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Risk factors for surgical site infections in horses following emergency laparotomy at the equine hospital of the VetMedUni Vienna between 2015 and 2020

Diploma Thesis

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submitted by

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Abbreviations

AMD = antimicrobial drugs

BCS = body condition score

BEVA = British Equine Veterinary Association

BMI = body mass index

CBC = complete blood count

CRT = capillary refill time

HE = high enema

E. coli = Escherichia coli

GI = gastro-intestinal

JC = jejunocaecostomy

JJ = jejunojejunostomy

MAP = mean arterial pressure

MRSA = methicillin resistant staphylococcus aureus

PCV = packed cell volume

PFE = pelvic flexure enterotomy

PO = post-operative

POC = post-operative colic

POF = post-operative fever

POR = post-operative reflux

SAA = serum amyloid A

SIRS = systemic inflammatory response syndrome

SSI = surgical site infection

TP = total protein

WBCC = white blood cell count

1. Introduction

The term colic describes a condition of abdominal pain and is one of the most common causes for equine emergencies, with a described annual incidence ranging up to 26% (Hines 2018). Up to 41% of colic cases need to be addressed surgically, bearing numerous risks (Southwood et al. 2017).
With a reported prevalence ranging from 9%-40%, (Costa-Farré et al. 2014, Scharner et al. 2017) surgical site infections (SSI) are a common complication following colic surgeries. In this context, SSI describes an infection that can be superficial and includes only the skin and subcutaneous tissues at the incision site, or deep, which includes underlying layers like muscles and fascia (CDC 2021). It leads to increased patient discomfort and often necessitates prolonged treatment, which is commonly associated with increased treatment costs (French et al. 2002, Colbath et al. 2014, Scharner et al. 2017).

Several studies have shown that SSI also predisposes to skin or abdominal wall dehiscence, which can lead to a catastrophic failure of the abdominal wall closure (Anderson et al. 2015).
According to two studies that explored the long-term complications of colic surgeries, horses that developed SSI were at a 10-20 fold increased risk of developing a ventral hernia (Mair und Smith 2005, Scharner et al. 2017) and horses that developed an incisional hernia were less likely to return to their intended use and performance level (Christophersen et al. 2011, Davis et al. 2013). Hernia formation also often requires prolonged treatment, such as the application of a hernia belt or the need for a surgical repair of the body wall (Davis et al. 2013, Immonen et al. 2017, Gardner et al. 2019).

A recent study has shown that multidrug resistance is not only common but was present in all isolates of Enterobacteriaceae and coagulase-positive Staphylococci cultures from SSI after laparotomy (Dziubinski et al. 2020). The zoonotic potential of certain nosocomial bacteria might pose a risk for people in close contact with infected horses. MRSA (Methicillin resistant Staphylococcus aureus) is a bacterium commonly transmitted between horses and humans and has been found in infected wounds (Cuny et al. 2008, van Duijkeren et al. 2010, Parisi et al. 2017).

Not only SSI development and its associated morbidities but also potential additional complications resulting from the presence of SSI highlight the need to identify and mitigate factors asscoiated with SSI development.
In the past decades a number of such potential risk factors for SSIs has been evaluated in the literature and will be addressed individually in this chapter.

Age

When examining horse age as a potential risk factor, studies have not been consistent in their results. Several authors could not find an association between age and the risk of developing SSI (Bischofberger et al. 2010, Tnibar et al. 2013, Colbath et al. 2014, Darnaud et al. 2016, Isgren et al. 2019). The findings of Gazzero et al. show that geriatric horses (≥20 years) have a similar duration recovery and amount of short-term post-operative complications as younger horses (Gazzerro et al. 2015). On the other hand Scharner et al. found, that horses ≥20 years of age were at a 17.9-fold greater risk of developing a SSI than younger horses (Scharner et al. 2017). However, the geriatric group in this study included only 11 animals, which might have been a limitation, due to small sample size.
Based on the results of these studies advanced age appears to be rather unlikely to increase the risk for SSI and that geriatric horses without concurrent diseases do not necessarily require a special treatment regarding this complication.

Sex

None of the studies found an association between sex and SSI development. (Tnibar et al. 2013, Colbath et al. 2014, Darnaud et al. 2016, Isgren et al. 2017, 2019, Scharner et al. 2017)

Weight

Studies in humans have found an increased risk for SSI in obese patients following abdominal surgery (de Oliveira et al. 2006, Waisbren et al. 2010). This might be true for horses as well, as a recent study found a tendency towards increased risk for SSI in horses with higher body mass index (BMI), which did not prove significant in the final multivariable analysis (Hill et al. 2020). In human studies percent body fat proved to be a better indicator for risk of SSI than BMI (Waisbren et al. 2010). However, this parameter is difficult to measure in horses and little validated methods are available (Ferjak et al. 2017, Greco-Otto et al. 2018).
Isgren et al. found that a higher body weight increases the risk of SSI slightly. They hypothesize that an increased weight of the abdominal content poses a greater tension on the suture and therefore might compromise the vasculature of the wound margins (Isgren et al. 2017). Since in this study only the bodyweight was measured and not the body condition score (BCS) it remains unclear, whether the increase in bodyweight can be traced back to the greater weight of the bowels or a higher proportion of muscles or fat outside the abdominal cavity.
However, other studies found no association between bodyweight and SSI (Coomer et al. 2007, Tnibar et al. 2013, Anderson et al. 2015, Darnaud et al. 2016, Isgren et al. 2019). This could be due to the great variety of sizes, and therefore weight of horses. It is possible, that patient weight is not a precise enough parameter and further research on BCS, BMI or percent body fat for their association with SSI should be conducted.

Breed

According to Darnaud et al. Standardbreds, draft horses, Arabians, ponies and Crossbreeds were less likely to develop SSI, whereas Thoroughbreds, Warmbloods and American breeds developed it more commonly. They hypothesized that either genetic predisposition or body conformation could be causative to this finding (Darnaud et al. 2016).

Interestingly, several other authors failed to find an association between breed and the risk of developing an SSI (Colbath et al. 2014, Isgren et al. 2017, Scharner et al. 2017). Possibly, the predisposition for SSI of certain breeds was not detected in these studies because the numbers of horses in some categories were small and certain breeds were not involved at all. In order to determine the extent of the influence of the breed on SSI, it needs to be subjected to further research.

Cleanliness on Arrival

Several authors had the hypothesis, that horses with dirtier coats and therefore higher bacterial contamination of the skin would be at an increased the risks for SSI. This did not hold true in three studies, that investigated the effect of the coat and skin condition prior to surgery (Coomer et al. 2007, Darnaud et al. 2016, Isgren et al. 2017).

Degree of Colic Symptoms

Prolonged duration and increased severity of colic signs was associated with higher likelihood of developing SSI (Smith et al. 2007). The authors hypothesize that a higher pain level may lead to more gross contamination from laying on the ground and rolling and that delayed surgical intervention results in more compromised bowel at the time of surgery, necessitating more lengthy surgical procedures that would cause more trauma to the incisional edges. This stands in contrast to the abovementioned findings concerning the cleanliness of the coat.
However, a number of other studies showed no significant associations between factors that reflect pain, such as heart rate or respiratory rate on arrival (Bischofberger et al. 2010, Tnibar et al. 2013, Darnaud et al. 2016, Isgren et al. 2017, 2019). These parameters reflect only the condition at admission to the referral facility and do not necessarily take the condition of the horse prior to referral and the effect of potentially administered pain medication by the referring veterinarian into account. Since the association between severity of colic signs and duration of colic surgery has not been investigated, this aspect requires further investigation.

