degenerating bowel likewise, but obviously no intestinal dilation.

In the current study (Chapter 3.1), the incidence of POI in SI cases at risk (surviving > 24h postoperatively) was 33%. In previous studies, incidence rates of POI in SI colic horses were 47% (Blikslager et al. 1994); 10% (Freeman et al. 2000); 36% (Roussel et al. 2001); 50% (Cohen et al. 2004); 34% (Mair and Smith 2005a) and 27% (Holcombe et al. 2009). Apparently no substantial decrease in mortality rates occurred over the last decade. Freeman et al. (2000) attribute their low incidence partially to a shorter duration of symptoms at admission and also suggest that the surgeon's experience may account for the wide range of incidences in different studies. In our study, the duration of symptoms before admission ranged from 1-72 hours (median 8 h). Detailed information on colic duration is lacking in other studies but Freeman et al. (2000) report durations ranging from 4 to 36 hours. In line with earlier studies (Mair and Smith 2005a; Blikslager et al. 1994; Roussel et al. 2001), we found that SI resection was significantly associated with POI, in contrast to SI strangulation. This implies that an earlier referral could contribute to the prevention of ileus, in agreement with findings by Freeman et al. (2000) who

report less resections and higher survival rates in horses with a shorter duration of colic. The importance of early treatment is also illustrated by the results on heart rate and PCV.

Early treatment also implies a rapid identification of surgical cases, which may be challenging in some cases. The selection of a surgical case is first of all based upon a thorough clinical examination, including a rectal palpation and nasogastric intubation, and upon observation of the degree of pain, the progression of the case and the response to medical therapy. Abdominal ultrasonography can be very useful as it is a means to detect structural abnormalities. Additional information can be gained from blood work and the evaluation of peritoneal fluid. Increased PCV values as well as blood gas or electrolyte changes indicate cardiovascular compromise, while peritoneal fluid changes may point to intestinal strangulation, inflammation or peritonitis (Gaughan and Van Harreveld 2002; White et al. 2005). A biochemical parameter that receives increasing attention is the blood or peritoneal fluid lactate concentration as a marker for ischemia. In colic horses, increased lactate concentrations have been associated with the need for surgery, the need for a small intestinal resection and the probability of POI or death (Delesalle et al. 2007). The availability of reliable portable devices for lactate determination has made this parameter accessible for both referring veterinarians and clinicians (Delesalle et al. 2007; Sloet van Oldruitenborgh-Oosterbaan et al. 2008). Peritoneal D-lactate, a stereoisomer that is produced by bacterial fermentation, could be a suitable novel biomarker. A recent study in colic horses showed that this parameter has high sensitivity but moderate specificity in the diagnosis of ischemic intestine (Yamout et al. 2011).

Although the time to referral and the experience of the surgeon are hard to influence on short-term, another notable finding of the Freeman et al. (2000) study is the early feeding protocol applied after surgery. Water is offered at 12 to 18h and the first roughage at 18 to 24h after surgery. Another study, with a very high POI incidence of 50% in SI cases, reports the first feeding only after 35h (median) postoperatively and even later for horses that developed POI (Cohen et al. 2004).

Unfortunately no information was gathered on feeding regimen in the current study. Generally at the Ghent University clinic, the first water and roughage are offered to SI colic horses only after evaluation of gastric emptying by abdominal ultrasound, usually the morning after surgery but > 24h postoperatively in cases operated during the night.

Fasting by itself inhibits intestinal motility in horses (Mitchell et al. 2005; Naylor et al. 2006) and the relation of postoperative fasting with POI development seems an interesting subject for further research. It has been demonstrated that the body's response to food deprivation changes in the presence of disease or inflammation. In contrast to the situation in food deprived but healthy animals, diseased animals quickly convert to a catabolic state, with utilization of proteins as principal source of energy. This condition is related to poor wound healing and a compromised immune function (Carr and Holcombe 2009; Langhans 2002; Romijn 2000). Moreover, enteral nutrient depletion has been associated with a delayed recovery of the intestinal epithelial barrier function in humans, manifested by degenerative changes in the intestinal mucosa and increased transmucosal penetration of bacteria (Hernandez et al. 1999; Saito et al. 1987). In addition to enteral and systemic nutrient depletion, a lack of cephalic stimulation of the GI tract might also contribute to the negative effects of fasting. In humans, sham feeding by gum chewing after abdominal surgery decreases POI duration. The positive effects of sham feeding on motility are probably mediated directly by sensory and mental inputs (the sight, smell and even thought of food) that lead to the cephalic stimulation of vagal activity. Indirect effects of sham feeding are an increased release of GI hormones and digestive secretions (Asao et al. 2002). Early water intake has the same positive effects but is not tolerated by all patients (Stewart et al. 1998). These effects have not been studies in depth in horses. The common opinion used to be that food deprived horses merely suffered from negative stress related effects when seeing other horses in the premises being fed. However, horses might experience comparable beneficial effects from sham feeding as human patients, e.g. on GI motility.

Positive clinical effects of early enteral feeding in human medicine (Roberts and Zaloga 2000), promote the evaluation of early postoperative feeding protocols in horses. Currently for colonic surgery, feeding small quantities of grass or high quality

grass hay is advised, starting already a few hours after anesthetic recovery. Horses operated for SI problems may be fed with small volumes of highly digestible, low bulk feeds like alfalfa leaves, fresh grass or a complete pelleted diet. Frequent feeding of very small amounts may be started within a few hours postoperatively if no symptoms of POI are present (Magdesian 2003). Additional parenteral nutrition should be considered if limited or no oral intake is possible, aiming at supplying at least the horse's basal energy needs.

Unfortunately, the highly fatal character of POI was confirmed by the current results: only 34% of POI cases survived to discharge, compared to 85% of horses at risk without ileus. The odds for non-survival increased 28-fold in POI horses (Chapter 3.1). Survival rates in POI horses were low compared to reported rates of 50% (Mair and Smith 2005a); 59% (Cohen et al. 2004); 63% (Morton and Blikslager 2002) and even 84% (Roussel et al. 2001) in other studies. Survival rates for horses without POI however, were comparable.

The high POI mortality rates compared to other studies are hard to explain. The difference could be due to the use of alternative definitions of POI. In the current study, horses were not classified as POI cases unless they had > 8 liters of reflux in one occasion or > 20 liters in 24h. This ruled out some less pronounced cases that would be attributed to the ileus group by other authors (Mair and Smith 2005a; Morton and Blikslager 2002).

