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Risk factors for surgical site infection following laparotomy: Effect of season and perioperative variables and reporting of bacterial isolates in 287 horses

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Summary

Reasons for performing study: Surgical site infection (SSI) is an important cause of post operative morbidity following laparotomy.
Objectives: To investigate risk factors for SSI, including effect of season and surgery performed outside normal working hours, and to report bacterial isolates and antimicrobial resistance patterns.
Study design: Retrospective cohort study.
Methods: Data were obtained from horses that had undergone exploratory laparotomy over a 3-year period (2010–2013) in a UK hospital population. SSI was defined as any purulent or serous discharge from the laparotomy incision of >24 h duration that developed during hospitalisation. Multivariable logistic regression was used to identify associations between pre-, intra- and post operative variables and altered likelihood of SSI.
Results: Surgical site infection developed in 73/287 (25.4%) horses during hospitalisation. Horses of greater bodyweight (odds ratio [OR] 1.002, 95% confidence interval [CI] 1.0002–1.005, P = 0.03), increased packed cell volume (≥48%) on admission (OR 3.03, 95% CI 1.32–6.94, P = 0.01), small intestinal resection (OR 2.27, 95% CI 1.15–4.46, P = 0.02) and post operative colic (OR 2.86, 95% CI 1.41–5.79, P = 0.003) were significantly associated with increased likelihood of SSI in a multivariable model. SSI was also significantly more likely to occur during winter (OR 3.84, 95% CI 1.38–10.70, P = 0.01) and summer (OR 5.63, 95% CI 2.07–15.3, P = 0.001) months in the model. Three-layer closure of the incision was protective (OR 0.31, 95% CI 0.16–0.58, P<0.001) compared to 2-layer closure. There was no effect of surgery being performed outside normal working hours (P = 0.5). The most common bacterial isolates were *Escherichia coli* (59.5%), *Enterococcus* spp. (42.4%) and *Staphylococcus* spp. (25.4%). Penicillin resistant isolates accounted for 92% (96/104) of isolates while 18% (21/119) of isolates were gentamicin resistant.
Conclusions: Laparotomy during winter and summer months was associated with increased likelihood of SSI but there was no effect of surgery performed outside normal working hours. This information assists in identifying horses at high risk of SSI and informing development of preventive strategies.

Keywords: horse; colic; exploratory laparotomy; surgical site infection; season; bacterial isolates

Introduction

Surgical intervention or euthanasia is required in around 9% of colic cases that occur in the general equine population [1]. Surgical site infection (SSI) of the abdominal incision has been reported in 10–37% of horses following laparotomy [2], with the prevalence depending on the population studied and the definition of SSI used. SSI is a common cause of post operative morbidity and results in increased duration of hospitalisation and costs of treatment, together with an increased risk of incisional hernia formation [3,4]. The latter is 4–9 times more likely to develop following SSI [3,4]. Horses that developed an incisional hernia have been reported to be significantly less likely to return to their previous use compared to those that did not [5,6]. Therefore, knowledge of ways in which the risk of SSI can be minimised is important.

A number of risk factors for incisional SSI have been identified. These vary between studies due to inconsistencies in definitions of SSI used and the duration of follow-up. Risk factors for SSI following laparotomy include preoperative factors such as weight [7], age [7], duration of colic signs prior to presentation, severe pain or elevated heart rate on admission [8], and elevated preoperative peritoneal fibrinogen concentration [9]. Intraoperative risk factors for SSI that have been reported include increased duration of surgery [7], hypoxaemia ($PaO_2 < 80$ mmHg) [10], lesion type [8], performing an enterotomy [9], surgeon performing incisional closure [11], high surgery room contamination [12] and method of incisional closure [9–11,13]. Application of a stent bandage over the incision [14] and placement of an abdominal bandage post operatively [8] have been identified to have a protective effect. The latter intervention was implemented in the authors' hospital to reduce the likelihood of SSI following laparotomy.

In human medicine, an association between season and SSI has been reported following spinal surgery [15]. In the latter study, patients were at increased risk of SSI when surgery was undertaken during the summer and autumn months. Surgery performed outside normal working hours has also been reported to result in higher post operative complication rates in both human and equine studies compared to those performed within normal working hours [16,17]. The effects of season and surgery being performed outside normal working hours have not been investigated as risk factors for SSI following laparotomy in horses.