Blood Parameters on Admission

Isgren et al. found, that an increased packed cell volume (PCV) on admission of >48% would enhance the risk for SSI (Isgren et al. 2017), but a later study by the same research group and others could not reproduce this finding (Tnibar et al. 2013, Darnaud et al. 2016, Isgren et al. 2019).

Further, total protein (TP), lactate level and white blood cell count (WBCC) on admission do not seem to play a predictive role either, suggesting that blood parameters on admission do not seem to act as a risk factor for SSI (Bischofberger et al. 2010, Darnaud et al. 2016, Isgren et al. 2019).

Surgeon and Surgeon Experience

A study performed at two referral clinics in the UK showed no association between individual surgeons and SSI occurrence (Coomer et al. 2007) and neither did two others (Bischofberger et al. 2010, Tnibar et al. 2013).

Interestingly, Torfs et al. found an association between the amount of experience of the surgeon closing the incision and SSI occurrence. Horse incisions closed by first- or second-year residents were significantly more likely to develop SSI than horses operated by third-year residents and senior surgeons. The authors hypothesize, that possible explanation for this could be the size of the bites taken and the amount of tension put on the incision edges. If the bites are too big and the tension too high, blood supply to the wound edges would be compromised and therefore predispose to dehiscence and wound suppuration. On the other hand, not enough tension would allow peritoneal fluid to leak out of the abdomen and form subcutaneous pockets, predisposing to bacterial growth (Torfs et al. 2010).

Similarly, Darnaud et al. found surgeon experience significantly influences SSI occurrence (Darnaud et al. 2016). However, one important inconsistency between these two studies are the differing definitions of “experienced” vs “unexperienced”. Torfs et al. defined <2 years of surgical experience as “unexperienced” and due to the high case load in their clinic >2 years as “experienced” (Torfs et al. 2010), while Darnaud et al. set the cutoff point at 5 years of surgical experience.

Colbath et al. found no difference in SSI prevalence between individual surgeons (Colbath et al. 2014). In their study the subcutaneous tissue and skin were closed by unexperienced staff, such as junior residents, interns or students after the linea alba was closed by the primary surgeon. Since the prevalence of SSI in their study (21.8%) was comparable to those of others, it leaves room to speculate about the importance of the quality of linea alba and subcutaneous/ skin closure for development of SSI.
Overall, there seems to be a tendency for less surgical experience to be a risk factor, especially when comparing senior surgeons to inexperienced, young staff. Further, it would be worth investigating, whether less surgical experience leads to increased surgery time, since this has also been reported to be a risk factor.

Incision Length and Wound Edema

Bischofsberger et al. also found that horses developing no wound edema 24-36 hours after surgery had a significantly shorter incision, compared to those experiencing moderate wound edema (Bischofberger et al. 2010). The presence of (moderate) wound edema was strongly associated with development of incisional complications, when compared to no or mild edema (Coomer et al. 2007, Scharner et al. 2017). Since wound edema might compromise blood flow by compressing vasculature and thereby tissue oxygenation and increase local inflammation at the incision site, it may predispose to infection. Further, incisions longer than 27cm were found to increase the risk for SSI 3.7-fold in another study (Darnaud et al. 2016). Based on the findings of these studies, incisions should be kept as short as possible and horses developing pronounced wound edema should be monitored closely for signs of wound infection.

Primary Lesion

The site and/or type of the primary lesion (clinically most relevant lesion) do not appear to act as a risk factor for SSI (Bischofberger et al. 2010, Tnibar et al. 2013, Colbath et al. 2014, Costa-Farré et al. 2014, Anderson et al. 2015, Darnaud et al. 2016, Scharner et al. 2017).

Procedure Type

Darnaud et al. found an association between the type of procedure and SSI occurrence, when re-categorizing procedures based on the amount of contamination in the direct proximity of the incisional edges as light contamination (Jejunojejunostomy/ jejunocaecostomy (JJ/JC), pelvic flexure enterotomy (PFE), high enema (HE)) and heavy contamination (multiple enterotomies, other enterotomies (small intestinal, typhlotomy, small colon), large colon resection, and other). With this categorization horses undergoing “heavily contaminating” procedures were 12-times more likely to develop SSI, than horses undergoing less contaminating procedures (Darnaud et al. 2016).

In support of the above findings, Isgren et al. found that a small intestinal resection posed a higher risk for SSI (Isgren et al. 2017). However, positive bacterial culture results from samples taken intra-operatively have proven not to be predictive for SSI (Rodriguez et al. 2009). Hence the authors surmise, that the need to perform a small intestinal resection may have rather been a marker for other factors, like systemic inflammatory response syndrome (SIRS) or pronounced cardiovascular derangement, which were also found to be associated with SSI in this study (Isgren et al. 2017).

Additionally, numerous other authors found no association between procedure type and SSI (Coomer et al. 2007, Bischofberger et al. 2010, Torfs et al. 2010, Tnibar et al. 2013, Colbath et al. 2014, Costa-Farré et al. 2014, Anderson et al. 2015, Scharner et al. 2017, Isgren et al. 2019).

This finding is interesting, since one could argue, that different procedures would not only influence the amount of possible contamination, but also the duration of surgery. However, two authors that identified duration of surgery as a significant factor did no find that the same held true for the type of surgical procedure (Costa-Farré et al. 2014, Anderson et al. 2015).

It is further worth mentioning that a prospective cohort study performed by Isgren et al. that examined the bacterial load on ventral midline incisions found that positive bacterial cultures from samples taken from the linea alba intraoperatively, after its closure, were not predictive for SSI. In cases where SSI occurred, it was due to a different bacterial isolate than the one obtained during surgery (Isgren et al. 2019). These findings may indicate, that if contamination of the surgical site leads to SSI, it likely occurs after the surgery.

Since there are considerable discrepancies regarding this factor, further research could focus on the type of protection used for the incisional edges during high-risk procedures.

Intraoperative Parameters

Hypotension is a common complication in horses undergoing anesthetic procedures, which may also lead to lower peripheral tissue oxygenation. A study that examined the impact of the lowest mean arterial blood pressure (MAP) and its duration during anesthesia found no association with SSI (Darnaud et al. 2016). However, the duration of the lowest pressure might not be indicative of the total time spent hypotensive, which could be a better parameter from which to draw conclusions on tissue oxygenation.

The impact of hypoxemia during anesthesia was examined by Costa-Farré et al. According to their prospective study, horses that experienced severe hypoxemia (<80 mmHg PaO_2) had an increased risk of developing SSI, especially if combined with prolonged anesthesia times (>2h) (Costa-Farré et al. 2014). The defense mechanisms of neutrophils against pathogens are based on oxidative killing, which is compromised if tissue oxygenation is insufficient. Impaired tissue oxygenation has also been described to be a risk factor for SSI in humans (Ragheb and Buggy 2004). Subcutaneous tissue oxygenation in humans post-operatively was also influenced by the quality of pain control. Pain evokes a sympathetic stress response that may lead to arteriolar vasoconstriction, thereby compromising tissue perfusion and oxygenation (Ragheb and Buggy 2004, Hopf and Rollins 2007). These findings aid in supporting appropriate pain control as a vital therapeutic measure pre-, intra- and post-operatively, since equine colic patients commonly suffer severe abdominal pain but also to potentially reduce the risk of SSI development (Gardner et al. 2019).

Further research is necessary to determine, whether hypotension during surgery is a risk factor for SSI, or whether immediate post-operative tissue oxygenation is a more crucial factor.