Applied treatment protocols were similar to those described in literature. Only regarding the placement of an indwelling nasogastric tube, POI treatment at the Ghent University equine clinic may differ from protocols used in other clinics. Indeed, in our clinic veterinarians tend to leave the tube in place in refluxing horses, allowing for regular gastric decompression without the need for repeated intubation. However, an indwelling tube was reported to delay gastric emptying in a small study on 6 horses (Cruz et al. 2006), although this was not confirmed by another study (Lammers et al. 2005). Disadvantages of the alternative –intermittent intubation– are pharyngeal irritation, additional stress for the patient and the risk of gastric rupture, making close monitoring required.

Another possible explanation for higher fatality rates of POI is an increased choice for elective euthanasia. Unfortunately, the vast majority of horses in Belgium is still uninsured, a situation that is not in favor of the long-term application of intensive, costly and prognostically dubious treatments. Illustratively, in one study (Cohen et al. 2004) the occurrence of POI almost doubled the total hospital bill.

In conclusion, in spite of improved surgical and postoperative care for colic horses, POI still has a high morbidity and mortality. It remains unclear why the horse is so susceptible to severe GI motility disturbance. Early referral and –if needed– prompt surgical treatment of colic horses remain imperative. Resolution of the GI obstruction before the development of marked intestinal dilation and systemic changes will prevent a number of POI cases. In horses at risk of developing ileus, however, medical treatments are still needed.

PHARMACOLOGICAL TREATMENTS

The second objective of this thesis was to assess the prophylactic efficacy of two prokinetic drugs, lidocaine and metoclopramide.

INTRAVENOUS LIDOCAINE

Lidocaine is usually applied in the prophylaxis and treatment of equine postoperative ileus as a 1.3 mg/kg IV bolus followed by a CRI of 0.05 mg/kg/min (pharmacokinetics: see below). It can be administered either intra- or postoperatively or both, and it has been clinically applied as a prophylactic drug or as a curative treatment for POI (Van Hoogmoed et al. 2004). At the Ghent University equine clinic, lidocaine is used both intra- and postoperatively and it is primarily intended for the prevention of ileus. Only in rare cases lidocaine treatment was only started after the horse developed POI (curative treatment). Prophylactic lidocaine

was most often administered as a sole treatment, sometimes combined with metoclopramide.

The factor "postoperative lidocaine treatment" was evaluated in a multivariable model together with other risk factors for POI development and for non-survival (Chapter 3.1). Lidocaine administration that was initiated after the onset of POI was logically excluded for the outcome parameter POI development. Our results quantified for the first time the clinical benefits of prophylactic lidocaine treatment. Only 21% of horses that received prophylactic IV lidocaine developed POI, compared to 51% of untreated horses. Lidocaine prophylaxis was a significant protective factor against POI in both the univariable and multivariable model (P = 0.013; OR = 0.31 (95% CI 0.12-0.78). Only a few other studies have evaluated the clinical effects of lidocaine treatment. Cohen et al. (2004) found limited multivariable evidence (P = 0.169) for a prophylactic effect of intraoperative lidocaine. No results on postoperative prophylactic lidocaine were reported. Brianceau et al. (2002) evaluated lidocaine administered during surgery and 24h postoperatively. They observed an improvement of some ultrasound parameters for contractility, but no difference in clinical intestinal motility (e.g. presence of reflux, defecation, intestinal sounds) or in outcome. Malone et al. (2006) report variable results in POI horses treated with lidocaine: although the clinical course of the complication improved, no differences in survival were observed. The apparently disappointing results of lidocaine, compared to the present study, could be explained by the fact that Cohen et al. (2004) did only evaluate intraoperative lidocaine; both other studies (Brianceau et al. 2002; Malone et al. 2006) were limited in sample size (28 and 32 horses respectively, compared to 126 in the current study) and the Malone group evaluated only curative lidocaine treatment.

In contrast to the findings reported by Malone et al. (2006), we could demonstrate a significant positive effect of postoperative lidocaine on short-term survival. The odds for survival increased 3.3-fold in horses that received lidocaine (P = 0.047; 95% CI for OR 0.09-0.98). Even though POI was a very important risk factor for non-survival (OR = 28.2), lidocaine treatment remained a significant protective

factor against non-survival in the multivariable model with POI. This is an indication for a positive effect of lidocaine on survival that goes beyond the effect exerted through decreased occurrence of ileus. Findings of Peiro et al. (2010) did recently demonstrate protective effects of lidocaine against the effects of endotoxin in horses, namely better clinical scores and lower activity of pro-inflammatory TNF-α. This might additionally explain the positive effect on survival observed in the current study.

Different mechanisms have been suggested for the positive effects of lidocaine on POI development and survival. A potential direct stimulating effect on intestinal smooth muscle has been proposed, but a lack of *in vitro* and *in vivo* prokinetic effect in healthy tissue (Nieto et al. 2000; Milligan et al. 2007) makes this hypothesis less probable.

Another possibility is the attenuation by lidocaine of the neurogenic inhibition of the intestinal motility. Its analgesic properties might inhibit autonomic reflexes or decrease nociceptive activation of the sympathetic nervous system. Intravenous lidocaine did indeed provide pain relief in humans, accompanied by a lower cytokine response and earlier return of bowel function (Kuo et al. 2006). However, evaluation of its analgesic effects in horses showed that IV lidocaine provided somatic, but no visceral nociception (Robertson et al. 2005).

The third and most sustained hypothesis is related to the anti-inflammatory effects of lidocaine. Lidocaine has inhibitory effects on various inflammatory cell types, including neutrophils, monocytes and macrophages (Hollmann et al. 2004). It decreases the production of superoxide anions and several adhesion molecules by the neutrophil (Azuma et al. 2000; Lan et al. 2004a; Lan et al. 2004b) and inhibits macrophage NO production (Shiga et al. 2001; Wang et al. 2011). The inhibition of nuclear factor (NF)-κB and cytokine release might be an important pathway leading to the observed anti-inflammatory effects (Lee et al. 2008). Whereas the analgesic effects exerted by local anesthetics are mediated by sodium channel blocking, recent findings indicate that their anti-inflammatory properties additionally result from

actions on other receptors like G-protein coupled receptors (Hollmann et al. 2001; Hollmann et al. 2004).
In human medicine, lidocaine was demonstrated to inhibit neutrophil and endothelial cell adhesion molecule expression after ischemia-reperfusion injury (Lan et al. 2004a). It inhibited cytokine responses and enhanced the recovery of bowel function after colonic surgery (Kuo et al. 2006) and decreased the duration and symptoms of postoperative ileus (Marret et al. 2008; Rimbäck et al. 1990).
In horses, lidocaine was demonstrated to decrease the *in vitro* adhesion and migration of neutrophils (Cook et al. 2009a). It decreased COX-2 expression and neutrophil counts after intestinal ischemia-reperfusion (Cook et al. 2009b) and attenuated the inhibitory effects on mucosal recovery exerted by the non-selective COX-inhibitor flunixin (Cook et al. 2008). Recently, *in vitro* effects of lidocaine on equine smooth muscle motility after ischemia-reperfusion challenge have been evaluated. Both after *in vitro* and *in vivo* administration, lidocaine was demonstrated to improve contractility and basic cell function, the latter measured by the release of creatine kinase (Guschlbauer et al. 2010; Guschlbauer et al. 2011).

