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(Article begins on next page)

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Original Article

Are horse age and incision length associated with surgical site infection following equine colic surgery?

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Highlights

- Surgical site infection (SSI) occurred in approximately 20% of horses undergoing colic surgery
- SSI was significantly different between breeds (PO.05) but no age difference was observed (P>0.05).
- SSI was associated with procedures likely to involve heavier contamination of the surgical site.
- Horses with longer incisions and with signs of colic postoperatively had a higher SSI rate.
- Horses that received extended antimicrobial prophylaxis (>24 h) did not have a lower SSL

Abstract

It is our clinical impression that age and incision length are more strongly associated with surgical site infection (SSI) following colic surgery than skin closure or wound protection method. Therefore, the objective of this observational clinical cohort study was to identify the risks for SSI in horses undergoing colic surgery. Data collection included pre-, intra-, and postoperative variables. Variables with P <0.2 following univariable analysis were used in a logistic regression multivariable model. Variables with P<0.05 were included in the final model. Odds ratios (OR; 95% confidence intervals, 95% CI) were determined. The area under the curve (AUC) for the receiver-operator characteristic was calculated.

The final multivariable model included breed (P=0.008), incision length (P=0.004), surgical procedure classification (PO.001), and postoperative (PO) colic (P=0.037; overall model P<0.001, and AUC was 0.81 [excellent discrimination between SSI vs. no SSI]). Warmbloods (OR 12.0; 95% CI 2.7-74.8), American breeds (OR 6.4; 95% CI 1.2-43.0), and Thoroughbreds (4.5; 95% CI 1.1-25.5), more commonly had SSI than other breeds (ponies/miniature horses, Draft breeds, Standardbreds, Arabians, and Crossbreeds [referent]). A higher SSI rate was associated with incision lengths >27 cm (3.7; 95% CI 1.5-9.9), heavily contaminated procedures (12.0; 95% CI 3.3-49.9), and horses with PO colic (2.7; 95% CI 1.1-6.8). SSI appeared to be more common after heavily contaminated procedures and in horses with PO colic, which probably resulted in more incisional contamination and trauma. Some breeds appeared to have higher odds of SSI. Age was not associated with SSI. The risk of developing SSI was higher for horses with an incision >27 cm; therefore, surgeons are encouraged to use the minimum incision length required to accomplish the necessary abdominal exploration and bowel manipulation in the safest manner possible.

Keywords: Colic; Horse; Incision; Infection; Laparotomy

Introduction

Incisional surgical site infection (SSI) following colic surgery is typically reported in 15-25% of horses (Ingle-Fehr et al., 1997; Mair and Smith, 2005a; Coomer et al., 2007; Torfs et al., 2010; Colbath et al., 2014; Anderson et al., 2014; Isgren et al., 2015); however, reports vary from as low as 3% (Tnibar et al., 2013) to > 40% (Wilson et al., 1995; Durward-Akhurst et al., 2013; Costa-Farré et al., 2014). The frequent occurrence of SSI after colic surgery requiring prolonged treatment, increased likelihood of hernia formation (Ingle-Fehr et al., 1997; Mair and Smith, 2005b; Smith et al., 2007), and consequent delayed return to athletic activity (Davis et al., 2013) make SSI an important complication.

Conflicting findings from retrospective studies on SSI following colic surgery have led to lack of consensus among surgeons. While it seems logical that the proportion of horses developing SSIs would be higher following a clean/contaminated procedure, most studies report no association between enterotomy/enterectomy and SSI (Phillips and Walmsley, 1993; Ingle-Fehr et al., 1997; Galuppo et al., 1999; Coomer et al., 2007; Torfs et al., 2010; Colbath et al., 2014; Anderson et al., 2014). However, high operating room environmental bacterial colony forming units (CFU) and high post-recovery skin bacterial CFU were associated with SSI in one study (Galuppo et al., 1999). Other associations include large intestinal lesions (Phillips and Walmsley, 1993), wound closure performed by an inexperienced surgeon (Torfs et al, 2010), longer duration of surgery and hypoxaemia (Costa-Farré et al., 2014). Skin closure and wound protection methods have also been investigated. In one study, SSI did not differ when two-layer (body wall and skin suture) vs. three-layer closure was performed (Coomer et al., 2007), but in another a three-layer closure was protective (Isgren et al., 2015). Use of a modified subcuticular

suture pattern decreased SSI (Colbath et al., 2014), but subcutaneous closure with polyglycolic acid increased SSI (Costa-Farré et al., 2014). Skin stapling (vs. skin suture) was associated with SSI (Torfs et al., 2010). Using stent bandages increased SSI in one study (Mair and Smith, 2005a) but decreased it in another (Tnibar et al, 2013). The use of an abdominal bandage during the postoperative (PO) period appeared to decrease SSI (Smith et al, 2007). Younger horses and Standardbreds have also been reported to have fewer SSI (Wilson et al., 1995). These contradictory findings might result from the definition of SSI used, whether or not long-term follow-up was obtained, and differences in variables included in the analysis and how the variables were defined or categorised. Wide variations in SSI rates between veterinary hospitals and their inherent multifactorial associations make it difficult to form conclusions regarding optimal equine patient care.

It is our clinical impression that young horses tend to have fewer SSI than mature horses, and horses with long incisions extending into the cranial abdomen are more likely to develop SSI than horses with short, caudal abdominal incisions. We hypothesised that age and incision length would be more strongly associated with SSI than methods of wound closure and wound protection. To address our hypothesis, the objective of this study was to compare pre-, intra- and post-operative clinical variables between horses that did and did not develop a SSI following colic surgery.