Duration of Surgery/ Anesthesia

There is little consensus in the literature regarding this factor. Some authors found significant correlations between increased surgery/ anesthesia time and an increased risk of SSI (Smith et al. 2007, Freeman et al. 2012, Costa-Farré et al. 2014, Anderson et al. 2015). These findings are supported by human literature (Lake et al. 2013, Waltz und Zuckerbraun 2017). Prolonged surgery time could be a result of more complex surgical procedures, leading to more trauma to the incision site and thereby increase the risk for SSI. Also prolonged anesthesia is associated with patient hypothermia, changes in perfusion and immune-mediating pharmacological effects (Waltz und Zuckerbraun 2017).

However a number of other authors could not find any association between surgical duration and SSI occurrence (Coomer et al. 2007, Bischofberger et al. 2010, Torfs et al. 2010, Tnibar et al. 2013, Darnaud et al. 2016, Isgren et al. 2017, Scharner et al. 2017). Due to the mostly retrospective nature of these studies, some discrepancies might be due to differences in the anesthesia protocols used and intra-operative anesthetic management. More research is needed, to investigate the effect of different anesthesia protocols and duration of surgery on SSI incidence.

Suture Technique

There are various findings on the impact of different suture techniques on risk of SSI development.

When compared to a 3-layer suture, a modified 2-layer subcuticular pattern showed protective tendencies against SSI. However, only the comparison of the 2-layer technique with a 3-layer suture with the skin layer consisting of another pattern than a simple continuous proved statistically significant (Colbath et al. 2014). The modified subcuticular suture consisted of a closure of the linea alba in one layer and of the subcutis and dermis in another layer, without penetration of the skin. According to the authors, this resulted in a reduction of dead space and a good apposition, while not creating a possibility for bacteria to ascend into the wound via the suture material (Colbath et al. 2014).

Coomer et al. compared a traditional 3-layer suture to a 2-layer suture. The 2-layer suture consisted of the suture of the linea alba, which incorporated some subcutaneous tissue, and a skin suture. A Ford interlocking pattern was chosen for the skin in each case. These authors did not find a difference between the two techniques and conclude that a 2-layer closure is sufficiently stable, while not posing a higher risk on wound suppuration (Coomer et al. 2007).

However, a later study performed at the same hospital detected a protective function of a 3-layer suture versus a 2-layer suture. (Isgren et al. 2017) The authors do not describe the 2-layer method further, other than stating that it included a linea alba and a skin suture, which makes it difficult to interpret. They explain this discrepancy with the fact that in the later study, the follow up period was longer and thus they were able to detect infections that occurred after discharge.

Regarding suture material, closure of the skin with staples was identified as a risk factor, when compared with the closure with a monofilament material and a continuous suture pattern. (Torfs et al. 2010) This finding stands in contrast to two other studies, where skin staples were not identified as a risk factor. (Colbath et al. 2014, Darnaud et al. 2016)

Suture material coated with antimicrobial drugs (triclosan) showed no positive effect on SSI. (Bischofberger et al. 2010)

Since the results of these studies are very heterogenous (Coomer et al. 2007, Colbath et al. 2014, Isgren et al. 2017) there is still no clear consensus in literature regarding this factor. Hence, the influence of suture pattern on SSI occurrence needs to be investigated prospectively, in a large enough and adequately distributed sample, to reliably determine its effect.

Protective Dressing of the Incision Site

The effect of the use of stent bandages was investigated in a study that compared the SSI occurrence in horses that wore a stent bandage for 5 days after surgery, to that of horses that did not receive one. In the “stent” group, the stent was sutured over the incision and protected by an adhesive drape for recovery, which was removed directly afterwards. The incision sites of horses in the control group were covered by an adhesive drape only, which was also removed after recovery. Horses, that received a stent bandage were significantly less likely to develop an SSI (Tnibar et al. 2013). The authors concluded that the beneficial effect of stent bandages results from the pressure applied to the incision site, the reduction of tension posed on the skin suture and the adequate wound oxygenation while still warranting protection from gross contamination.

Similarly, Smith et al. found that horses wearing abdominal bandages from the time they recovered from general anesthesia, until 14 days after discharge were 12,5 times less likely to develop incisional drainage than horses that did not wear an abdominal bandage at all in one study (Smith et al. 2007).

In other studies, the usage of an abdominal bandage did not influence SSI occurrence (Torfs et al. 2010, Costa-Farré et al. 2014, Darnaud et al. 2016). It has to be taken into account, that in these studies, the use of abdominal bandages was not standardized. This may have biased to the use of bandages to horses considered at risk to develop SSI (e.g., high anticipated abdominal wall tension, not perfectly aligning incisional edges…) or already showed signs of wound healing complications, like swelling or heat, prior to developing an infection. This limitation of retrospective analysis may have influence on the outcome and further prospective, standardized research is necessary to evaluate the protective benefit and the ideal application regimen of stents and abdominal bandages.

Quality of Recovery

Horses experiencing a poor recovery had an increased risk of SSI development, compared to horses with a good recovery phase in one study (Freeman et al. 2012). A possible explanation could be that rough recoveries lead to more stress and tension on the wound and increase contamination in the recovery period (Freeman et al. 2012).

In another study, horses with an excellent or good recovery were more likely to keep their protective dressing (adhesive or stent bandage) fully in place, compared to horses with a poor recovery. However, there was no association between the grade of protection at the end of the recovery, or the quality of recovery and a positive bacterial culture result. Additionally, a positive bacterial culture result immediately after recovery did not predict SSI development (Isgren et al. 2019).
Further the duration of recumbency during recovery did not show an effect on SSI development (Darnaud et al. 2016).
Taking all the above into consideration, the effect of quality of recovery and early wound contamination remains questionable.

Regimen and Duration of Antimicrobial Drug Use

The antimicrobial drugs (AMD) most commonly used for equine surgical prophylaxis are a combination of Penicillin and Gentamycin (Southwood 2014). Penicillin, as a beta-lactam antibiotic has a good effect against many important gram-positive bacteria but has limited effect against some gram-negative bacteria associated with the gastro-intestinal (GI) tract and hence combined with Gentamycin (an aminoglycoside), which is effective against gram-negative bacteria. Additionally, these two classes of antibiotics are known to have synergistic effect when combined (BEVA 2021).
Therefore it is little surprising, that the use of the combination of potassium penicillin and gentamycin was associated with a lower risk for SSI (Isgren et al. 2019). Horses receiving potassium penicillin and gentamycin/enrofloxacin showed significantly less SSI than those that received either potassium penicillin or gentamycin or no AMDs (Darnaud et al. 2016).
In a study performed by Freeman et al. horses that received AMD closer to time of first incision tended to be less likely to develop SSI than horses receiving AMD earlier (Freeman et al. 2012). Pre- and peri-operative AMD dosing aims at achieving effective tissue concentrations at the time of incision. Consistent with human surger, if the first dose of prophylactic antimicrobials is given too early before surgery, or only

started post-operatively, the infections occur at the same rate, as when no antimicrobials are used (Southwood 2014).
No impact on SSI rate with different dosages of potassium penicillin (21,456–27,806 IU/kg bodyweight i.v.) and gentamycin (6.8–8.7 mg/kg bodyweight i.v.) was found (Freeman et al. 2012). The authors of this study stated that it was difficult to draw conclusions on the effect of peri-operative AMD re-dosing during long surgeries, because the majority of patients in this retrospective study were not re-dosed according to current guidelines.
The duration of post-operative AMD use showed no significant influence on SSI (Freeman et al. 2012, Durward-Akhurst et al. 2013, Darnaud et al. 2016, Aitken et al. 2019, Isgren et al. 2019). Further, there was no significant difference in SSI prevalence between horses that received AMD for <36 hours and >36 hours. (Freeman et al. 2012) Since the findings of these studies are clear and consistent, they show that prolonged prophylactic antimicrobial therapy can be abandoned. This is consistent with the recommendations of international guidelines on antimicrobial use in equine patients, such as promoted in the “Protect ME” toolkit by the British Equine Veterinary Association (BEVA 2021) or in the Australian Veterinary Prescribing Guidelines (AVPG 2021). For clean contaminated procedures (in which category most exploratory laparotomies would fall is), the AVPG recommends the use of Penicillin and Gentamycin and to stop administration within 24 hours, unless there is ischemic damage to the gastro-intestinal GI tract, then the same drug combination can be administered for 3 days. Further, they recommend administration of intravenous drugs less than 60 minutes prior to surgery, and that of intramuscular Procaine Penicillin within 3.5 hours of surgery. Penicillin should be re-dosed every 80 minutes (two half-life times of the drug) during surgery (AVPG 2021). For clean surgical procedures BEVA recommends post-operative administration of Penicillin only, for 24 hours. For procedures classified as contaminated, they recommend pre-operative administration of penicillin and post-operative use of the combination of Penicillin and Gentamycin for 3 days (BEVA 2021).
More prospective and standardized research is needed to evaluate the potential beneficial effects of anti-microbial drug use on SSI development in colic patients.