The pharmacokinetics and side effects of IV lidocaine at a bolus of 1.3 mg/kg followed by CRI of 0.05 mg/kg have been studied in horses. Reported therapeutic levels are 1000-2000 ng/ml (Malone et al. 2006) and toxicity may occur at levels above 1850 ng/ml (Meyer et al. 2001). It was demonstrated that the therapeutic levels are reached after administrating the mentioned dosage regimen in colic horses undergoing an exploratory laparotomy (Feary et al. 2006). Increasing plasma concentrations have been reported after several hours of administration (Milligan et al. 2006); however, during a prolonged infusion of 96 hours, lidocaine accumulation was not observed and potentially toxic concentrations of > 1850 ng/ml never reached. On the contrary, plasma concentrations were often slightly below the target therapeutic level of 980 ng/ml (Dickey et al. 2008). An observed accumulation of a potentially toxic metabolite of lidocaine, glycinexylidide, warrants for caution, but toxic levels have not yet been determined and no clinical signs of toxicity were apparent (Dickey et al. 2008). Lidocaine is reported to be about 50% plasma protein bound. Highly protein bound drugs like ceftiofur have been observed to displace

lidocaine and cause increased free concentrations (Milligan et al. 2006). No changes in plasma lidocaine concentrations were observed after concurrent administration of flunixin, which is also highly protein bound and often applied in combination with lidocaine in a clinical situation (Waxman et al. 2012).
The narrow therapeutic index and fast metabolism (Engelking et al. 1987) of lidocaine make regulation of the infusion with fluid pumps advisable. Observed adverse effects are muscle fasciculation, ataxia, collapse, mild sedation or anxiety and alteration in visual function (Brianceau et al. 2002; Malone et al. 2006; Meyer et al. 2001). Its short half-life of 40 minutes leads to a quick resolution of side effects after discontinuation of the infusion (Engelking et al. 1987).

It can be concluded that lidocaine has important beneficial effects on equine intestinal integrity and motility. Although the current results on ileus prophylaxis and short-term survival in SI surgical colic horses are very promising, more clinical studies are needed. The benefits of IV lidocaine in POI prophylaxis have to be confirmed and information must be gained on its potential for the treatment of POI cases; information that was lacking in the current study. Furthermore, the clinical effects of lidocaine in endotoxemic horses are promising and warrant further investigation. The observed effects in the multivariable survival analysis indicate that this drug might also be useful in large intestinal colic cases or other pathologies involving endotoxemia.

METOCLOPRAMIDE

No evidence was found for the benefit of metoclopramide administered IM at a dose of 0.05 mg/kg every 6h (Chapter 3.1). Although indications for a prokinetic effect in horses were found in several studies, univocal evidence on its clinical effectiveness is still lacking. The occurrence of extrapyramidal side effects, caused by the effect on central D_2 receptors, makes that this drug has to be used with caution. Some authors have advised domperidone, a D_2 antagonist with only minimal penetration through the blood brain barrier, as a safer alternative (Reddymasu et al. 2007). Positive

effects of domperidone in GI electromechanical activity and coordination were demonstrated in a small clinical study (n = 3) (Gerring and King 1989). More information on the effectiveness and possible side effects is needed before considering this drug as a valid alternative. At the moment, neither metoclopramide, nor domperidone can be advocated for the treatment or prophylaxis of ileus.

PLASMA SEROTONIN CHANGES

SEROTONIN DETERMINATION

Serotonin is a major regulator of GI motility and also exerts functions inside the brain and in the circulatory system. In colic horses, platelet activation as well as EC cell serotonin release could affect plasma serotonin. Serotonin concentrations in platelet free plasma are in the nanomolar range which makes them difficult to quantify. In equine serotonin research, mostly HPLC and sometimes ELISA or RIA methods have been used. The highly variable reference values for equine PPP serotonin reported in literature (2.5 to 90 ng/ml; Chapter 3.2), indicate a need for a highly reliable quantitation method. Therefore the third objective of this thesis was to develop a solid method for equine PPP serotonin measurement and, concurrently, to evaluate results of a more accessible test. We developed and validated a suitable LC-MS/MS method and compared the results with those of a commercial ELISA test kit (Chapter 3.2). The advantage of the LC-MS/MS method is its high specificity. The sample clean-up step turned out to be the pitfall of this analysis. Analogous problems in other reported chromatographic methods could lead to decreased serotonin recovery, contributing to the highly variable reference values reported. We were able to solve this problem adjusting the pH with sodium hydroxide, followed by a liquid extraction with ethyl acetate.

The method, as described in Chapter 3.2, could be applied for serotonin determination in various conditions and for studying normal serotonin physiology in the horse. The linearity was validated in a rather limited range of 3-100 ng/ml;

suitable for PPP serotonin determination. Serum samples contain much higher serotonin concentrations compared to PPP, therefore additional validation studies in serum would extend the applicability of this method even further.

The ELISA kit (Serotonin ELISA, Genway Biotech, San Diego, CA) was evaluated as a practical alternative for the LC-MS/MS method. Although less accurate than LC-MS/MS, ELISA tests are much easier to apply in a clinical setting. A drawback of the commercial kits is their expense, which might limit their use for individual patient evaluation. The observed ELISA serotonin recovery rates were acceptable and ELISA and LC-MS/MS results were highly correlated (R^2 = 0.94). However, test agreement was limited, with consistently higher results for the ELISA test. This points out the risks of comparing results of different tests, and the need for method specific reference values. Researchers need to be aware of this when comparing different literature results for plasma serotonin. In conclusion, the serotonin ELISA could be used as an alternative for LC-MS/MS for the determination of serotonin in horses.

PLASMA SEROTONIN CHANGES IN SMALL INTESTINAL SURGICAL COLIC HORSES

The fourth objective of this thesis was to quantify plasma serotonin changes in SI surgical colic horses and to study serotonin as a potential parameter for predicting POI development and non-survival. Therefore PPP serotonin concentrations were measured at several time points pre- and postoperatively and compared to values in healthy horses. The finding that serotonin concentrations were significantly decreased in colic horses, was somewhat unexpected. Indeed, it has been demonstrated in other species that intestinal ischemia-reperfusion causes a serotonin release from the EC cells (Choudhury et al. 1996; Galmarini et al. 1997; Kaihara et al. 1997; Matia et al. 2004; Nakamura et al. 2001). However, the evidence of a subsequent increase in plasma serotonin is limited to one study in rats, that only demonstrated a short lived increase (Teramoto et al. 1998). On the other hand, Delesalle et al. (2008) were able to demonstrate increased plasma serotonin

concentrations in strangulating SI colic horses. They suggest activated platelets as an important source of this PPP serotonin.