Materials and methods

The study was an observational clinical cohort study. Informed consent was obtained at the time of hospital admission. Horses admitted to New Bolton Center for colic and recovering

from exploratory abdominal surgery through a ventral midline laparotomy were included. Data was collected prospectively (Supplementary Information 1) between December 2006 to May 2008 (group 1) and from August 2011 to September 2012 (group 2). Additional information pertaining to PO care and complications was obtained for both groups retrospectively from the medical record. Some data from group 1 were used in a separate study (Freeman et al., 2012); however, when specific variables associated with SSI were analysed using data from group 1, a number of trends towards significance were observed, prompting the collection of data prospectively (and retrospectively) from additional cases (group 2). Horses admitted during the intervening period were not included because prospective information (Supplementary Information 1) was not routinely or reliably part of the medical record and therefore was unavailable.

SSI was defined as drainage of serous, purulent or serosanguineous fluid from the incision after the initial 48 h PO that persisted for at least 36 h (Freeman et al., 2012), either during hospitalisation or following discharge from hospital. Horses were classified as having (a) normal wound healing, or (b) SSI. SSI was the end-point of interest; any horse with a SSI at any time point was included but horses were required to have at least 12 days (for rationale, see Results) without signs of infection to be classified as normal wound healing. Horses without SSI that were euthanased or underwent repeat laparotomy within 12 days of surgery were excluded, as were horses without at least 12 days PO follow-up, unless they developed a SSI within the period available. Horses that had a second laparotomy after 12 days were included in the study for their first surgery only. Post-discharge information pertaining to incisional healing and complications including SSI was obtained by telephone questionnaire with the owner/caregiver.

Signalment and bodyweight was recorded. The prospective data collection sheet is provided in Supplementary Information 1 and details of the standard surgical site preparation in Supplementary Information 2. The effects of antimicrobial drug (AMD) re-dosing were investigated in surgeries ongoing 120 min after AMD administration corresponding to approximately twice the half-life of IV penicillin G (53 min in horses; Dürr, 1976).

Primary surgeon was classified as experienced (ACVS board-certified > 5 years) vs. inexperienced (senior surgery resident, ACVS board-eligible or board-certified < 5 years). The procedure was categorised as exploratory laparotomy (needle decompression/repositioning); jejunojejunostomy/jejunocaecostomy (JT/JC); pelvic flexure enterotomy (PFE); high enema (HE); other enterotomy (small intestinal, typhlotomy, small colon); multiple enterotomies; large colon resection; or other. HE referred to intra-operative intraluminal lavage of the small colon with a nasogastric tube via the rectum. Because the type of surgical procedure was considered clinically important, for multivariable analysis, procedures were re-categorised based on the amount of contamination immediately adjacent to the incision as light contamination (JJ/JC, PFE, and HE) and heavy contamination (multiple enterotomies, other enterotomies, large colon resection, and other). Methods of skin closure were staples, suture, cyanoacrylate skin glue or none (subcutaneous tissue only). Skin incision length was measured after wound closure using the ruler on the scalpel blade handle. Incisional protection during recovery from general anaesthesia was classified as iodine-impregnated incise drape, stent or none. Use of an abdominal bandage during the PO period was recorded. A PO critical illness score (Freeman et al., 2012) was used to evaluate status during the first 3 days PO (score 0 to 3 based on heart rate, mucous membranes,

borborygmi, packed cell volume/total plasma protein). PO complications, duration of PO AMD administration, and length of hospital stay (LOS) were recorded.

Statistical analysis

Distributions of continuous variables were analyzed. If data were normally distributed or could be normalised using log or other transformation, they were analysed continuously. If they could not be normalised and appeared multimodal, they were categorised based on univariate normal mixture decomposition estimation to determine the number of normal distributions and their share. A cutoff point was constructed based on the upper limit of the 95% confidence interval (CI) for the distribution with the major share. All categorical variables were analysed using a Chi-squared test or a Fisher's exact test. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated. At minimum, the prospective data sheet (Supplementary Information 1) was completed and the medical record available. No assumptions were made regarding missing data points and they were left blank in the statistical analysis. The number of data points for each variable was recorded.

Variables with P <0.2 at the univariable stage were included in a multivariable analysis performed using a logistic fit and likelihood ratio tests. When the multivariable model was evaluated, variables with P >0.2 were sequentially removed such that the final model included variables with P<0.05. Clinically relevant two- and three-way interactions between significant variables were also evaluated. OR and 95% CI were calculated. Clinically relevant variables were forced back into the model to determine if there was any improvement in the model with their inclusion. Overall model fit, area under the curve (AUC) of the receiver operator

characteristic (ROC), the corrected Akaike Information Criterion (AICc), and Goodness-of-fit test were used to evaluate each iteration of the model. Statistical analysis was performed using JMP Pro 12 (SAS) and STATA 14.1 (Statacorp).

Results

Supplementary Information 3 provides a flow diagram of the study protocol. There were a total of 238 colic surgeries from which horses recovered during the study period. Two horses were admitted a second time for colic, at 12 and 49 days PO, and only admission one was included; therefore, there were 236 potentially eligible horses. Forty-one of the 236 potentially eligible horses (17%) developed a SSI; 35/227 (15%) horses underwent single and 6/9 (67%) horses underwent repeat laparotomy. In this study, SSI occurred between 2 and 21 days following surgery. The 75 th percentile was 12 days, which was used as the decision point for including horses in the study population or not, and for designating horses with no signs of SSI as having normal wound healing.

Ultimately, 185 of the 236 potentially eligible horses fulfilled the study inclusion criteria. Exclusion reasons were as follows: death within 12 days PO (n=23); lack of follow-up *(n=2l);* and repeat laparotomy within 12 days PO (n=7). Groups 1 and 2 included 96 and 89 horses, respectively. Among horses that fulfilled the inclusion criteria, 36/185 developed a SSI (20%). SSI occurrence was similar in group 1 (19%) and group 2 (20%). Statistical comparisons and effect size comparisons were made between groups 1 and 2 for selected variables. As no significant differences were identified *(P=0.082-0.800),* the two groups were combined. Data for 42 clinically relevant non-significant variables are included in Supplementary Information 4.