Earlier publications reported an overall SSI rates of 2.6, 3.3 and 1.7% at 3 equine hospitals for all surgical procedures [18]. Penicillin sensitive *Staphylococcus* and *Streptococcus* were the most common bacterial isolates cultured [18] and Day 7 post operatively was the median day SSI was first noticed [18]. Common bacterial isolates from laparotomy incisions include *Staphylococcus* spp., β-haemolytic *Streptococcus* and *Escherichia coli* [19,20]. A prospective pilot study, reporting on sequential midline culture in horses following colic surgery, found clinical cases of SSI to be associated with significant midline bacterial growth after anaesthetic recovery and at 24 h post operatively [19]. Other authors have reported poor correlation between perioperative culture results and subsequent development of SSI [20,21] with SSI predicted in only 20% [21].

The aims of this study were to: 1) investigate pre-, intra- and post operative risk factors for SSI during hospitalisation in horses in which an abdominal bandage was routinely applied post operatively; 2) determine whether there was an effect of season or the timing of surgery in relation to normal working hours on the likelihood of SSI; and 3) report the prevalence of SSI together with bacterial isolates and antimicrobial resistance patterns identified in a UK hospital population.

Materials and methods

Study design and case selection

The records of all horses that underwent exploratory laparotomy at the Philip Leverhulme Equine Hospital, University of Liverpool, UK for investigation and treatment of colic and those undergoing other emergency abdominal procedures over a 3-year period (July 2010–July 2013) were reviewed. Development of SSI during hospitalisation was the outcome of interest. SSI was defined as any purulent or serous discharge from the laparotomy incision of >24 h duration. Horses that did not survive to hospital discharge or those undergoing repeat laparotomy within 4 weeks of the first surgery were excluded. A standard protocol to protect the incision was routinely used in horses undergoing laparotomy. Immediately following closure of the skin, a Primapore adhesive dressing[a] and Opsite incise drape[a] were placed over the incision and a textile abdominal recovery bandage (OrthoHorse)[b] was applied over these for anaesthetic recovery. Once the horse was moved from the anaesthetic recovery box, the dressings were removed and a single use abdominal dressing was placed. This consisted of an absorptive contact layer (Melonin)[a] followed by elastic cohesive dressing (Co-Plus)[c]. The bandage was examined daily for slippage or evidence of incisional discharge through the bandage layers. Bandages were changed at 48 h intervals until hospital discharge, when the incision was left uncovered. Standard medical therapy consisted of flunixin meglumine administration for least 48 h (1.1 mg/kg bwt i.v. q. 12 h) and antimicrobial therapy with penicillin (12 mg/kg bwt i.m. q. 12 h) or penicillin and gentamicin (6.6 mg/kg bwt i.v. q. 1 h) for 3–5 days based on clinician preference. Cases at high risk of post operative ileus (POI) were placed on a lidocaine infusion (1.3 mg/kg bwt bolus given over 15 min followed by 0.05 mg/kg bwt/min continuous rate infusion) [22] and polymixin B (5000 iu/kg bwt i.v. q. 12h) was administered to horses likely to develop endotoxaemia/systemic inflammatory response syndrome (SIRS) based on clinician preference.

Clinical signs of abdominal pain necessitating additional analgesia were classified as post operative colic (POC). POI was defined as >2 l net reflux obtained on at least 2 occasions and pyrexia was defined as a rectal temperature >38.6°C on at least one occasion. Serous drainage from the incision within the first 24 h post operatively and where no subsequent discharge occurred was considered normal. Incisions that developed SSI were routinely swabbed and submitted for aerobic and anaerobic bacterial culture. Bacterial culture consisted of direct plating onto 5% sheep blood agar[d] and the plates were incubated aerobically and anaerobically for 2–7 days. Microorganisms isolated from positive cultures were identified using API kits[e] and GNID Sensititre Identification plates[f]. Out of hours (OOH) was defined as surgery performed between the hours of 5 pm and 9 am Monday–Friday and at any time over the days of Saturday or Sunday [17].