Although the number of horses tested in our study may be limited, the collection of several samples per colic horse led to a total of 117 colic samples and 24 control samples. Serotonin values in colic horses were consistently decreased at different perioperative time points. The confirmation of these results with both LC-MS/MS and ELISA also contributes to the reliability of the findings. In comparison to many equine serotonin studies, considerable effort was done in our study as well as the Delesalle (2008) study to prevent and monitor serotonin changes by *in vitro* platelet activation. Although the observed decrease of plasma serotonin concentrations has to be confirmed in larger studies, our results lead to the conclusion that perioperative increases are not expected, or at least very variable, in surgical SI colic horses. Peripheral serotonin does not turn out a suitable marker for intestinal ischemia in horses. Our results on survival and POI development, neither being significantly associated with PPP serotonin concentrations, contribute to this conclusion.

Although serotonin is an important molecule exerting multiple physiological functions, many circumstances seem to affect its plasma and serum concentrations, as summarized in the introduction section of Chapter 3.2. At the moment, plasma serotonin changes do not seem a key factor in the pathogenesis or prediction of POI. Therefore future pathophysiological studies should rather focus on local serotonin changes in the intestinal wall. It would be useful to assess whether those changes can be demonstrated in horses, whether they are associated with POI development and whether they are accompanied by an altered intestinal response to serotonin agonists.

CONCLUSIONS ON POI AND FUTURE DIRECTIONS

Postoperative ileus is a multifactorial problem. Therefore, strategies applied to decrease its incidence and mortality must also be multimodal. In human medicine, a significantly improved patient outcome and shorter hospitalization period are obtained by combining single-modality evidence based care principles into a multimodal recovery program (Rawlinson et al. 2012).

The first aim should be the prevention of ileus by avoiding the etiologic risk factors: circulatory compromise and intestinal dilation. Horse owners, referring veterinarians and clinicians need to be aware of the urgency involved with small intestinal colic. Signs of SI distention upon rectal examination are a sufficient ground for referral to a clinic. Abdominal ultrasound examinations should be performed more often, even by the attending veterinarian. They can significantly accelerate the detection of gastric distention with fluid or dilated small intestines (Beccati et al. 2011; Busoni et al. 2011), especially in regions too distant for rectal palpation. Another useful aid is the determination of the blood or peritoneal fluid lactate concentration (Delesalle et al. 2007).

A second strategic focus must be the prevention and treatment of intestinal inflammation. Increasing evidence shows that IV lidocaine has prophylactic properties. It may also have curative effects. Lidocaine may be combined with the NSAID flunixin for analgesic and anti-inflammatory purposes. Future research should be aimed at unraveling the early, molecular pathophysiologic pathways with the goal of suppressing intestinal inflammation at an early stage. It could also be interesting to evaluate the effects of corticosteroid administration to horses at risk of developing POI. In human medicine several large studies demonstrate a prophylactic effect of a single preoperative dose of dexamethasone on postoperative nausea and vomiting (Apfel et al. 2004; Feo et al. 2006; Henzi et al. 2000; Wang et al. 2000). No adverse effects on wound healing or other complications were observed (Feo et al. 2006; Henzi et al. 2000). However the exact mechanism of the anti-emetic effect is unknown and positive and adverse effects in horses are to be evaluated.

A third goal should be preventing the neurological inhibition of motility. The suppression of intestinal inflammation will already contribute to this. Local ENS inhibiting reflexes can be avoided by the prevention of intestinal dilation and by surgical decompression of dilated intestines. Appropriate surgical techniques, without excess mesenteric traction, help to prevent sympathetic activation. Drugs antagonizing α_2-adrenergic receptors are available, but unambiguous evidence for their effectiveness was never found.

A last strategy is the active stimulation of motility. Although many researchers have focused on finding the key drug to cure postoperative ileus, it must be realized that inducing motility in severely damaged, inflamed intestines is basically impossible, and that a morphologically healthy intestine on the other hand, will generally display normal motility. Therefore prokinetic treatment is intentionally the last item in this section. At the moment there is a lack of effective, evidenced based prokinetics in horses.

In view of the importance of motilin as a determinant of the MMC, erythromycin or other motilin receptor agonists would be interesting subjects for future equine motility studies (Binder 2009; Sasaki and Yoshihara 1999). Motilin receptors have been demonstrated in the equine duodenum, jejunum, pelvic flexure and cecum, with the highest number occurring in the more proximal regions (Koenig et al. 2002). There is some experimental evidence in horses for the prokinetic effect of motilin (Sasaki and Yoshihara 1998) and the motilin receptor agonist erythromycin (Lester et al. 1998; Ringger et al. 1996; Roussel et al. 2000). However, the observation that the number of motilin receptors is decreased after intestinal ischemia or dilation (Koenig et al. 2006) points out the need for clinical studies. In human medicine, failure of motilin agonists in clinical trials has been attributed to problems of receptor desensitization and impaired gastric accommodation. The latter can be avoided by selection of the appropriate dose, emphasizing again the importance of elaborate clinical trials. A number of second generation motilin agonists are currently under evaluation in humans (De Smet et al. 2009).

Another interesting molecule is the rather recently discovered ghrelin. This multifunctional hormone has a structural similarity with motilin and is co-secreted

by motilin producing cells. Its positive effects on gastric emptying and on the MMC complex were shown in several studies (summarized in: De Smet et al. 2009) and several promising ghrelin agonists are currently under development for human POI treatment. Advantages of ghrelin are its concurrent cholinergic anti-inflammatory and appetite stimulating effects (De Smet et al. 2009).

In human medicine serotonergic drugs, in particular 5-HT_4 receptor agonists and 5-HT_3 receptor antagonists, have been studied extensively. Besides their effect on motility, these drugs can also interact with serotonin receptors on the EC cells. In guinea pigs, 5-HT4 agonists and 5-HT3 antagonists were demonstrated to inhibit the mucosal serotonin release (Gebauer et al. 1993). This could be a relevant feature in attenuating the ischemia-reperfusion induced 5-HT release, if the latter is present in horses. Serotonergic drug research in horses should focus on gaining more information on the equine serotonin receptors and on the *in vitro* and *in vivo* effects of the drugs, before proceeding to clinical studies.

Besides the administration of medication, feeding also has stimulating effects on intestinal motility. However, it may be hard to decide in the early postoperative stage as well as in POI horses if the horse can tolerate food. Empirically, at the Ghent University clinic feeding is started if less than 4l of gastric content could be retrieved over an 8h period. However, there are no evidence based criteria on when to stop emptying the stomach. Indeed, even in healthy horses a considerable amount of duodenal reflux towards the stomach is considered normal (Merritt 1999).