Post-operative Colic

All studies, that investigated the effect of post-operative colic on SSI occurrence found, that horses showing signs of colic post-operatively are at an increased risk of developing SSI (Coomer et al. 2007, Anderson et al. 2015, Darnaud et al. 2016, Isgren et al. 2017). The authors hypothesize that a higher level of activity, such as pawing or rolling would increase the stress on the laparotomy site and prolonged time spent in recumbency would increase the likelihood of contamination of the wound. Also, ongoing signs of abdominal discomfort after surgery might result in the need for a re-laparotomy, which is associated with higher SSI rates (Dziubinski et al. 2020). Further, prolonged withholding of feed, as a reaction to colic symptoms might influence wound healing capacities and immunity.

Post-operative Fever

The occurrence and/or duration of elevated post-operative rectal temperatures were significantly associated with a higher risk for SSI (Smith et al. 2007, Anderson et al. 2015, Scharner et al. 2017).

Slight pyrexia (38.3-38.8 °C) was present in 85% of surgical colic patients in one study (Freeman et al. 2012). Horses developing infections (pneumonia, thrombophlebitis, peritonitis, salmonellosis, clositridiosis or SSI) had higher peak rectal temperatures (>39.2°C), longer duration between surgery and peak rectal temperature (>48h) and longer duration of pyrexia (>48h) than horses without infections.

However, all horses that develop fever post-operatively should be monitored closely for signs of incisional infection (Anderson et al. 2015).

Season

Horses that underwent colic surgery during summer and winter months were significantly more likely to develop SSI, than those operated in spring (Isgren et al. 2017). A longer winter coat and higher skin contamination could account for the higher incidence in winter. However, the factor “unclean coat” did not prove statistically significant in the same study. Another hypothesis would be that during summer, higher ambient temperatures could create a favorable environment for bacterial growth and lead to more sweating of the horse under the protective dressing, which may predispose for SSI (Isgren et al. 2017).

Since colic surgeries are emergency interventions, they cannot be scheduled to season. However, with the knowledge about individual climate conditions, clinicians can take extra measures for horses operated in risk seasons, like more frequent abdominal bandage or stent changes or the use of more breathable abdominal bandages in summer.

Post-operative Serum Amyloid A (SAA) Concentration

A recent study, that examined SAA concentrations in post-operative colic horses found that SAA was elevated in all horses in the early post-operative period. The increase was more marked in horses experiencing complications but did not differ between horses having complications associated with infection (e.g., SSI, thrombophlebitis) and those with other complications (e.g., PO colic, PO reflux), while SAA concentrations at the time of discharge were predictive for development of SSI (Aitken et al. 2019).

Based on these findings, referring clinicians and horse owners can be advised to monitor horses with elevated SAA in the post-operative period more closely.

There are a few limitations to the abovementioned literature. First the prevalence, but also possible risk factors are greatly influenced by the definition of SSI. If SSI was defined as purulent discharge, cases with serous or serosanguineous discharge would have gone unnoticed.

Second, the period after surgery in which it is considered normal for wound discharge to occur varies from 12 to 72 hours (Smith et al. 2007, Costa-Farré et al. 2014, Darnaud et al. 2016).

Third, the sample size varies greatly between the studies, with some studies only investigating as much as 75 cases (Anderson et al. 2015). Many of the studies that investigate risk factors for SSI are retrospective, bearing limitations, such as missing or incomplete information in medical records, lack of uniformity of certain procedures (e.g., scrubbing of the surgical site, use and type of bandages, antimicrobial drug dosing…) and are often conducted at a single hospital. This may limit the ability to generalize the applicability of the obtained results in many cases and has to be kept in mind when interpreting them in the individual hospital context. For example, it is possible that the season only proves to be a risk factor in countries with pronounced temperature or humidity changes throughout the year.

Further, some studies took only the time the patient was present at the hospital into account, whereas others provided long-term follow-ups up to 3 months (Smith et al. 2007). Including cases only until discharge could underestimate the occurrence of SSI, since in some horses SSI only develops after discharge (Anderson et al. 2015). In addition, an unbalanced sample and a small sample size might lead to very small numbers within some categories, which makes it difficult to gain significant data.

All these factors likely account for some of the discrepancies between studies, regarding individual risk factors for SSI.

This retrospective study was conducted to determine the prevalence and risk factors of SSI at the equine clinic of the University of Veterinary Medicine Vienna. Discrepancies in literature regarding various factors show, that risk factors may vary and emphasize the need to evaluate specific risk factors that apply to individual institutions, their management practices and patient population.

Identification of risk factors specific for our institution may lead to the ability to adress these directedly and subsequently reduce SSI occurence in our operative colic patients in the future.

We hypothesized, that the prevalence of SSI at our institution is comparable to that in previously published literature (9%-40%, Costa-Farré et al. 2014, Scharner et al. 2017) and that the occurrence of SSI significantly increases the duration of hospitalization and the associated costs. Further, we hypothesized that longer duration of surgery, decreased quality of recovery, presence of post-operative colic and fever and surgical procedures during warmer months would be associated with an increased risk of SSI.

2. Material and methods

Medical records of horses that underwent ventral midline celiotomy for acute colic signs at the University of Veterinary Medicine Vienna between August 2015 and August 2020 were reviewed.
All horses older than 8 weeks were included and of those horses where a repeat celiotomy was performed less than 8 weeks after the initial surgery, only the first surgery was included in the analysis. Horses were excluded if they survived less than 10 days after surgery or if they underwent a repeat celiotomy within 10 days after the initial surgery, without developing SSI prior to the second surgery/ euthanasia.
SSI was defined as any purulent discharge from the incision site, that was described in the medical records more than once (Mair and Smith 2005b, Espinel-Rupérez et al. 2019).
During the study, information was obtained on pre-, intra- and post-operative parameters, that were previously described as risk factors for SSI development. Recorded variables included age (years), breed, weight (kg), sex (mare/gelding/stallion), severity (mild/moderate/severe) and duration of colic symptoms (hours), rectal temperature (°C), heart rate (beats per minute) and respiration rate (breaths per minute), color of mucous membranes (pale pink, mildly/moderately/severely congested/anemic/cyanotic/icteric), capillary refill time (seconds), packed cell volume (PCV, %), total protein (TP, g/dl) and blood lactate levels (mmol/L). Breed was subcategorized as Warmblood, Icelandic horse, American breed (American Quarter Horse, Appaloosa, American Paint horse), ponies (any breed with a standard whither height <1,48 meters), Thoroughbred-type (Thoroughbred, Arabian), Iberian breeds (Lusitano, Andalusian, Criollo).
Further parameters obtained were the date of the surgery, whether it was performed in or out of regular hours (regular hours are Monday – Friday, 8am – 4pm), primary surgeon, primary surgeon experience (first/second/third year resident/diplomate or >5 years of experience), primary lesion (exact diagnosis based on intra-operative findings and whether the lesion was strangulating or not), site of primary lesion (stomach, small intestine, caecum large colon, small colon, other gastro-intestinal