Data collection

Data were collected for pre-, intra- and post operative variables considered *a priori* as risk factors for SSI (see Supplementary Items 1–5 for full details). Preoperative variables investigated included weight, breed, age, sex and coat condition (clean clipped, clean unclipped, moderate, filthy), clinical parameters on admission (heart rate, packed cell volume [PCV], total protein [TP] and peripheral lactate), if surgery was performed OOH, month and season of admission: spring (March–May), summer (June–August), autumn (September–November) and winter (December–February). Intraoperative data included duration of anaesthesia and surgery, surgeon, surgical lesion (location and strangulating/nonstrangulating), small intestinal resection performed and type of anastomosis, clean contaminated surgery (opened viscus), if an enterotomy was performed, abdominal incision closure (2 layer [*linea alba* and skin] or 3 layer [*linea alba*, subcutis and skin]), type of suture material and pattern, and anaesthetic recovery score [23]. Postoperative data collected included heart rate, PCV and TP at 24 and 48 h, antimicrobial type and duration, development of POC, POI or pyrexia, and administration of lidocaine or polymixin B. The results of microbiological culture of incisional discharge, when SSI was first identified and duration of hospitalisation were also recorded.

Data analysis

Surgical site infection during hospitalisation was the outcome of interest. All variables were screened using descriptive statistics and those with >30% missing values were excluded from initial analysis. Categorical variables with small numbers of observations in one or more categories or where the reference category contained relatively few individuals were re-coded to create fewer categories or a different reference category. The functional form of the relationships between continuous predictor variables and the log-odds of SSI were investigated using smoothed scatterplots and generalised additive models [24]. Variables that demonstrated significant nonlinear relationships were categorised using biologically plausible cut-off points. Univariable associations between explanatory variables and outcome were examined using a chi-squared test for categorical variables and univariable logistic regression for continuous variables. Variables with a univariable P value of ≤0.25 were considered for inclusion into a multivariable logistic regression model. Preoperative, intraoperative and post operative variables were analysed in 3 separate multivariable models. Models were built in a forwards stepwise approach and variables were retained if their manual inclusion resulted in a likelihood ratio test statistic of P<0.05. The retained variables were then forced into a single multivariable model and all remaining variables considered for inclusion (including those with missing values) were then forced back into the model to ensure that no significant or confounding variables had been excluded. The effects of biologically plausible interaction terms were also tested in the final model. The fit of the model was evaluated by the Hosmer-Lemeshow goodness of fit test statistic and influential data points were examined in a delta-beta scattered plot. Data analysis was performed using Stata 9.2[g] and S-plus 6 statistical software[h]. A critical probability of 0.05 was used for all analyses.

Results

Descriptive statistics and univariable analysis

Of 511 horses that underwent exploratory laparotomy during the study period, 287 fulfilled the study inclusion criteria. SSI developed in 73 horses (25.4%) during hospitalisation and was identified a median of 7 days (interquartile range [IQR] 6–8) post operatively. Horses that did not develop SSI were hospitalised significantly shorter time (median 8 days, IQR 7–9) compared to those that developed SSI (median 10 days, IQR 9–13). Variables significantly associated with SSI on univariable analysis (P<0.05) are presented in Supplementary Item 4. The estimated relationship (smooth) between likelihood of SSI and horse weight is shown in Figure 1. The proportion of SSI diagnosed during the different seasons is shown in Figure 2. Descriptive statistics and univariable analysis of categorical and continuous variables are available in Supplementary Items 1 and 2. There was no significant association between SSI and surgery performed OOH (P = 0.5).

Multivariable analysis

A final multivariable logistic regression model is shown in Table 1. Increased likelihood of SSI was associated with greater bodyweight, PCV on admission of >48%, small intestinal resection and post operative colic. A 3-layer incisional closure was protective compared to a 2-layer closure. There was an apparent seasonal effect with increased likelihood of SSI in horses undergoing laparotomy in the summer and winter months compared to the spring. Model diagnostics did not identify any influential data points and the Hosmer-Lemeshow goodness-of-fit test showed that the model was a good fit (P = 0.5).