SURGICAL SITE INFECTIONS

The fifth and last objective of this PhD thesis was to study the incidence of surgical site infections in view of the applied skin closure technique. We found a general incidence of 15%, which was in line with equine literature results. Infections in horses occur much more frequently than in other species. In human medicine, a large multicenter study showed that gastrointestinal surgery is associated with a low SSI incidence of 1.3%; colorectal surgery had a higher SSI rate which is still limited to 4.1%. Certain procedures, like a small bowel resection, had a higher incidence of

6.2% (De Lissovoy et al. 2009). In dogs and cats, 5 to 6 % SSI have been reported after comparable surgical procedures (Brown et al. 1997; Nicholson et al. 2002; Mayhew et al. 2012). Evidence on SSI incidence in cattle is limited. In one study, an incidence of 2.4% has been reported for standing laparotomies. Although the number of cases was rather limited, the ventral abdominal approach led to a significantly higher incidence of 36% (5 out of 14) (Desrochers et al. 1996). Contaminated surgery like caesarian section led to 22% wound infections (Seger et al. 1994).

As discussed in the introduction section, the susceptibility to SSI depends on the number of bacteria in the wound, bacterial virulence and the innate host resistance. Therefore explanations for the high incidence of SSI in horses should be sought in the above parameters.

Equine SSI do not seem to involve especially virulent bacteria. Our results confirm the importance of facultative pathogens: the majority of wounds were infected with *Staphylococci* and enterobacteriaceae. This is in line with infections occurring in humans (Cheadle et al. 2006). Although infections involving multiresistant bacteria were limited, methicillin-resistant *S. aureus* did occur in 4 out of 33 positive cultures. Routine perioperative broad spectrum antibiotics were administered for 3-5 days, which is long compared to human guidelines (Bratzler and Houck 2004) and protocols applied by veterinary surgeons in the USA (Traub-Dargatz et al. 2002). Although prolonged treatment may be warranted in horses with increased risk of septicemia, peritonitis or other severe infections, the duration of routine administration of antibiotics in uncomplicated cases needs to be reconsidered.

Several studies indicate that bacterial contamination occurs mainly in the immediate postoperative period (Freeman et al. 2012; Galuppo et al. 1999; Ingle-Fehr et al. 1997). However, rinsing the linea alba with sterile saline before closure of the subcutaneous tissues was identified as a significant protective step in our study. This indicates that intraoperative contamination is also contributing. Postoperative bacterial contamination could be facilitated in horses by the relatively violent anesthetic recoveries, compared to other species, sometimes involving intensive

contact of the surgical incision with the recovery floor and the horse's hind legs. Accurate protection of the incision is imperative. The influence of the contaminated stable environment, illustrated by the protective effect of an abdominal bandage (Smith et al. 2007), could also account for the difference between horses and other species. The higher incidence of SSI in ventral incisions in cattle may support this hypothesis.

The host resistance does not only involve the innate immune system but also tissue properties and local mechanical factors. Another explanation for the higher SSI incidence in horses could be a species-specific connective tissue structure or impaired lymphatic drainage from the abdominal wall, favoring the formation of fluid pockets. Indeed horses are prone to ventral edema formation, compared to other species. Increased traction on the sutures during the anesthetic recovery may also predispose horses to fluid pocket formation. The observed positive effects of an abdominal bandage could partially be related to the applied external pressure, which may decrease fluid accumulation.

Deeper infections could be facilitated by an inadequate blood supply. The tendinous tissue of the linea alba is typically rather avascular. In combination with the suture tension needed in a heavy animal like the horse, local ischemia and even necrosis might occur. Proper bite size and tension of the linea alba suture layer may help to avoid this (Trostle et al. 1994). Our finding that the use of skin sutures rather than staples is protective against SSI, might be attributed to a better skin apposition. This could prevent bacterial contamination of subcutaneous layers, but it may also decrease dead space formation. The significant influence of surgical experience illustrates the technical skills involved in proper abdominal closure avoiding dead space formation, tissue ischemia and excessive presence of suture material.

A number of SSI is probably unavoidable and horses seem to be at higher risk. Fortunately, the increasing knowledge of risk factors and protective factors may help to decrease the incidence of SSI as much as possible. Rinsing the linea alba before applying the next suture layer and the use of skin sutures instead of staples are two simple but effective techniques for the reduction of surgical site infections.

GENERAL CONCLUSION

Complications after colic surgery may occur at various time points. In the first days postoperatively, postoperative ileus has high morbidity and mortality, especially in small intestinal cases. Results from this PhD study emphasize the importance of timely surgical treatment. Evidence was found for the benefits of prophylactic lidocaine on POI and short-term survival, the latter possibly mediated by a protective effect against the effects of endotoxemia, another important short-term complication. We developed a solid LC-MS/MS method for platelet poor plasma serotonin quantification. Although changes in intestinal or platelet serotonin release may occur, plasma serotonin concentrations do not seem invariably associated with surgical colic and they are not suitable as a predictor for ileus or non-survival.

Surgical site infections are a major complication at the end of the hospitalization period. Among many potential risk factors, we found three important associations with SSI. The importance of technically perfect suturing was illustrated by the influence of the surgeon's experience. Lavage of the linea alba after closure decreases bacterial contamination and removes debris that might act as a nidus for bacterial growth. Finally the use of skin sutures instead of staples is advocated. Their significant positive effect on wound healing is probably caused by a better skin apposition.

Important long-term complications, occurring after discharge, are recurrent colic episodes, adhesions and hernia formation. Although these complications were not a subject of this thesis, POI and SSI were identified before as risk factors for adhesion formation; SSI are highly associated with abdominal herniation (Mair and Smith 2005b). Prevention of POI and SSI will thus also decrease problems in the long term.

We believe this thesis provided interesting new insights that will contribute to the final goal: an increased number of horses experiencing an eventless and complete recovery after colic surgery.

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SUMMARY

Gastrointestinal colic is a very common problem in the horse. Due to advances in surgical techniques and perioperative care, exploratory laparotomy has become a valuable treatment option in cases that do not respond to medical treatment. Because of improved technical possibilities, an increasing number of horses with severe pathologies can be surgically treated. However, and even in spite of better postoperative care, the incidence of postoperative complications has not decreased over the years.

Numerous complications of colic surgery have been described in the literature and there are great differences in their occurrence and severity. Complications with a high morbidity and low mortality, such as surgical site infections or thrombophlebitis, primarily increase treatment costs and prolong the hospitalization period. Other complications like postoperative ileus or endotoxemic shock are potentially fatal. Compared to intraoperative elective euthanasia, postoperative death or euthanasia is associated with a much higher financial, but also emotional toll for the owner.