structure and non gastro-intestinal structure), abdominal closure technique (information on suture material, patterns and number of layers), whether or not a stent and/or abdominal bandage was applied, duration of surgery and anesthesia (minutes), the lowest MAP (mmHg) and arterial partial oxygen pressure (PaO2, mmHg) and recovery score (defined as excellent/good/rough/wild).
Post-operative variables included the use of abdominal bandages, antimicrobial drug (AMD) regimen and duration of administration (hours), signs of colic (any mention of signs of abdominal discomfort, that were medically addressed), presence of reflux (>2 liters, more than once), duration of fever (>38.5°C) and peak rectal temperature, white blood cell count (cells/µl) and time a complete blood count (CBC) was performed after surgery (hours). Further, duration of hospitalization (days) and costs (euro) were also collected in this study. Since SSI was the endpoint of interest, post-operative variables were recorded to the point that SSI was first mentioned in the medical records, in those horses that developed SSI.

Statistical analysis was performed using Stata IC 15.1 (StataCorp LLC, College Station, Texas, USA). In total, 209 horses were included in the statistical analysis. Descriptive statistics was performed on all recorded variables. For categorical data, absolute and relative (%) frequency was calculated. For continuous data mean/median and standard deviation/interquartile range were determined.
Fisher’s exact test was used for comparison among groups of categorical data and continuous data were analyzed using a t-test.
To examine the potential univariable associations between all independent variables and the dichotomous outcome SSI/non-SSI, simple logistic regression models were applied.
Predictive variables with a univariable p-value of <0.25, a sufficient number of datapoints and considered clinically relevant were considered for inclusion in the multivariable logistic regression model, with the presence or absence of SSI as the dichotomous dependent variable. Variables included were: breed, body weight, TP on admission, rectal temperature on admission, capillary refill time on admission, month of surgery, surgery in/out of hours, strangulation of lesion, surgical procedure

type, pattern of skin suture, duration of surgery and anesthesia, post-operative WBCC, post-operative reflux, post-operative colic, post-operative fever and its duration.

A stepwise backward elimination strategy was used, and significance level was set at $p<0.05$. Two-way interactions between independent variables were evaluated. Model performance was assessed and area under receiver operator characteristic curve was determined, as a summary statistic of discriminating power.

3. Results

During the study period of 5 years, 352 horses underwent ventral midline laparotomy for signs of acute colic. 117/352 (33.2%) horses survived less than 10 days after surgery. Of these, 84/117 (71.8%) were euthanized during surgery, 9/117 (7.7%) during recovery, 17/117 (14.5%) horses were euthanized because of worsening/progression of colic symptoms, 6/117 (5.1%) because of concurrent issues/complications and 1/117 (0.8%) horse died less than 10 days after surgery. 20/352 (5.7%) horses underwent a second laparotomy within 10 days after the first one, however one developed an SSI before, so only 19/352 (5.4%) were excluded. 7/352 (2.0%) horses were less than 8 weeks old.
Finally, 209 horses met the criteria and were included in the statistical analysis.
Of these horses 34.5% (72/209) were mares, 59.3% (124/209) were geldings and 6.2% (13/209) were stallions. The average age was 14 years (±7 years). Breeds were distributed as follows: 53.4% (111/206) warmblood, 20.4% (42/206) pony, 8.7% (18/206) Icelandic, 4.4% (9/206) draft breeds, 1.9% (4/206) Standardbred, 3.4% (7/206) American breeds, 3.9% (8/206) Spanish breeds and 3.4% (7/206) Thoroughbred. The average weight was 488 ± 147 kg.
14.8% (31/209) horses developed an SSI. On average, first signs of an infection were noticed on day 10 ± 5 since surgery. Horses were hospitalized an average of 13 ± 10 days.

Signalment and Pre-operative Parameters

Of the factors regarding signalment of the horse, only the association between bodyweight and SSI occurrence approached significance (p=0.057). Horses with SSI had a mean bodyweight of 535 ± 122kg and those without 480 ± 150kg.
Age, breed, and sex were not associated with increased SSI occurrence.
Body condition scores (BCS) were only recorded for 78 patients. 25.6% of the horses were obese, 48.7% fat, 1.3% normal, 18.0% skinny, and 6.4% thin. This factor was not associated with SSI occurrence (p=0.656). Further, there was no correlation between bodyweight and BCS (p=0.712).

Except for TP on admission (p=0.030), no other pre-operative parameters were significantly associated with an increased SSI rate.

The month in which the surgery was performed was significantly associated with SSI occurrence, with most SSI developing in between January and March (Figure 1).

Results of signalment and pre-operative parameters are shown in table 1.

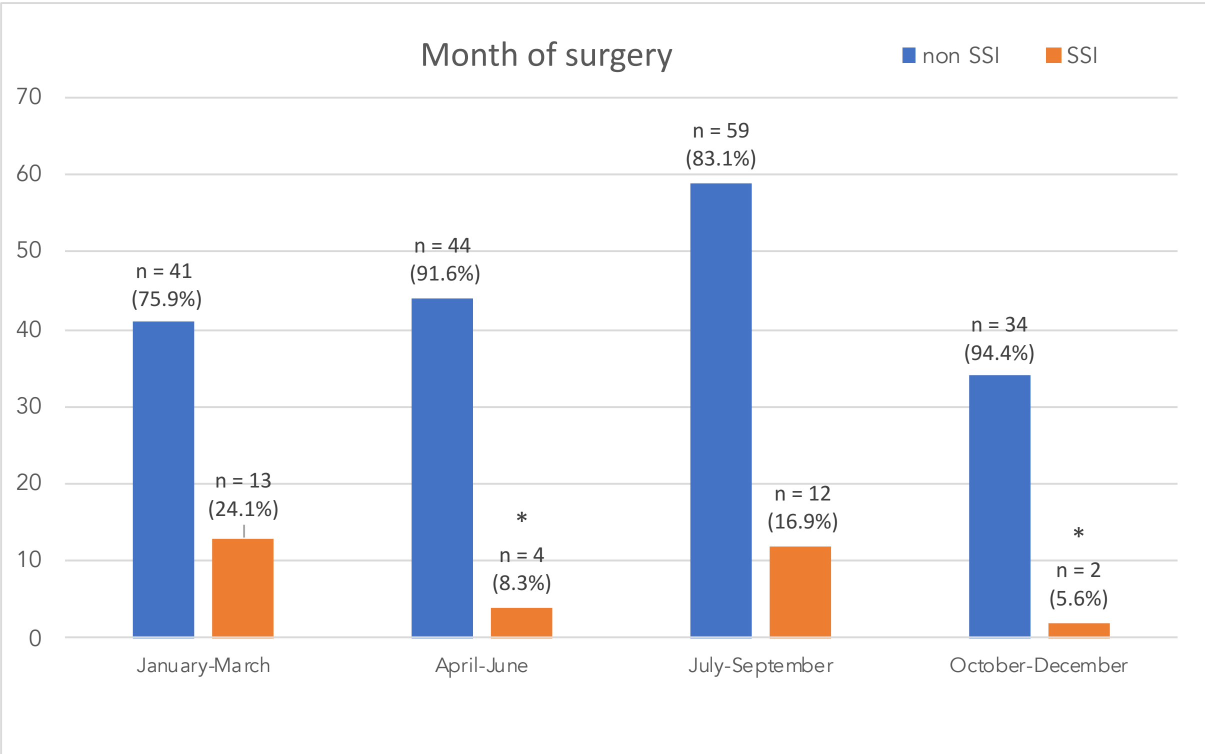

Figure 1, proportions of horses with and without SSI in relation to the month of the surgery
Month of surgery was significantly associated with SSI occurrence (p=0.039).
** Horses operated in the months April-June and October-December were significantly less likely to develop SSI (p=0.041 and p=0.034) compared to horses operated between January and March. No difference was found for the months July-September.*

Table 1, Univariable association between signalment and pre-operative variables and SSI occurrence in 209 horses undergoing emergency laparotomy at the University of Veterinary Medicine Vienna, 2015-2020, based on simple logistic regression models.