Results of univariable analysis are shown in Table 1. Because age had a multimodal distribution, cut-off values were determined for categorical analysis. SSI occurred more frequently in horses 12 to 19 years old than other age categories (P=0.001). Ponies/miniature horse (n=0/l 1) and Draft horse (n=0/7) categories had no SSI and could not be included in the multivariable analysis. Furthermore, because Standardbreds (n=l/22), Arabians (n=l/7), and Crossbred horses (n=l/6) each had only one horse with SSI, they were grouped together with ponies/miniature horses and Draft horses as 'other' for the multivariable analysis and used as the referent. Antimicrobial prophylaxis was based on the clinical judgement of individual surgeons. Horses administered potassium penicillin and gentamicin or enrofloxacin (to provide gram-negative coverage in two horses with azotaemia) had fewer SSI than horses given either penicillin *(n=2)* or gentamicin (n=2) or neither (n=l; P=0.047). Incisional length had a bimodal distribution therefore a length of 27 cm was used as the cut-off value for categorical analysis. SSI occurred more frequently in horses with an incisional length >27 cm than in horses with an incisional length < 27 cm (P=0.006).

SSI and procedure performed as originally categorised (Materials and methods) were not associated (P=0.522). However, procedures were re-categorised because preliminary examination of the data suggested that SSI occurred more frequently following procedures potentially associated with more contamination immediately adjacent to the incision vs. procedures with light contamination or contamination not immediately adjacent to the incision. SSI occurred more frequently in horses undergoing procedures re-categorised as heavily contaminated i.e. multiple enterotomies («=1/3; 33%), enterotomies other than at the pelvic

flexure *(n=2/5;* 40%), procedures categorised as other *(n=3/l0;* 30%), and large colon resection (n=2/3; 66%) than in horses undergoing procedures with light contamination or contamination not immediately adjacent to the incision, i.e. exploratory laparotomy with or without repositioning/decompression (n=16/87; 18%), JJ/JC (n=4/26;15%), PFE (rc=8/48; 17%), or HE only (rc=0/3;Table 1; P=0.036).

Horses with PO colic (P=0.059) and diarrhoea (P=0.072) during hospitalization were more likely to have SSIs (Table 1). Horses with SSI had a longer (log)LOS compared to horses without a SSI (P=0.003). The mean ± SD were 10.5 ± 7.4 and 7.2 ± 6.3 days, respectively. Because it is not possible to discern retrospectively whether increased LOS was a cause or an effect of SSI, it was not included in the final multivariable model (Isgren et al., 2015). That being said, when included in one iteration of the model, there was an interaction between PO colic and LOS (P=0.021). Horses with PO colic had increased odds (OR 8.5; 95% CI 1.4-52) of developing SSI for each increase in (log)day (3 actual days) of LOS. In horses without PO colic, longer LOS did not increase the odds of developing a SSI. There were no significant interactions between any other variables. The final multivariable model included breed, incision length, surgical procedure category, and PO colic (Table 2).

Discussion

Similar to previous reports of SSI occurrence following both equine exploratory laparotomies (Ingle-Fehr et al., 1997; Mair and Smith, 2005a; Coomer et al., 2007; Torfs et al., 2010; Colbath et al., 2014; Anderson et al., 2014; Isgren et al., 2015) and elective colorectal surgery in humans (26%, Smith et al., 2004), SSI occurrence in this study was 20%. The

development of SSI was significantly associated with heavily contaminated surgical procedures, PO colic, incision length >27 cm, and certain breeds. Age, bodyweight, methods of skin closure and wound protection, and duration of AMD use were not associated with SSI (Supplementary Information 4). Therefore, we accept part of our general hypothesis in that incision length was more closely associated with SSI than were methods of skin closure and wound protection; however, younger horses did not have fewer SSI compared to mature horses. Area under the ROC curves generated using the current dataset indicated that the final model we describe was excellent at discriminating between horses with and without SSI after colic surgery. However, without evaluating this model with a different dataset, the broad applicability of our findings are undetermined. Our intent is to perform a multicenter, prospective model evaluation.

There was a significant association between type of procedure(s) performed and SSI.
Most studies have reported no association between SSI and enterotomy/enterectomy, in agreement with our results. However, in our study, horses that underwent procedures categorised as likely to be heavily contaminated immediately adjacent to the incision were significantly more likely to develop SSI than horses that underwent procedures with light contamination or contamination not immediately adjacent to the incision. Therefore, categorising all enterotomies/enterectomies together is probably inappropriate when evaluating potential associations with SSL Procedures categorised as heavily contaminated might have had a higher operating room and surgical site contamination because these enterotomies tended to be performed closer to the incision, and possibly had longer times with the bowel open, particularly when multiple enterotomies were performed.

Surgical duration was not associated with SSI (Supplementary Information 4); however, surgical duration does not necessarily correlate with the length of time the bowel is open. High operating room environmental and post-recovery skin bacterial CFU have been associated with SSI (Galuppo et al., 1999). These variables were not investigated in the current study, thereby preventing further interpretation, which is a study limitation. Of note is that JC in our hospital were performed using a stapled side-to-side technique, usually using an ILA-100 for both the resection and anastomosis, which limited contamination (i.e. the bowel is only open at the stab incision to insert the stapling device for the anastomosis). Although JJ were performed using a hand sewn end-to-end technique, there was minimal to no gross contamination compared to an enterotomy, because the lumen was occluded adjacent to the resected ends. During more complicated procedures, higher contamination usually cannot be avoided. Therefore, protecting the incision throughout the procedure and effectively communicating with the owner/caregiver that horses that have undergone multiple or complicated procedures have higher risk of SSI is important.

Breed, but not age or bodyweight, was associated with SSI. As reported previously (Wilson et al., 1995), we found that Standardbreds had fewer incisional complications than other breeds. Although interpretation is challenging, one could speculate that genetic predisposition and body conformation, particularly relative abdominal size, could possibly play a role.