By decreasing the occurrence of postoperative complications, colic surgery will become increasingly acceptable and affordable to a larger proportion of horse owners. Survival rates will increase and horses will have a more comfortable and faster recovery. This research project focused on two of the most important complications of colic surgery: postoperative ileus and surgical site infections.

First (Chapter 3), the incidence of postoperative ileus (POI) and risk factors associated with its development were studied and the effects of two prophylactic treatments evaluated. The next part of the research focused on the pathophysiology of POI, more specifically on the plasma serotonin changes occurring in colic horses. The study of surgical site infections (SSI) occurring after equine colic surgery, is described in the final part of this dissertation (Chapter 4). Possible risk factors for SSI were analyzed, including different skin closure techniques.

In Chapter 3, first the current incidence of postoperative ileus was determined. A retrospective study was conducted on colic horses operated on between March 1,

2004 through December 31, 2006 at the Ghent University equine clinic. Since POI usually onsets at the first day postoperatively and mainly occurs in small intestinal (SI) cases, the study population was limited to SI colic horses that survived surgery for at least 24h. In this group of 126 horses at risk, a POI incidence of 33% was recorded. Risk factors for POI development were preoperative reflux (> 8 liters), preoperative tachycardia and a small intestinal resection. All three of them emphasize the importance of timely referral and treatment of horses with colic, in order to prevent endotoxemia, intestinal dilation and ischemia-reperfusion lesions as much as possible. In the POI group, only 34% of horses survived to discharge, compared to 85% of non-POI horses. Postoperative ileus increased the odds for non-survival 28-fold. These results confirm that for SI colic surgery, POI is still a frequently occurring and highly fatal complication (Chapter 3.1).

The effectiveness of intravenous lidocaine and intramuscular metoclopramide, both used as prophylactic drugs for POI, was evaluated in the same retrospective study (Chapter 3.1). Although metoclopramide did not exert a significant protective effect, postoperative treatment with intravenous lidocaine (a 1.3 mg/kg bolus followed by a continuous rate infusion of 0.05 mg/kg/h) led to a clear and significant decrease in POI occurrence. The odds ratio (OR) for POI in horses receiving lidocaine prophylaxis was 0.31 (95% confidence interval [CI] 0.12-0.78; P = 0.013). Lidocaine administration also exerted significant positive effects on short term survival (OR 3.33; 95% CI 1.02-11.1; P = 0.047) apart from the effects on POI. Anti-inflammatory and anti-endotoxemic properties of IV lidocaine possibly account for this positive effect on survival.

The next part of the research focused on plasma serotonin changes in surgical colic horses. Serotonin is an important intestinal neurotransmitter and a major regulator of the intestinal motility. Major human prokinetic drugs, some of which are also used in horses, act on serotonin receptors. Furthermore, serotonin is involved in platelet activation and is also released by activated platelets. All of this makes serotonin an interesting molecule in POI research. A serotonin release from ischemic bowel has been demonstrated in different species and platelet activation is

commonly observed in severe colic cases. Therefore we investigated changes in free plasma serotonin concentrations in surgical colic horses. This was done by determining the serotonin concentrations in platelet poor plasma (PPP) at several perioperative time points.

First, a reliable method for the quantification of serotonin in equine PPP had to be developed. In Chapter 3.2, the development and validation of a suitable LC-MS/MS method is described. For this method deuterated serotonin was used as an internal standard. The sample preparation was based upon a liquid extraction into ethyl acetate and chromatographic separation was performed with an acetic acid–acetonitrile mobile phase gradient elution. The method's linearity was demonstrated for serotonin concentrations ranging from 3 to 100 ng/ml. The limit of quantification was 3 ng/ml and the limit of detection 0.10 ng/ml.

The results of a commercial human serotonin ELISA kit, more suitable for use in a clinical situation, were compared with the LC-MS/MS results. Although the ELISA serotonin recovery rates were acceptable and the correlation between the two tests was excellent, Passing–Bablok regression and Bland–Altman plotting showed a poor agreement between the methods, with an increasing difference within the higher range of measurements. This emphasizes the need for in house determination of method specific reference values.

Subsequently, both quantification methods were used to study the evolution of PPP serotonin concentrations in surgical colic horses during the perioperative period (Chapter 3.3). The concentrations found in surgical colic horses preoperatively, immediately postoperatively and the next morning were compared to those in morning samples taken in healthy horses. During sample handling, measures were taken against inadvertent platelet activation and additional tests were performed to evaluate possible effects of platelet activation on serotonin concentration. Significantly lower PPP serotonin concentrations were found in all three samples of the colic horses, compared to controls. However, there were no associations with POI development or with non-survival. Free plasma serotonin concentrations are influenced by multiple mechanisms, but basically they mirror the ratio of serotonin release by enterochromaffin cells and –to a lesser extent– platelets, and plasma clearance, mainly by platelet uptake. Since findings on PPP serotonin changes are

inconsistent and there is a lack of association of PPP serotonin concentrations with POI or non-survival, it can be concluded that PPP serotonin does not seem a suitable marker in POI research. It might be more useful to focus on local intestinal serotonin changes in the future.

The next chapter of this dissertation (Chapter 4) describes a retrospective study of surgical site infections in the horse. A total of 356 horses that underwent colic surgery between March 1, 2004 and December 31, 2007 and survived at least 2 weeks postoperatively were included in the study. An SSI incidence of 15% was observed, with positive bacterial cultures in the majority of cases (33 out of 40). Multivariable regression analysis showed that surgical site closure by an inexperienced surgeon was a risk factor (OR 2.20 [95% CI 1.16-4.18]; $P = 0.016$) for SSI development. Lavage of the linea alba with sterile saline solution after closure was a protective factor (OR 0.38 [95% CI 0.20-0.74]; $P = 0.004$). The use of staples for skin closure was an important risk factor (OR 3.85 [95% CI 2.04-7.29]; $P < 0.001$) compared to monofilament sutures. Despite the fact that skin staples are easy to apply and their use may slightly decrease the total anesthesia duration, their association with SSI development makes them less suitable for use in equine celiotomies.

The final chapter (Chapter 5) contains the general discussion and the main conclusions. For the treatment and prevention of POI a multimodal approach is advised. Our results emphasize, once again, the importance of early referral. The use of IV lidocaine can be advocated as a prophylactic treatment for POI. Future research should be conducted to examine its curative potential once POI has developed.
Plasma serotonin did not turn out to be a suitable marker or predictor for POI. Further pathophysiologic POI research could rather focus on mucosal serotonin changes. However, the LC-MS/MS test we developed may be used for other serotonin research purposes. If LC-MS/MS is not available, ELISA is a useful alternative but rather for clinical purposes than for exact quantifications.

Our work on surgical site infections revealed three factors associated with their development. Together with earlier identified risk factors and protective factors, these results could be applied in strategies for the prevention of SSI.