Variable (unit) (n)	**Category**	**Surgical site infection**		**OR**	**95% CI**	**p-value**
		no	**yes**			
Age (years) (209)		14.1 ± 7.0	13.7 ± 5.9	0.99	0.94-1.05	0.760
Breed (206)	Warmblood	81.8% (90)	18.1% (20)	referent		0.450 [a]
	Icelandic	77.8% (14)	22.2% (4)	1.29	0.38-4.32	0.684
	Pony	92.9% (39)	7.1% (3)	0.35	0.10-1.23	0.102
	Other	88.9% (32)	11.1% (4)	0.56	0.17-1.77	0.325
Sex (209)	Mare	83.3% (60)	16.7% (12)	referent		0.666 [a]
	Gelding	85.5% (106)	14.5% (18)	0.85	0.38-1.88	0.687
	Stallion	92.3% (12)	7.7% (1)	0.42	0.05-3.51	0.421
Body weight (kg) (208)		480 ± 150	535 ± 122	1.00	1.00-1.01	0.057
Body condition score (78)	good to obese	79.7% (47)	20.3% (12)	referent		0.656 [a]
	skinny	84.2% (16)	15.8% (3)	0.73	0.18-2.94	0.663
Severity of symptoms (181)	mild + moderate	87.6% (141)	12.4% (20)	referent		0.646 [a]
	severe	80.0% (16)	20.0% (4)	1.76	0.54-5.80	0.351
Duration symptoms to surgery (h) (190)		18 ± 20	15 ± 12	0.99	0.96-1.02	0.402
Colour of mucous membranes	hyperemic	86% (121)	14% (20)	referent		0.597 [a]
on admission (161)	other	90% (18)	10% (2)	0.67	0.14-3.12	0.612
Capillary refill time on	1-2 sec	89.2% (99)	10.8% (12)	referent		0.074 [a]
admission (196)	3-4 sec	80.0% (68)	20.0% (17)	2.06	0.93-4.59	0.076
Rectal temperature on admission (°C) (195)		37.7 ± 0.6	37.9 ± 0.5	1.85	0.91-3.76	0.088
Heart rate on admission (bpm) (205)		55 ± 14	55 ± 16	1.00	0.97-1.02	0.898
Respiratory rate on admission (breaths/min) (203)		23 ± 9	22 ± 7	1.00	0.95-1.04	0.822
PCV on admission (%) (199)		36.9 ± 6.2	35.8 ± 5.8	0.97	0.91-1.03	0.348
TP on admission (g/dl) (196)		6.56 ± 0.77	6.23 ± 0.67	0.54	0.30-0.95	0.031
WBCC on admission (cells/µL) (42)		7590 ± 3209	9553 ± 4773	1.00	1.00-1.00	0.187
Lactate on admission (mmol/L) (157)		2.97 ± 2.70	2.78 ± 2.81	0.97	0.81-1.16	0.762
Month (209)	January-March	75.9% (41)	24.1% (13)	referent		0.039 [a]
	April-June	91.6% (44)	8.3% (4)	0.29	0.09-0.95	0.041
	July-September	83.1% (59)	16.9% (12)	0.64	0.27-1.55	0.323
	October-December	94.4% (34)	5.6% (2)	0.19	0.04-0.88	0.034
Year (209)	2015	75% (15)	25% (5)	referent		0.499 [a]
	2016	84.2% (32)	15.8% (6)	0.56	0.15-2.14	0.399
	2017	91.9% (34)	8.1% (3)	0.26	0.06-1.25	0.094
	2018	86.6% (39)	13.3% (6)	0.46	0.12-1.74	0.254
	2019	80.8% (38)	19.1% (9)	0.71	0.20-2.47	0.591
	2020	90.9% (20)	9.1% (2)	0.30	0.05-1.76	0.183
Surgery in/out of hours (209)	out	87.6% (134)	12.4% (19)	referent		0.116 [a]
	in	78.5% (44)	21.4% (12)	1.92	0.87-4.28	0.109

p[a] – overall p-value for categorical data with more than two categories, based on univariable logistic regression analysis.
OR odds ratio, CI confidence interval, PCV packed cell volume, TP total protein, WBCC white blood cell count

Intra-operative Parameters

SSI incidence varied between 0.0% (0/14) and 35.3% (6/17) among individual surgeons. However, the overall difference among the 14 surgeons was not significant (p=0.790). Surgeons experience (p=0.703), as well as the location of the primary lesion (p=0.682) and whether the lesion was strangulating or not (p=0.192) were not significantly associated with SSI occurrence.

Surgery time was significantly longer for strangulating lesions than for non-strangulating ones (143 ± 54 minutes vs. 123 ± 35 minutes, p=0.002).

Horses undergoing longer surgical procedures were more likely to develop an SSI (average 150min vs. 124min, p=0.002) (Figure 2).