Results of culture and sensitivity tests

Of the 73 horses that developed SSI, samples were submitted for bacterial culture and sensitivity testing in 61 horses (84%). Bacteria were cultured in

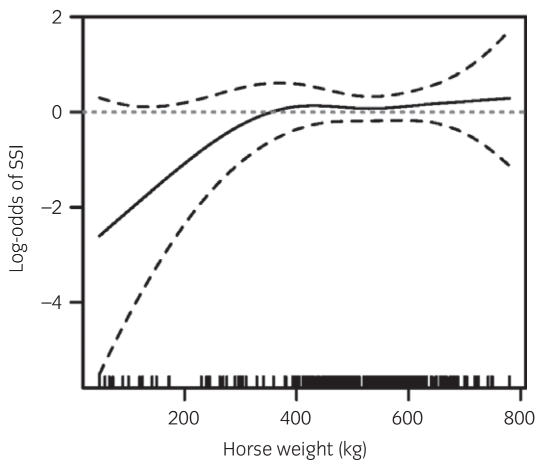

Fig 1: Generalised additive model used to demonstrate the functional form of the relationship between the weight of horses that survived to hospital discharge following exploratory laparotomy and the outcome (log odds of surgical site infection [SSI]). The plot shows the fitted curve with 95% confidence intervals (dashed line) and the rug plots along the *x* axis represents the number of data points. The P value (P = 0.16) is the chi-squared test for nonlinearity.

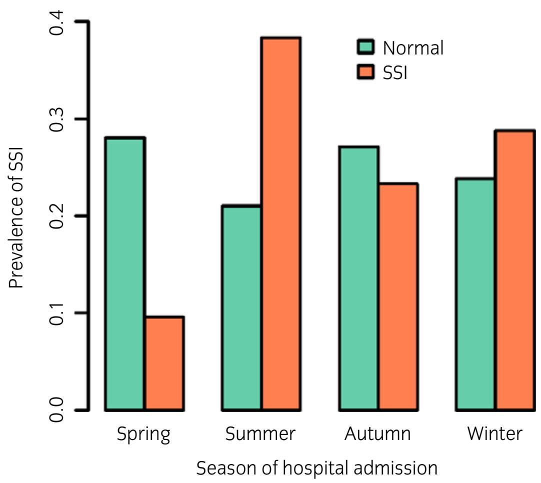

Fig 2: The proportion of surgical site infections (SSI) diagnosed during Spring (March–May), Summer (June–August), Autumn (September–November) and Winter (December–February) months.

59 samples (96.7%); there was no bacterial growth from 2 samples. A total of 120 bacterial isolates were identified, of which 69/120 (57%) were Gram-negative and 51/120 (43%) were Gram-positive. A mixed growth of bacteria was found in 63% (37/59) of SSI sampled compared with 37% (22/59) in which only a single bacterial species was cultured. The three most commonly isolated bacteria were *E. coli, Enterococcus* spp. and *Staphylococcus* spp., which were cultured in 59.5, 42.4 and 25.4% of horses with SSI respectively. Please refer to Supplementary Item 3 for frequency of bacterial isolates from cultures in 59 horses that developed SSI during hospitalisation.

Antimicrobial sensitivity testing revealed a predominance of penicillin resistant isolates (96/104; 92%) with 95/96 of the isolates cultured despite prior penicillin administration. Only 18% (21/119) of isolates were gentamicin resistant with 10/21 of the isolates cultured despite prior gentamicin administration. Seventeen isolates (17/104; 16.3%) were resistant to both penicillin and gentamicin including 2 methicillin-resistant *Staphylococcus aureus* isolates. Out of these 17 isolates, 41.2% (7/17) and 94.1% (16/17) of the isolates were cultured despite prior gentamicin and penicillin administration, respectively.

Discussion

This study identified a significant effect of season on the likelihood of SSI following laparotomy, which has not previously been reported. There was no effect of surgery being performed outside normal working hours. Risk factors for SSI identified in this study add to knowledge about identification of horses at high risk of SSI and can be used to inform development of strategies to minimise SSI development.

No previous equine studies have investigated an effect of season on SSI. In the current study, horses were more likely to develop SSI if laparotomy was performed during the summer and winter months compared to the spring months. Autumn was not significantly associated with altered likelihood of SSI. In man, increased risk of SSI in children undergoing cerebrospinal fluid shunt surgery during the months of July and August [25] and following spinal surgery during the summer and autumn months have been reported [15]. The latter findings were considered to be due to increased ambient temperature and seasonal staffing changes. Increased risk of SSI has been reported in spring compared to other seasons in human surgery, with gastrointestinal surgery carrying the highest risk of SSI [26]. The reason for the seasonal differences in likelihood of SSI identified in the present study is unclear. Horses admitted during the winter months had a high prevalence of coats classified as 'unclean' and increased likelihood of bacterial contamination around the surgical site could potentially account for this difference compared to those admitted during spring months. However, this variable was not significant in the multivariable model. This theory might also make increased likelihood of SSI in the summer months counterintuitive. It is plausible that increased ambient temperatures may provide an optimum environment for the bacteria to colonise the abdominal incision. This finding requires further investigation and season/month and average ambient temperature should be considered in other studies investigating risk factors for SSI.