Horses with an incision lengths >27 cm had higher SSI rates. While incision length could be confounded by size of the horse, lesion site, and procedure performed, it remained significant in the multivariable analysis, even when such variables and their interactions were forced back

into the model despite their lack of significance. To the authors' knowledge, no other studies in human or veterinary medicine have demonstrated higher SSI rates with longer incisions, except studies comparing laparoscopic to laparotomy techniques. Interestingly, most studies that have investigated associations between SSI and laparotomy techniques have not included incision length as a variable, possibly because it is often more uniform in humans and other species for specific procedures. Horses may be somewhat unique compared to other species because of their large body size, the laparotomy incision being made on the ventral midline, the variability in the length of laparotomy incisions, and the immediate requirement for prolonged standing and ambulation.

Surgeon experience could potentially be a confounding variable with incision length but, in contrast to another study (Torfs et al., 2010), surgeon experience was not a statistically significant variable in our study. Shorter laparotomy incisions have been associated with less incisional edema (Bischofberger et al., 2010) and, therefore, may help limit the occurrence of SSI (Coomer et al., 2007). The incision for ventral midline laparotomy typically starts at the umbilicus and extends cranially. Studies of equine linea alba have shown that it is thinner in the cranial abdomen (Trostle et al., 1994; Chism et al., 2000) and subject to a relatively higher visceral load, which can result in increased tension on the skin/linea, which in turn could compromise wound healing. Incisions <27 cm typically remain within the 30-cm fenestration of the drape in our hospital and longer incisions require cutting the drape to extend the fenestration. Unfortunately, we did not record the initial drainage site or the length of the incision relative to the xiphoid-umbilicus length. Future studies should identify the location of initial drainage, the relative length of the incision compared to the animal size, and whether or not the incision

extends into the thinner part of the linea alba. Even so, surgeons should carefully consider their approach to a ventral midline laparotomy and, to potentially minimise the risk of laparotomy wound complications, should limit skin incisions to <27 cm in adult horses. That being said, a small incision is not recommended for exteriorising a distended or compromised colon or caecum because of risks associated with rupture.

Horses with PO colic were more likely to have SSI, particularly those horses with a longer LOS. Anderson et al. (2014) and Isgren et al. (2015) also recently reported an association between SSI and PO colic. PO colic can increase SSI through surgical site trauma associated with rolling/recumbency, tension associated with abdominal distension, and prolonged withholding of feed could influence wound healing and immunity. The role of LOS on SSI warrants further investigation.

Other results of interest were lack of association between SSI and many variables previously associated with SSI, including methods of skin apposition, surgical site protection, and age (Supplementary Information 4). Similar to our findings, in humans there was no difference in SSI when the skin was apposed with staples vs. subcuticular sutures following open gastrointestinal surgery (Tsujinaka et al., 2013). We also found no association between duration of PO AMD administration and SSI, which argues against the use of extended antimicrobial prophylaxis (>24 h, Supplementary Information 4).

Limitations of this study include the fact that it was performed at only one veterinary hospital and that, despite a large number of horses, some of the groups eventually had small

numbers because of categorisation and some missing data points. Horses were required to have at least 12 days follow up to be classified as healing normally (e.g. horses with no evidence of a SSI with only 10 days postoperative follow up were excluded), whereas all horses with SSI were included. These criteria were established to avoid excluding a large number of horses without SSI while not misclassifying horses with insufficient follow up as healing normally. The 75* percentile was chosen because in data that are not normally distributed, the lower and upper percentiles (25l and 75l percentiles) remain stable, whereas the outliers are more labile. If anything, this may have slightly overestimated the SSI rate in this study; when considering all potentially eligible horses (excluding horses undergoing repeat laparotomy) the SSI was 15% vs. 20% for the final study population. Other published studies, such as Isgren et al. (2015), have only considered the hospitalisation period for classifying horses as having normal healing (vs. SSI). We believe that our study design, while still risking some bias, was a reasonable compromise between maximising case inclusion and accurately classifying horses as having normal healing. Investigating the usefulness of the model at other veterinary hospitals, exploring the association between breed and postoperative complications, and further defining SSI based on severity, location of drainage, and bacteria isolated, and identifying associated variables with specific SSI characteristics are some future directions. The relationship between LOS and SSI also warrants further investigation with regard to cause and effect.

Conclusions

Horses that underwent procedures likely associated with heavy contamination adjacent to the incision were more likely to have SSI; care should be taken to protect the incision during such procedures. Ponies/miniature horses, Draft breeds, Standardbreds, Arabians, and

Crossbreeds appeared to have a lower risk of SSI compared to Warmbloods, American breeds, and Thoroughbreds, but younger horses do not appear to have fewer SSI compared to mature horses. Horses with incisions <27 cm which remained within the fenestration of the drape and possibly within the thicker portion of the linea alba had fewer SSL Therefore, whenever possible, an incision of <27 cm is recommended. However, we do not recommend attempting to remove a distended and heavy colon through a small incision because of the risk of viscus perforation. Horses showing signs of colic PO were more likely to have SSI, particularly where LOS was prolonged.

Conflict of interest statement

None of the authors has any other financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Appendix A: Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi

Supplementary Information 1 : Detailed data collection form used to obtain information prospectively from cases.

Supplementary Information 2: Details of surgical site preparation

Supplementary Information 3: Flow diagram

Supplementary Information 4: Clinically important variables not significantly associated with SSL

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Table 1

Results of univariable analysis for categorical variables included in the initial iteration of the multivariable analysis for a study of the association of horse age and incision length with surgical site infection following equine colic surgery.