In conclusion, the research conducted for this PhD study has led to increased knowledge on two of the most important complications after equine colic surgery. These recommendations can be implemented in evidence based protocols aimed at improving the results of colic surgery in the future.

SAMENVATTING

Gastro-intestinale koliek is een veelvoorkomend probleem bij het paard. Dankzij steeds verbeterende chirurgische technieken en perioperatieve zorg is exploratieve laparotomie een waardevolle optie geworden voor gevallen die niet reageren op een medicamenteuze behandeling. Door de toegenomen technische mogelijkheden kunnen steeds meer paarden met ernstige darmletsels toch operatief behandeld worden. De incidentie van postoperatieve complicaties is echter, ondanks de verbeterde postoperatieve zorg, de laatste decennia niet verminderd.

Er zijn talrijke complicaties van koliekchirurgie bekend, met grote variaties in hun ernst en mate van voorkomen. Complicaties met een hoge morbiditeit en lage mortaliteit, zoals postoperatieve wondinfecties en thromboflebitis, veroorzaken een stijging van de behandelkosten en een verlenging van de hospitalisatieduur. Andere complicaties, zoals postoperatieve ileus (POI) en endotoxemische shock, gaan bovendien dikwijls gepaard met postoperatieve sterfte of euthanasie. In vergelijking met een intra-operatieve euthanasie, leidt postoperatieve sterfte tot veel hogere kosten en dikwijls ook tot een grotere emotionele belasting van de eigenaar.

Het terugdringen van de incidentie van postoperatieve complicaties zal ertoe leiden dat koliekchirurgie door een groter deel van de paardeneigenaars als een acceptabele en betaalbare optie beschouwd wordt. De overlevingspercentages van koliekchirurgie zullen toenemen en geopereerde paarden zullen sneller herstellen en minder ongemak of pijn ondervinden. Dit onderzoeksproject is gericht op twee van de belangrijkste complicaties van koliekchirurgie: postoperatieve ileus en infecties van de buikwonde.

Eerst (Hoofdstuk 3) werd een studie uitgevoerd naar de incidentie van POI en de risicofactoren voor deze complicatie. Ook werd de effectiviteit onderzocht van twee preventieve behandelingen tegen POI. Het volgende deel van het onderzoek was gericht op de pathofysiologie van ileus, in het bijzonder op veranderingen van de plasma-serotonineconcentratie bij koliekpaarden. In het laatste deel van deze dissertatie wordt het onderzoek naar wondinfecties beschreven (Hoofdstuk 4). Er werd een risicofactor analyse uitgevoerd, waarbij onder andere de resultaten van twee technieken om de huid te sluiten, vergeleken werden.

In het eerste deel van Hoofdstuk 3 werd de epidemiologie van POI onderzocht. Een retrospectieve studie werd uitgevoerd op koliekpaarden die tussen 1 maart 2004 en 31 december 2006 geopereerd werden aan de Faculteit Diergeneeskunde van de Universiteit Gent. Omdat POI meestal pas de dag na de operatie begint en vooral voorkomt in paarden met dunne darmkoliek, werden enkel paarden die na een operatie voor dundarmkoliek minimaal 24u overleefden, geselecteerd voor de studie. In deze groep van 126 paarden was de incidentie van POI 33%. Risicofactoren geassocieerd met de ontwikkeling van POI waren preoperatieve reflux (> 8 liter), preoperatieve tachycardie en een dunne darmresectie. Hieruit blijkt het belang van tijdig doorverwijzen en operatief behandelen van erge koliekgevallen. Zo kan de ontwikkeling van endotoxemie, opzetting van darmen en het ontstaan van letsels door ischemie en reperfusie zoveel mogelijk voorkomen worden. Van de paarden die POI ontwikkelden, overleefden maar 34% de hospitalisatieperiode, in vergelijking met 85% bij de niet-POI groep. De kans op sterfte is extreem verhoogd bij POI-paarden (odds ratio [OR] 28.2). De beschreven resultaten bevestigen dat POI nog altijd een veelvoorkomende en vaak fatale complicatie is van chirurgie voor dundarmkoliek bij het paard (Hoofdstuk 3.1).

Het effect van intraveneus toegediende lidocaine en intramusculair ingespoten metoclopramide, beide gebruikt als preventieve medicatie tegen POI, werd onderzocht in dezelfde retrospectieve studie (Hoofdstuk 3.1). Er kon geen significant effect van metoclopramide aangetoond worden. Postoperatieve behandeling met lidocaine, door middel van een bolus van 1.3 mg/kg gevolgd door een continu infuus aan 0.05 mg/kg/u, leidde echter tot een significante vermindering van het aantal POI gevallen (OR 0.31; 95% betrouwbaarheidsinterval [BI] 0.12-0.78; P = 0.013). De toediening van lidocaine was ook geassocieerd met een significante stijging van de overlevingskans (OR 3.33; 95% BI 1.02-11.1; P = 0.047). Deze stijging kon niet louter door het effect op ileus verklaard worden. De anti-inflammatoire en anti-endotoxemische effecten van lidocaine liggen waarschijnlijk aan de basis van dit resultaat.

Het volgende deel van het onderzoek was gericht op mogelijke veranderingen van de plasma-serotonineconcentratie in de perioperatieve periode. Serotonine is een belangrijke neurotransmitter in de darm en één van de voornaamste regulatoren van de darmmotiliteit. Belangrijke humane prokinetica, waarvan sommige ook bij het paard gebruikt worden, werken in op serotoninereceptoren in de darm. Bovendien is serotonine betrokken bij de activatie van bloedplaatjes en wordt het ook vrijgesteld door geactiveerde plaatjes. Dit alles maakt serotonine een interessante molecule in het POI onderzoek. Bij verschillende diersoorten is reeds aangetoond dat ischemische darmen serotonine vrijstellen; activatie van bloedplaatjes wordt dikwijls gezien bij erge koliekgevallen. In dit kader werden veranderingen in de hoeveelheid ongebonden serotonine in het bloedplasma bij koliekpaarden opgevolgd. De serotonineconcentratie in plasma arm aan bloedplaatjes (platelet poor plasma; PPP) werd hiertoe gemeten op verschillende tijdstippen in de perioperatieve periode.

Eerst moest een betrouwbare methode ontwikkeld worden om serotonineconcentraties te meten in PPP bij het paard. In Hoofdstuk 3.2 wordt de ontwikkeling en validatie van een geschikte vloeistofchromatografie-tandem massaspectrometrie (liquid chromatography-tandem mass spectrometry; LC-MS/MS) methode beschreven. Er werd gebruik gemaakt van gedeutereerd serotonine als interne standaard. De monstervoorbereiding was gebaseerd op een vloeistofextractie in ethylacetaat en de chromatografische scheiding werd uitgevoerd door middel van gradiënt-elutie met een mobiele fase van azijnzuur-acetonitril. De lineariteit van de methode werd aangetoond voor serotonineconcentraties van 3 tot 100 ng/ml. De kwantificatielimiet was 3 ng/ml en de detectielimiet 0.10 ng/ml.