Marked preoperative pain has been identified as a risk factor for SSI [8] but POC has not been reported as a risk factor in existing published studies. The precise reason for this is unclear. POC may result in increased frequency and duration of recumbency [27] and may increase the likelihood of physical contact between the floor and associated pathogens. It may also result in disruption of the protective abdominal dressing, exposing the laparotomy incision, particularly if also associated with more physical activity such as rolling. The latter may also disrupt incisional healing and increase inflammation at the incision site. Increased horse weight was associated with increased likelihood of developing SSI and is consistent with findings from other studies [7]. It is plausible that the relatively greater weight of the abdominal contents in horses of greater weight places greater force on the incision, which may potentially compromise the local vasculature. Human studies have identified an increased risk of SSI following abdominal surgery in patients with a greater body mass index [28] and it is plausible the same applies in horses. A recent equine study identified incision length ≥24 cm as a risk factor for SSI [29]. Neither body condition score nor incision length were measured in the present study but should be considered for further investigation in future studies.

Horses with increased PCV (>48%) on admission were found to be at increased likelihood of SSI, which is consistent with other studies [4,8]. In addition, increased likelihood of SSI was associated with horses that had undergone small intestinal resection. Performing an enterotomy [9] and intraperitoneal contamination [3] were reported previously to be associated with increased risk of SSI and together with our results this may imply that contamination at surgery was the reason for such a relationship. However, in the current study pelvic flexure enterotomy (P = 0.05), enterotomy (P = 0.17), and clean contaminated surgery (P = 0.37) were not significantly associated with the risk of SSI. In addition, bacterial cultures obtained from perioperative sampling including from peritoneal fluid, enterotomy and anastomosis sites and *linea alba* have previously failed to predict development of SSI post operatively [20,21]. Therefore, small intestinal resection in the current study may be a marker for other factors associated with SSI such as cardiovascular derangements and SIRS. These findings emphasise the importance of early surgical treatment of colic prior to the development

TABLE 1: Multivariable logistic regression model of variables association with surgical site infection in horses that survived to hospital discharge following exploratory laparotomy

Variable	Coefficient	Standard error	Odds ratio	95% confidence interval for the odds ratio	Wald P-value	Likelihood ratio test P-value
Weight	0.002	0.001	1.002	1.0002–1.005	0.03	0.02
Season of admission						
Spring	Ref.					
Summer	1.726	0.509	5.63	2.07–15.3	0.001	
Autumn	1.027	0.533	2.79	0.98–7.951	0.05	
Winter	1.348	0.521	3.84	1.38–10.70	0.01	0.003
PCV (%) on admission						
<48	Ref.					
≥48	1.110	0.422	3.03	1.32–6.94	0.009	0.009
Small intestinal resection						
No	Ref.					
Yes	0.821	0.344	2.27	1.15–4.46	0.017	0.02
Abdominal incision closure						
Two layers	Ref.					
Three layers	-1.154	0.316	0.31	0.16–0.58	<0.001	<0.001
Postoperative colic						
No	Ref.					
Yes	1.053	0.359	2.86	1.41–5.79	0.003	0.004

Number of observations in the final model = 285, the Hosmer-Lemeshow goodness-of-fit test statistic (χ^2 = 6.96, P = 0.54). PCV = packed cell volume; Ref. = reference category.

of marked cardiovascular and intestinal compromise, including the need for intestinal resection, in minimising post operative morbidity and morbidity [4], including the risk of SSI.

A 3-layer compared to 2-layer closure of the abdominal incision was associated with reduced likelihood of SSI development during hospitalisation. This is in contrast to a randomised controlled trial previously conducted at the same hospital that found no difference in likelihood of development of incisional suppuration between 2- and 3-layer closure techniques [30]. However, risk factors for SSI vary depending on the time point at which SSI is measured [8] and the latter study followed-up horses over a longer time period post operatively, which may account for this difference. The findings of the present study are in agreement with a study that identified a protective effect for SSI with the use of a subcutaneous layer [8].