Variable Likelihood Ratio *P (n)*	Classification	Surgical Site Infection		Crude OR (95% CI)	*P*[a]
		Yes *n* (%)	No *n* (%)		
Age (years) *P* = 0.045 (185)	<4	6 (13%)	42 (87%)	Referent	
	4-11	15 (16%)	76 (84%)	1.3(0.5-3.8)	0.624
	12-19	14 (36%)	25 (64%)	3.9(1.3-11.5)	0.001
	>19	1 (14%)	6 (86%)	1.2(0.1-11.4)	1.000
Breed *P* = 0.006 (184)	Other[b]	3 (6%)	50 (94%)	Referent	
	Thoroughbreds	14 (21%)	54 (79%)	4.3(1.2-15.9)	0.033
	Warmbloods	11(29%)	27 (71%)	6.8(1.7-26.4)	0.003
	American breeds	8 (32%)	17 (68%)	7.8(1.9-33.0)	0.004
Preoperative AMD *P* = 0.038 (181)	Penicillin and gentamicin/ enrofloxacin	31 (18%)	145 (82%)	Referent	
	Penicillin or gentamicin or neither	3 (60%)	2 (40%)	7.0(1.1-43.8)	0.047
Incision length (cm) *P* = 0.006 (166)	<27	11(11%)	87 (89%)	Referent	
	>27	19 (28%)	49 (72%)	3.1(1.4-7.1)	0.006
Surgical procedure classification *P* = 0.033 (185)	Light contamination	28 (17%)	136 (83%)	Referent	
	Heavy contamination	8 (38%)	13 (62%)	3.0(1.1-7.9)	0.036
Postoperative colic *P* = 0.065 (185)	No colic	20 (16%)	107 (84%)	Referent	
	Colic	16 (28%)	42 (72%)	2.0(1.0-4.3)	0.059
Postoperative diarrhoea *P* = 0.079 (180)	No diarrhoea	21 (17%)	106 (83%)	Referent	
	Diarrhoea	15 (28%)	38 (72%)	2.0 (0.9-4.3)	0.072

OR, odds ratio; AMD, antimicrobial drugs; CI, confidence interval.

[a] Pearson chi-square statistic or Fisher's exact text comparing each category to the referent category;

[b] Other breeds include ponies/miniature horses, Draft horses, Standardbreds, Arabians, and Cross breeds. American breeds included Quarter horses, Paints, Appaloosas, Tennessee walking horses, Morgans and Rocky mountain horses. Warmbloods also included Irish sport horses.

Table 2

Results of the multivariable logistic analysis [a] for a study of the association of horse age and incision length with surgical site infection following equine colic surgery.

Variable Likelihood ratio *P*	Classification	Adjusted OR (95% CI)	Chi-square *P*
Breed P= 0.008	Other[b]	Referent	
	Thoroughbred	4.5(1.1-25.5)	0.038
	American breeds	6.4(1.2-43.0)	0.031
	Warmbloods	12.0(2.7-74.8)	<0.001
Incision length (cm) *P*= 0.004	<27	Referent	
	>27	3.7(1.5-9.9)	0.004
Surgical procedure Classification *P*< 0.001	Light contamination	Referent	
	Heavy contamination	12.0(3.3-49.9)	<0.001
Postoperative colic *P*= 0.037	No colic	Referent	
	l Colic	2.7(1.1-6.8)	0.037

OR, odds ratio; CI, confidence interval.

[a] This model had a good fit with an overall P<0.001, area under the receiver operator characteristic 0.81 (excellent discrimination between SSI vs. no SSI; AICc 137, Goodness-of-fit test P=0.53).

[b] Other breeds include ponies/miniature horses, Draft horses, Standardbreds, Arabians, and Cross breeds. American breeds included Quarter horses, Paints, Appaloosas, Tennessee walking horses, Morgans and Rocky mountain horses. Warmbloods also included Irish sport horses.

Supplementary Information 1:

COLIC STUDY – SURGICAL PROCEDURES

Patient Details

Case #		Admit Time: AM PM	Date:
Owner		OR	☐ General ☐ Special ☐ CMK
Animal Name		Pre-op Antimicrobials:	☐ KPen ☐ Gent ☐ Other
Classification	☐ Emergency ☐ Elective	Abx Dose(s)	
First Celiotomy?	☐ Yes ☐ No What # celiotomy?	Time Pre-op Abx Given:	AM PM

Surgery and Anesthesia

Surgeon(s)		Surgery Date (if different from admit date):		
Resident(s)		Anesthesia	**Start** AM PM	**End** AM PM
Anesthetist(s)		Surgery	**Start** AM PM	**End** AM PM
Prep Nurse(s)		Number of People in OR		**PLEASE CIRCLE NAMES OF PRIMARIES**

Condition of Patient

Presenting/Pre-Op Values: Heart Rate _____ bpm Resp Rate brpm Rectal Temp _____°F pH _____ Lactate _____ mmol/L PCV _____ % Total Protein _____ g/dl WBC K/µl	Length of Hair: ☐ Clipped, very short ☐ Short < ¼" ☐ Medium ¼-1" ☐ Long > 1" ☐ Hair on abdomen, length _____ "
Cleanliness: ☐ Clean, no visible mud/dirt anywhere on patient ☐ Slightly dirty, < 25% of patient visibly dirty ☐ Moderately dirty, 25-50% of patient dirty ☐ Very Dirty, > 50% of patient visibly dirty ☐ **Check if** dirt on abdomen ☐ **Check if** excessive smegma	Skin Condition: ☐ Normal, smooth, no visible lesions ☐ Lacerations ☐ Flaking ☐ Crusts or dermatitis ☐ **Check if** significant sweating ☐ **Check if** reaction to prep

Surgical Prep

Where Was Patient Clipped? ☐ Clipped prior to anesthesia ☐ Clipped in OR ☐ Vacuum used	Type of Final Abdominal (Incision Site) Prep: ☐ None ☐ Wiping incision site only ☐ Ioban with Vidrapespray