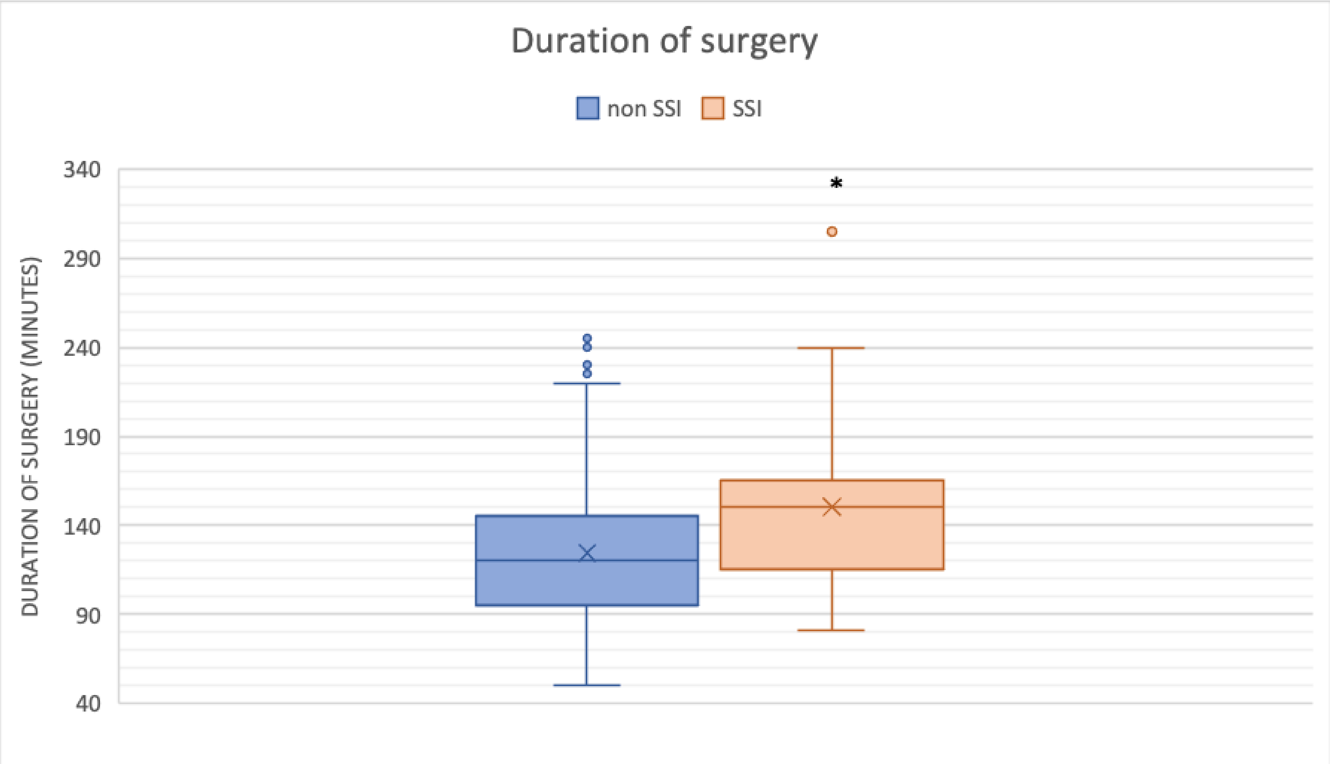

Figure 2, duration of surgery (minutes) in relation to SSI occurrence
Lines within box represent median duration of surgery, crosses within box represent mean duration of surgery. Upper and lower limit of box represent the 75th and 25th percentiles, upper and lower limit of the error bars represent the 90th and 10th percentiles. Dots represent outliers.
** Surgical duration was significantly (p=0.002) longer for horses that developed SSI.*

Similarly, a longer duration of general anesthesia, resulted in an increased risk for SSI. Horses that developed an SSI were anesthetized on average 26 minutes longer than horses that did not develop SSI (180 ± 57 vs. 154 ± 43 minutes, p=0.005).
The suture material used for the different layers was the same in the vast majority of horses, so that no statistical analysis was made for the lack of sufficiently large samples in the other categories. In 91.4% of the horses, Polysorb 2 was used for the suture of the linea alba and Monosyn 2/0 was used for the subcutaneous layer in 90.1% of horses and for the skin suture in 88.4% of horses.
None of the horses with intradermal and modified subcuticular skin suture patterns developed an SSI, making horses with these two kinds of skin suture significantly less likely to develop SSI (p=0.037) (Figure 3).

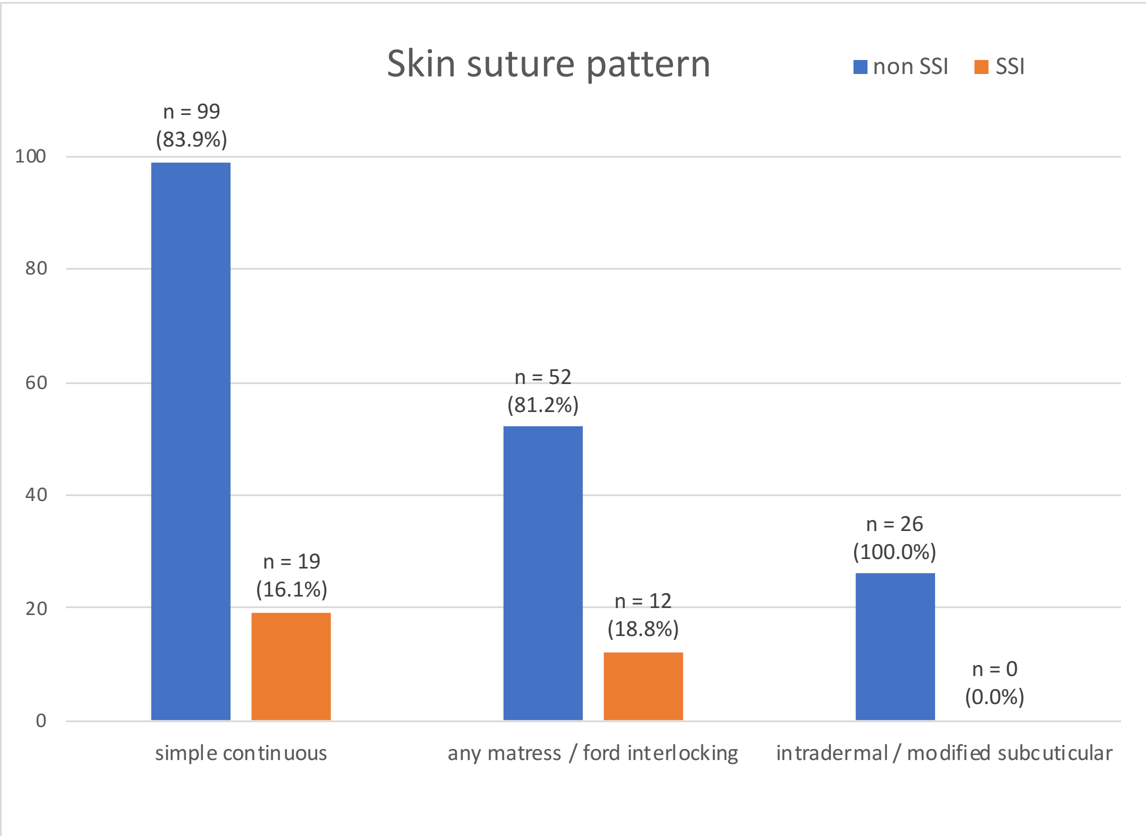

Figure 3, proportions of horses with and without SSI in relation to the skin suture pattern
Skin suture pattern was significantly associated with SSI occurrence (p=0.037). None of the horses in the intradermal/subcuticular skin suture pattern group developed SSI.

The majority of horses underwent surgery outside of normal working hours (73.2%, 153/209). This parameter was not significantly associated with SSI occurrence, but interestingly, 21.4% (12/56) of the horses that underwent surgery during normal working hours developed a SSI, while this was the case in only 12.4% (19/153) of the horses that underwent surgery out of normal working hours.
Results of all intra-operative parameters are shown in table 2.

Table 2, Univariable association between intra-operative variables and SSI occurrence in 209 horses undergoing emergency laparotomy at the University of Veterinary Medicine Vienna, 2015-2020, based on simple logistic regression models.