De resultaten van een humane serotonine ELISA kit, meer geschikt voor gebruik in een kliniekomgeving, werden vergeleken met de LC-MS/MS resultaten. Het terugvinden of de recovery van toegevoegd serotonine in de ELISA test was aanvaardbaar en de correlatie tussen de twee tests was zeer goed. Toch wezen Passing-Bablok regressie analyse en Bland-Altman plotting op een slechte overeenkomst tussen de twee methodes wat betreft de absolute waarden (“test agreement”). Het verschil nam toe bij hogere serotonineconcentraties. Dit geeft aan

dat laboratorium- en test-specifieke referentiewaarden nodig zijn voor een betrouwbare meting van PPP serotonine.
Tenslotte werden beide meetmethodes gebruikt om de evolutie van PPP serotonineconcentraties te volgen bij koliekpaarden tijdens de perioperatieve periode. Preoperatief, direct postoperatief en de daaropvolgende morgen werden stalen genomen. Referentiewaarden werden bepaald in stalen van gezonde paarden die steeds 's morgens genomen werden. Er werden maatregelen genomen om de activatie van bloedplaatjes tijdens het verwerken van de stalen te voorkomen. Mogelijke effecten van plaatjesactivatie op de serotonineconcentratie werden nagegaan door middel van afzonderlijke tests. De drie opeenvolgende stalen van de koliekpaarden bevatten elk significant lagere serotonineconcentraties dan de stalen van de gezonde paarden. Er waren echter geen associaties met de ontwikkeling van POI of met overleving. De vrije plasma-serotonineconcentratie wordt beïnvloed door allerlei factoren, maar weerspiegelt in principe de verhouding tussen enerzijds de serotoninevrijstelling uit enterochromaffiene cellen in de darm en in mindere mate de bloedplaatjes, en anderzijds de plasmaklaring, vooral door opname in bloedplaatjes. Omdat de bevindingen betreffende de veranderingen in PPP serotoninegehaltes niet consistent zijn en er geen associatie is met ileus of overleving, kan men concluderen dat PPP serotonine geen geschikte merker lijkt voor verder POI onderzoek. Wellicht is het nuttiger om zich in de toekomst te richten op veranderingen van de serotoninegehaltes in de darm zelf.

Het volgende hoofdstuk van deze dissertatie (Hoofdstuk 4) beschrijft een retrospectieve studie van buikwondinfecties na koliekchirurgie. Een groep van 356 paarden, geopereerd aan koliek tussen 1 maart 2004 en 31 december 2007 en met een postoperatieve overleving van minimaal 2 weken, werd geselecteerd voor de studie. De incidentie van wondinfecties was 15%. Bacteriologisch onderzoek was in de meeste gevallen (33 van de 40) positief. Multivariabele regressieanalyse toonde aan dat het sluiten van de buik door een onervaren chirurg een risicofactor voor infectie was (OR 2.20 [95% BI 1.16-4.18]; P = 0.016). Spoelen van de linea alba met steriele fysiologische zoutoplossing vóór het hechten van de volgende laag was een beschermende factor (OR 0.38 [95% BI 0.20-0.74]; P = 0.004). Het gebruik van

agrafen voor het sluiten van de huid was een belangrijke risicofactor (OR 3.85 [95% BI 2.04-7.29]; P < 0.001) in vergelijking met monofilament hechtdraad. Ondanks het feit dat agrafen gemakkelijk aan te brengen zijn en daardoor de totale anesthesieduur wat kunnen verkorten, maakt hun associatie met wondinfecties hen minder geschikt voor gebruik na een buikoperatie bij het paard.

Het laatste hoofdstuk (Hoofdstuk 5) bevat de algemene discussie en conclusies. Voor de preventie en behandeling van POI wordt een multimodale benadering voorgesteld. Onze resultaten onderschrijven het belang van tijdige behandeling van koliekpaarden en tonen positieve effecten aan van lidocaine als preventief medicament tegen POI. Verder onderzoek moet uitwijzen of dit medicament ook effectief is in de behandeling van paarden die al POI hebben ontwikkeld.
Plasma serotonine bleek geen geschikte merker of predictor te zijn voor POI. Verder pathofysiologisch onderzoek naar POI zou wellicht beter gericht zijn op de serotoninegehaltes in het darmslijmvlies. De ontwikkelde LC-MS/MS methode kan verder gebruikt worden voor het meten van serotonine in andere studies. ELISA is een alternatief als LC-MS/MS technologie niet beschikbaar is, maar dan eerder voor klinisch gebruik dan om absolute waarden te verkrijgen.
Het onderzoek naar buikwondinfecties heeft drie factoren geïdentificeerd die geassocieerd zijn met de ontwikkeling ervan. Gecombineerd met eerder beschreven risicofactoren en beschermende factoren passen ze in een strategie voor de preventie van buikwondinfecties.

Samengevat heeft deze doctoraatsstudie geleid tot een grotere kennis van twee van de belangrijkste complicaties na koliekchirurgie bij het paard. De gegeven aanbevelingen bieden mogelijkheden om de resultaten van koliekchirurgie in de toekomst te verbeteren.

CURRICULUM VITAE

Sara Torfs werd geboren op 12 september 1980 te Groningen. Na het beëindigen van het secundair onderwijs aan SSG De Rede in Terneuzen begon zij in 1998 met de studie diergeneeskunde aan de Universiteit Gent. In 2004 behaalde ze het diploma van dierenarts (optie paard met keuzevak kleine huisdieren) met grote onderscheiding.

Na een waarneming van enkele maanden in een gemengde praktijk voor paarden en kleine huisdieren startte zij in juni 2005 met een internship paard bij Dierenkliniek Emmeloord (Nederland). Na de voltooiing hiervan volgde een aanstelling als assistent bij de vakgroep Interne Geneeskunde en Klinische Biologie van de Grote Huisdieren op 1 juni 2006. Hier is zij betrokken bij het werk in de kliniek en de opleiding van laatstejaarsstudenten en volgt ze de opleiding tot specialist inwendige ziekten paard (ECEIM residency). Dit werd gecombineerd met een doctoraatsstudie over de complicaties na koliekchirurgie bij het paard. Ook vervolledigde zij in 2012 het trainingsprogramma van de Doctoral School of Life Sciences and Medicine van de Universiteit Gent.

Sara Torfs is auteur of coauteur van meerdere wetenschappelijke publicaties, gaf presentaties op verschillende internationale congressen en trad op als reviewer voor verschillende veterinaire tijdschriften.

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