Type of General Prep: ☐ Gross prep ☐ Sterile 5 minute prep ☐ Other type of prep ☐ Male: **check if** catheterized ☐ **Check if** prepuce sewn closed Other Comments:	☐ Ioban after wiping incision site Draping Procedure: ☐ 4 quarters then lap ☐ Lap then 4 quarters ☐ Proxima drape with 4 quarters ☐ Other – please specify

Surgical Findings and Procedures

Site of Lesion: ☐ Small intestine ☐ Jejunum ☐ Ileum ☐ Large Intestine ☐ Cecum ☐ Small Colon ☐ Other – please specify	Procedure Performed: ☐ Pelvic flexure enterotomy ☐ Jejunojejunostomy ☐ Jejunocecostomy ☐ Typhlotomy ☐ High enema ☐ Exploration & reposition to proper anatomical site ☐ Exploration only ☐ Decompression ☐ Decompression site sutured ☐ Other – please specify
Obstruction Type: ☐ Simple Obstruction ☐ Strangulating Obstruction	Intra-abdominal Instillation: ☐ CMC ☐ Antibiotics ☐ Other – please specify
Lesion Description:	

Closure

Suture Technique: ☐ Simple continuous for linea ☐ Interrupted for linea ☐ SubQ and skin all suture ☐ SubQ suture and skin staples ☐ SubQ suture only ☐ Nexaband used	Incision Site Protection: ☐ Ioban for recovery only ☐ Ioban left in place after recovery ☐ Stent for recovery only ☐ Stent left in place after recovery ☐ None
Incision: Length at closure _____ inches cm (circle units) ☐ Aluspray used ☐ Antibiotic lavage of incision	Other Comments:

Recovery

How Was Recovery?	Recovery Time:
☐ Excellent, stands at first attempt ☐ Good, stands after 2-3 coordinated attempts ☐ Rough, > 3 attempts or very uncoordinated attempts to stand Other Comments:	Time into recovery stall _____ AM PM Time sternal _____ AM PM ☐ With assistance ☐ No assistance Time standing _____ AM PM ☐ Sling used

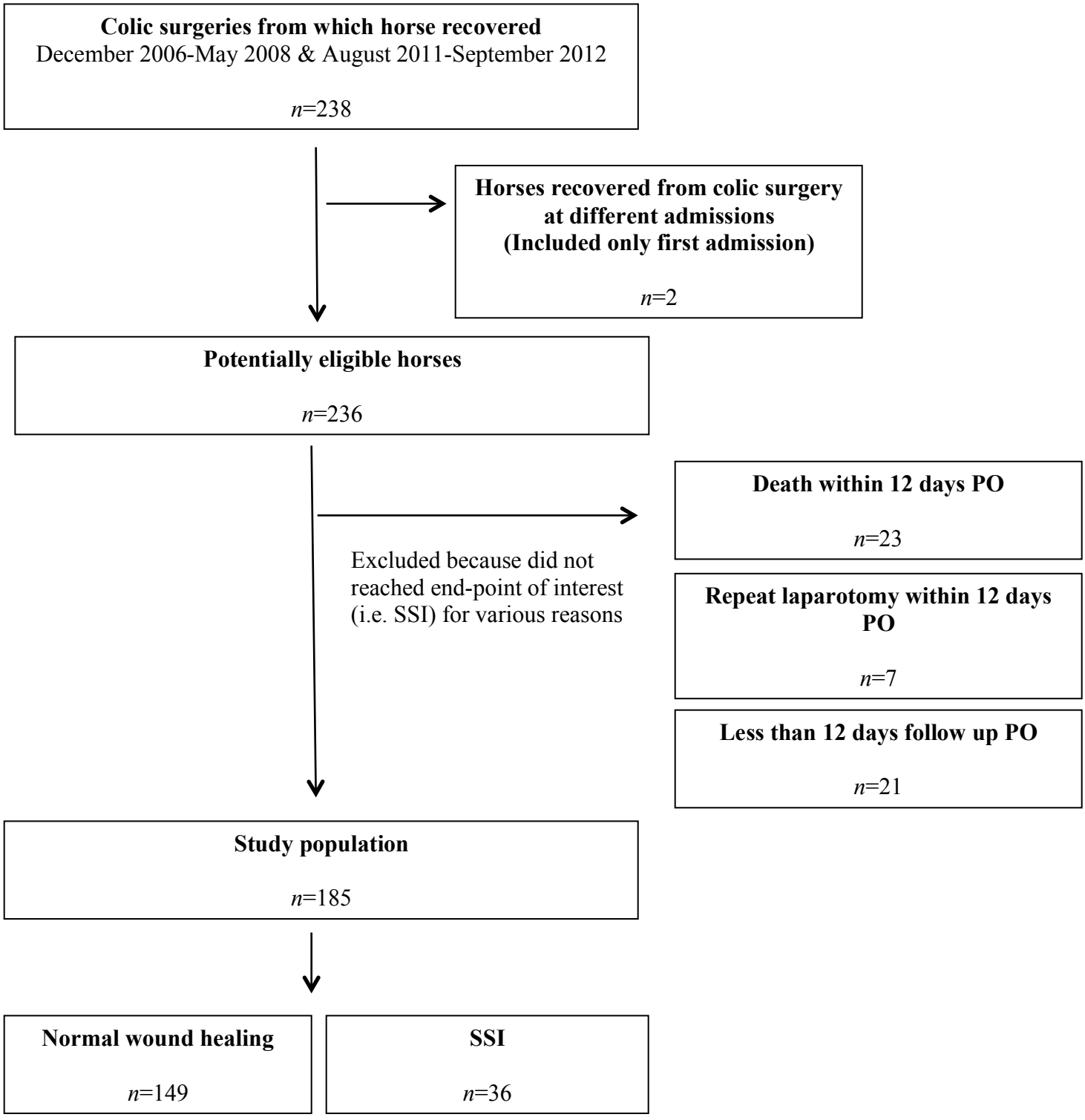
ADDITIONAL COMMENTS:

Supplementary information 2:

Details of surgical site preparation

Following induction of general anaesthesia, the prepuce and penis were cleaned, a urinary catheter passed, gauze sponge placed in the prepuce, and the prepuce sutured closed (males). The skin was cleaned using 4% chlorhexidine gluconate solution, rinsing with tap water and then wiping with 70% isopropyl alcohol soaked gauzes. This procedure was repeated until the white gauze was visibly clean. The 5 min sterile preparation used 4% chlorhexidine gluconate solution soaked sterile gauzes, which was rinsed with sterile 0.9% saline solution, sprayed with alcohol and then 2% chlorhexidine solution.