The prevalence of SSI (25.4%) in the present study is within the range of those previously reported (10–37%) [3,8,30,31]. The latter rates are variable and this is likely to be in part due to the definition of SSI used and the duration and quality of post operative follow-up. Some studies define SSI as any drainage from the incision [21], whereas in others it is confined to purulent drainage only [9]. In the present study, we utilised a definition of SSI [8,30] that has now become widely accepted and enables more accurate comparisons to be made between studies. Horses that developed SSI were significantly more likely to be hospitalised for a longer duration. SSI was a frequent reason for horses to be kept in for ongoing medical therapy (including topical therapy at the incision site) at the authors' hospital. Therefore this variable was not considered for inclusion in the multivariable model as this would be more likely to be an effect rather than a cause of SSI.

The current study identified that 57% of bacterial isolates were Gram-negative and 43% were Gram-positive. This contradicts previous studies that have obtained intraoperative incisional cultures where predominantly Gram-positive bacteria were cultured [21]. In addition, in the latter study multiple species were isolated in only 17% [21] of positive cultures compared to 63% of cultures in the present study. *Streptococcus* spp. (28%), *E. coli* (17.7%) and *Enterococcus* (13.6%) were the most common bacterial species identified from intraoperative peritoneal fluid and enterotomy sites and an equal proportion of Gram-positive and Gram-negative bacteria were isolated in a separate study [20]. Knowledge of bacterial species isolated from SSI and antimicrobial sensitivity patterns is important in terms of monitoring for antimicrobial resistance and nosocomial infections, and is a key part of hospital clinical audit [32]. Further studies utilising sequential perioperative sampling during different intra and post operative phases would be useful to establish when contamination of the surgical site occurs, including horses that have protective abdominal dressings placed to reduce the risk of SSI.

Limitations of the present study include the fact that these results were from a single equine hospital in the UK and may not represent other hospital populations. Development of SSI was not determined following hospital discharge due to concerns about accurate reporting of SSI by owners and may have led to under-reporting of the rates of SSI [12,33]. Three-layer closure of the abdominal incision was not performed at random and was based on the surgeon's personal preference and may therefore have introduced bias.

In conclusion, this study has identified a number of pre-, intra- and post operative risk factors for SSI following exploratory laparotomy. This study is the first to report a seasonal effect together with increased likelihood of SSI following small intestinal resection and in horses that develop POC during hospitalisation. These findings assist identification and monitoring of horses at increased risk of SSI. We have also identified areas that require investigation in future studies to determine whether other interventions can be developed to minimise the likelihood of this post operative complication occurring.

Authors' declaration of interest

No competing interests have been declared.

Ethical animal research

Ethical approval for the study was granted by the University of Liverpool Veterinary Research Ethics Committee (VREC172). Owners gave consent for their horses' inclusion in the study.

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Authorship

C.M. Isgren, N.B. Townsend and D.C. Archer contributed to study design. C.M. Isgren, S.E. Salem and F.C.F. Worsman contributed to study execution. S.E. Salem and D.C Archer contributed to data analysis and interpretation. C.M. Isgren, N.B. Townsend and D.C. Archer contributed to the preparation of the manuscript. All authors gave their final approval of the manuscript.

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[f]TREK Diagnostic Systems, West Sussex, UK.
[g]StataCorp LP, College Station, Texas, USA.
[h]Insightful Corporation, Seattle, Washington, USA.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Supplementary Item 1. Descriptive statistics and univariable analysis of categorical variables investigated for association with surgical site infection (SSI) in horses that survived to hospital discharge following exploratory laparotomy.

Supplementary Item 2. Descriptive statistics and univariable logistic regression analysis of continuous variables investigated for association with the risk of surgical site infection (SSI) in horses that survived to hospital discharge following exploratory laparotomy.

Supplementary Item 3. Frequency of bacterial isolates from cultures in 59 horses that developed surgical site infection (SSI) and survived to hospital discharge following exploratory laparotomy. MRSA = methicillin resistant *Staphylococcus aureus*.

Supplementary Item 4. Descriptive statistics and univariable analysis of categorical variables investigated for association with surgical site infection (SSI) in horses that survived to hospital discharge following laparotomy (variables with $P<0.05$).

Supplementary Item 5. The use of generalised additive models to demonstrate the functional form of the relationships between continuous pre, intra- and post operative variables and risk of SSI in 287 horses that recovered following exploratory laparotomy.