Supplementary Information 3 Flow Diagram



SSI, surgical site infection

Supplementary Information 4

Table 1: Associations between categorical variables and surgical site infection

Variable Likelihood ratio *P* (*n*)	Classification	Surgical site infection		Crude OR (95% CI)	*P*
		Yes N (%)	No N (%)		
Sex *P* = 0.9 (185)	Female	17 (20%)	69 (80%)	Referent	
	Male (stallions & geldings)	19 (19%)	80 (81%)	1.0 (0.5-2.0)	0.9
Hair length *P* = 0.7 (152)	Clipped/very short	6 (25%)	18 (75%)	Referent	
	Short (<0.5 cm)	16 (17%)	76 (83%)		
	Medium (0.5-2.5 cm)	4 (13%)	27 (87%)		
	Long >2.5 cm	1 (20%)	4 (80%)		
Cleanliness[a] *P* = 0.11 (153)	Clean	14 (25%)	42 (75%)	Referent	
	Slightly dirty (<25% body covered in dirt)	8 (11%)	62 (89%)		
	Moderately dirty (25-50% body covered in dirt)	2 (11%)	16 (89%)		
	Very Dirty (>50% body covered in dirt)	3 (33%)	6 (76%)		
Dirt on ventral abdomen *P* = 0.3 (153)	No	24 (17%)	119 (83%)	Referent	
	Yes	3 (30%)	7 (70%)	2.1 (0.5-8.8)	0.4
Excessive smegma *P* = 0.8 (153)	No	24 (18%)	112 (82%)	Referent	
	Yes	3 (19%)	13 (81%)		
Condition of skin *P* = 0.5 (154)	No visible lesions	23 (17%)	111 (83%)	Referent	
	Lacerations[b]	3 (30%)	7 (70%)		
	Flaking	1 (14%)	6 (86%)		
	Crusts/dermatitis	0	3 (100%)		
Excessive sweating *P* = 0.9 (154)	No	25 (18%)	117 (82%)	Referent	
	Yes	2 (17%)	10 (83%)	0.9 (0.2-4.5)	1.0
Skin reaction to surgical preparation *P* = 0.4 (154)	No	27 (18%)	125 (82%)	Referent	
	Yes	0	2 (100%)	NA	1.0
Timing pre-operative AMD (minutes before start of surgery) *P* = 0.9 (178)[c]	After surgery started	1 (14%)	6 (86%)	Referent	
	$\leq$ 30	16 (20%)	64 (80%)	1.5 (0.2-29.4)	0.7
	31-60	5 (14%)	31 (86%)	1.0 (0.1-20.3)	1.0
	61-120	7 (16%)	36 (84%)	1.2 (0.2-23.8)	0.9
	>120	3 (25%)	9 (75%)	2 (0.2-46)	0.6
Re-dose AMD when surgery >120 min *P* = 0.7 (109)	Yes	4 (15%)	23 (85%)	Referent	
	No	16 (19%)	66 (81%)	1.4 (0.4-4.6)	0.7
Ioban final prep	Yes	13 (22%)	45 (78%)	Referent	

P = 0.3 (72)	No	8 (15%)	47 (85%)	0.6 (0.2-1.6)	0.3
Surgeon experience *P* = 0.8 (183)	Experienced	13 (19%)	56 (81%)	Referent	
	Inexperienced[d]	23 (20%)	91 (80%)	1.1 (0.5-2.3)	0.8
Lesion site *P* = 0.8 (185)	Small intestine	10 (18%)	45 (82%)	Referent	
	Large intestine	25 (21%)	95 (79%)	1.2 (0.5-2.8)	0.7
	Other	1 (14%)	6 (86%)	0.75 (0.04-5.1)	0.8
	Peritonitis	0	3 (100%)	NA	
Obstruction type *P* = 0.5 (185)	Strangulating	13 (25%)	39 (75%)	Referent	
	Simple obstruction	21 (17%)	100 (83%)	0.6 (0.3-1.4)	0.3
	Inflammatory	2 (22%)	7 (78%)	0.9 (0.1-4.1)	0.9
	Other	0	3 (100%)	NA	0.2
Enterotomy/enterectomy *P* = 0.9 (185)	Yes	17 (20%)	68 (80%)	Referent	
	No	19 (19%)	81 (81%)	0.9 (0.5-1.9)	0.9
Intraperitoneal SCMC *P* = 0.2 (185)	Yes	29 (22%)	105 (78%)	Referent	
	No	7 (14%)	44 (86%)	0.6 (0.2-1.3)	0.2
Intraperitoneal AMD *P* = 0.4 (180)	Yes	4 (29%)	10 (71%)	Referent	
	No	30 (18%)	136 (82%)	0.6 (0.2-2.1)	0.4
Method of skin closure[e] *P* = 0.5 (185)	Skin staples	7 (15%)	41 (85%)	Referent	
	Suture	24 (22%)	83 (78%)	1.7 (0.7-4.5)	0.2
	Skin glue	2 (12%)	15 (88%)	0.8 (0.1-3.7)	0.8
	SQ only (no skin)	3 (23%)	10 (77%)	1.8 (0.3-7.7)	0.5
GA recovery *P* = 0.3 (179)	Excellent	19 (23%)	64 (77%)	Referent	
	Good	10 (14%)	59 (86%)	0.6 (0.2-1.3)	0.2
	Rough	7 (26%)	20 (74%)	1.2 (0.4-3.1)	0.7
Method of wound protection during GA recovery *P* = 0.5 (182)	Ioban	21 (17%)	100 (83%)	Referent	
	Stent bandage	8 (25%)	24 (75%)	1.6 (0.6-4)	0.3
	Stent bandage + Ioban	2 (50%)	2 (50%)	4.8 (0.6-35.7)	0.2
	Other	4 (20%)	16 (80%)	1.2 (0.3-3.9)	1.0
	None	1 (20%)	4 (80%)	1.2 (0.1-11.2)	1.0
Postoperative abdominal bandage *P* = 0.7 (183)	Yes	10 (21%)	37 (79%)	Referent	
	No	25 (18%)	111 (82%)	0.8 (0.4-1.9)	0.7
Duration postoperative AMD (h) *P* = 0.4 (176)[c]	≤ 12	6 (30%)	14 (70%)	Referent	
	13-24	7 (13%)	47 (87%)	0.3 (0.1-1.2)	0.1
	25-48	7 (18%)	33 (82%)	0.5 (0.1-1.8)	0.3
	49-72	4 (19%)	17 (81%)	0.5 (0.1-2.3)	0.4
	>72	11 (27%)	30 (73%)	0.9 (0.3-2.9)	0.8
PO septic peritonitis *P* = 0.5 (177)	No	34 (20%)	134 (80%)	Referent	
	Yes	1 (11%)	8 (89%)	0.5 (0.06-4.1)	0.7
PO pneumonia	No	33 (20%)	134 (80%)	Referent	

P = 0.5 (176)	Yes	1 (11%)	8 (89%)	0.5 (0.06-4.2)	1.0
Salmonellosis *P* = 0.3 (179)	No	29 (18%)	129 (82%)	Referent	
	Yes	6 (29%)	15 (71%)	1.8 (0.6-5.0)	0.3
Catheter-associated complication *P* = 0.5 (176)	No	31 (19%)	130 (81%)	Referent	
	Yes	4 (27%)	11 (73%)	1.5 (0.5-5.1)	0.5
PO reflux *P* = 0.9 (176)	No	28 (19%)	116 (81%)	Referent	
	Yes	6 (19%)	26 (81%)	1.0 (0.4-3.0)	0.9
Intravenous lidocaine *P* = 0.3 (185)	No	15 (16%)	77(84%)	Referent	
	Yes	21 (23%)	72 (77%)	1.5 (0.7-3.1)	0.4
Postoperative critical illness score *P* = 0.8 (169)	0	21 (23%)	72 (77%)	Referent	
	1	9 (15%)	50 (85%)	0.6 (0.3-1.4)	0.3
	2	3 (19%)	13 (81%)	0.8 (0.2-2.4)	0.7
	3	0	1 (100%)	NA	0.5

SQ, subcutaneous tissue; AMD, antimicrobial drugs; SCMC, sodium carboxymethylcellulose; Ioban, iodine-impregnate incise drape; GA, general anaesthesia; NA, not applicable because odds ratios cannot be calculated with a zero value; PO, postoperative.

[a] Cleanliness data were re-categorised based on preliminary analysis and clinical relevance into ≤50% body covered in dirt to >50% body covered in dirt (i.e. it did not make sense that clean horses had a higher SSI) and the relationship with SSI was no longer statistically significant *P* = 0.24 and it was not included in the multivariable model.

[b] Including clipper-associated injury

[c] See Table 2

[d] Some inexperienced surgeons appeared to have a higher SSI than others (range 0 to 33%)

[e] The majority (>95%) of horses had the linea alba closed using #2 polyglactin 910 in a simple continuous pattern and subcutaneous tissue apposed using 2-0 polyglactin 910 in a simple continuous pattern which precluded comparison with different techniques.

Table 2: Associations between continuous variables and surgical site infection.

Variable (N)	Surgical Site Infection		P-value
	Yes mean ± SD	No mean ± SD	
Body weight (kg) (184)	511 ± 22	492 ± 11	0.5
Admission heart rate (beats/min) (182)	52 ± 13	55 ± 18	0.6[1]
Admission respiratory rate (breaths/min) (170)	21 ± 7	24 ± 12	0.4[1]
Admission rectal temperature (oC) (168)	37.8 ± 0.6	37.8 ±0.7	0.8
Admission PCV (l/l) (184)	0.40 ± 0.67	0.39 ± 0.65	0.5[1]
Admission TPP (g/L) (183)	68.9 ± 9.3	66.1 ± 1.0	0.2
Admission WBCC ($x10^9$/L) (183)	8.97 ± 0.61	9.00 ± 0.30	0.7[1]
Admission blood lactate (mmol/L) (176)	2.3 ± 2.3	2.5 ± 2.5	0.7[1]
Lowest MAP under GA mmHg (180)	60.6 ± 1.6	62.3 ± 0.8	0.3
Duration lowest MAP (min) (180)	2.4 ± 0.9	2.8 ± 0.4	0.6
Surgery duration (min) (184)	124 ± 10	115 ± 5	0.5[1]
Number people in OR (154)	7.6 ± 0.4	7.5 ± 0.2	0.9
Duration in recumbency during GA recovery (min) (183)	57 ± 6	65 ± 3	0.3[2]
Timing of pre-operative AMD (min before start of surgery) (178)	46.8 ± 8.3	46.9 ± 3.9	0.8[1]
Duration postoperative AMD (hours) (176)	72.5 ± 10.1	52.7 ± 5.1	0.1

[1] data analysed following normalisation by log transformation of raw data. Raw data presented as mean ± SD

[2] data analysed following normalisation by square root transformation of raw data. Raw data presented as mean ± SD

SD, standard deviation, IQR, interquartile range, OR, operating room