Variable (unit) (n)	**Category**	**Surgical site infection**		**OR**	**95% CI**	**p-value**
		no	**yes**			
Surgeon (208)	1	84.6% (22)	15.4% (4)	referent		0.790 [a]
	2	87.5% (28)	12.5% (4)	0.79	0.18-3.50	0.752
	3	90.9% (10)	9.1% (1)	0.55	0.05-5.57	0.613
	4	83.3% (15)	16.6% (3)	1.10	0.21-5.64	0.909
	5	88.1% (37)	11.9% (5)	0.74	0.18-3.06	0.681
	6	87.5% (7)	12.5% (1)	0.79	0.07-8.24	0.841
	7	100.0% (1)	0.0% (0)	1.00		
	8	81.8% (9)	18.2% (2)	1.22	0.19-7.90	0.833
	9	88.9% (8)	11.1% (1)	0.69	0.07-7.11	0.753
	10	64.7% (11)	35.3% (6)	3.00	0.70-12.89	0.140
	11	72.3% (8)	27.3% (3)	2.06	0.38-11.31	0.404
	12	100.0% (14)	0.0% (0)	1.00		
	13	100.0% (1)	0.0% (0)	1.00		
	14	85.7% (6)	14.3% (1)	0.92	0.09-9.81	0.943
Surgeon's experience (208)	Diplomate/ very experienced	85.8% (139)	14.2% (23)	referent		0.703 [a]
	3rd or 4th year resident	80.0% (24)	20.0% (6)	1.51	0.56-4.10	0.417
	2nd year resident	87.5% (14)	12.5% (2)	0.86	0.18-4.05	0.852
Location of primary lesion (209)	open	66.7% (2)	33.3% (1)	referent		0.682 [a]
	small intestine	83.9% (52)	16.1% (10)	0.38	0.03-4.66	0.453
	caecum	93.3% (14)	6.7% (1)	0.14	0.01-3.31	0.225
	large colon	85.7% (102)	14.3% (17)	0.33	0.02-3.88	0.380
	small colon	87.5 % (7)	12.5% (1)	0.29	0.01-6.91	0.441
	non gastro-intestinal	50.0% (1)	50.0% (1)	2.00	0.05-78.25	0.711
(204)	small intestine	83.1% (52)	16.9% (10)	referent		0.572 [a]
	large intestine	86.1% (123)	13.9% (19)	0.80	0.35-1.84	0.606
Strangulation (204)	no	88.0% (125)	12.0% (17)	referent		0.175 [a]
	yes	81.6% (50)	19.4% (12)	1.76	0.79-3.96	0.169
Surgical procedure type (208)	Exploratory laparotomy/ needle decompression/ repositioning	92.3% (60)	7.7% (5)	referent		0.428 [a]
	Pelvic flexure enterotomy	84.3% (86)	15.7% (16)	2.23	0.78-6.42	0.136
	JJ + JI	82.4% (14)	17.6% (3)	2.57	0.55-12.06	0.231
	JC	66.7% (2)	33.3% (1)	6.00	0.46-78.24	0.171
	typhlotomy + caecumspitzenresekt	75.0% (3)	25.0% (1)	4.00	0.35-45.90	0.265
	large colon resection	0.0% (0)	100.0% (1)	1.00		
	multiple +SI + small colon enterotomy	78.6% (11)	21.4% (3)	3.27	0.68-15.72	0.139
	biopsies	50.0% (1)	50.0% (1)	12.00	0.65-222.08	0.095
Lowest intraoperative PaO2 (mmHg) (189)		113 ± 80	105 ± 86	1.00	0.99-1.00	0.616
Lowest intraoperative MAP (mmHg) (182)		65 ± 11	66 ± 14	1.00	0.97-1.04	0.796
Number of layers (208)	2	84.1% (58)	15.9% (11)	referent		0.768 [a]
	3	85.6% (119)	14.4% (20)	0.89	0.40-1.97	0.767
Pattern of skin suture (208)	simple continuous	83.9% (99)	16.1% (19)			0.037 [b]
	any matress/ford interlocking	81.2% (52)	18.8% (12)			
	intradermal/modified subcuticular	100.0% (26)	0.0% (0)			
Surgery time (min) (206)		124 ± 40	150 ± 49	1.00	1.00-1.02	0.003
Anesthesia time (min) (187)		154 ± 43	180 ± 57	1.01	1.00-1.02	0.007
		non-strangulating lesion	**strangulating lesion**			
Duration surgery time (min) (201)		123 ± 35	143 ± 54	1.01	1.00-1.02	0.002

p[a] – overall p-value for categorical data with more than two categories, based on univariable logistic regression analysis.
p[b] – p-value based on Fisher's exact test.
OR odds ratio, CI confidence interval, PaO2 partial pressure of arterial oxygen, MAP mean arterial blood pressure

Post-operative Parameters

A combination of Penicillin G and Gentamycin was used for 91.9% of horses for post-operative AMD treatment. Hence, no statistical analysis was performed, since the other categories did not hold a large enough sample size.

The presence of post-operative fever was significantly associated with SSI development (OR 2.46, 95% CI 1.09-5.52, p=0.032). Duration of fever was only recorded up to the point where signs of an SSI were first noticed, which might limit interpretability. Nonetheless, there was a tendency for horses that developed SSI to have a longer duration of fever (average 17 ± 15 hours vs. 12 ± 12 hours, p=0.102). Post-operative WBCC was increased in horses that developed SSI (7194 ± 4006 cells/µL vs. 5253 ± 2284cells/µL, p=0.001), which was due to increased neutrophil count (neutrophilia) (average 5680 ± 3483 cells/µL vs 3840 ± 2114 cells/µL, p=0.002). However, the time span between surgery and the performance of a CBC was significantly longer for horses that developed SSI as well (average 64 ± 52 hours vs. 35 ± 29 hours, p<0.001). No significant association was found between other post-operative variables and SSI occurrence.

Results of post-operative variables are shown in table 3.

Table 3, Univariable association between post-operative variables and SSI occurrence in 209 horses undergoing emergency laparotomy at the University of Veterinary Medicine Vienna, 2015-2020, based on simple logistic regression models.

Variable (unit) (n)	**Category**	**Surgical site infection** no	yes	**OR**	**95% CI**	**p-value**
Use of a stent bandage	no	86.4% (19)	13.6% (3)	referent		0.985 a
(209)	yes	84.9% (152)	15% (27)	1.12	0.31-4.04	0.865
	ioban plaster	85.7% (6)	14.3% (1)	1.06	0.09-12.14	0.965
Use of an abdominal bandage	none	89.7% (61)	11.3% (7)	referent		0.440 [a]
(209)	yes. before recovery	87.3% (55)	12.7% (8)	1.27	0.43-3.72	0.666
	yes. within 12h after recovery	78.6% (44)	21.4% (12)	2.38	0.87-6.52	0.093
	yes. later than 12h after recovery	75% (6)	25% (2)	2.90	0.49-17.25	0.241
	only hansapore/ioban/animal polster	85.7% (12)	14.3% (2)	1.45	0.27-7.86	0.665
Recovery score	excellent	80.0% (44)	20.0% (11)	referent		0.424 [a]
(172)	good	82.4% (70)	17.7% (15)	0.86	0.36-2.03	0.727
	rough	90.3% (28)	9.7% (3)	0.43	0.11-1.67	0.223
	wild	100.0% (1)	0.0% (0)	1.00		
Post-operative WBCC (cells/µL) (153)		5253 ± 2284	7194 ± 4006	1.00	1.00-1.00	0.004
Post-operative Neutrophil count (cells/µL) (129)		3840 ± 2114	5680 ± 3483	1.00	1.00-1.00	0.004
Post-operative Lymphocyte count (cells/µL) (129)		913 ± 649	1130 ± 729	1.00	1.00-1.00	0.198
Time between surgery and WBCC (hours) (153)		35 ± 29	64 ± 52	1.02	1.01-1.03	0.001
Post-operative reflux	no	87.1% (155)	12.9% (23)	referent		0.081 [a]
(209)	yes	74.2% (23)	25.2% (8)	2.34	0.94-5.86	0.068
Signs of post-operative colic	no	87.3% (130)	12.7% (19)	referent		0.177 [a]
(208)	yes	79.7% (47)	20.3% (12)	1.75	0.79-3.87	0.170
Post-operative fever	no	90.6% (96)	9.4% (10)	referent		0.025 [a]
(209)	yes	79.6% (82)	20.4% (21)	2.46	1.09-5.52	0.029
Duration of post-operative fever (hours) (103)		12 ± 12	17 ± 15	1.03	0.99-1.06	0.121
Duration of antimicrobial therapy (hours) (209)		81 ± 60	82 ± 44	1.00	0.99-1.01	0.887

p^a – overall p-value for categorical data with more than two categories, based on univariable logistic regression analysis.
OR odds ratio, CI confidence interval, WBCC white blood cell count

Outcome and Treatment Costs

SSI resulted in significantly increased (41.0 %) treatment costs (average 6148 ± 2196 € vs. 4360 ± 2196 €, p<0.0001) (Figure 4). Also, horses with SSI were hospitalized significantly longer (average 22 ± 15 vs. 12 ± 8 days, p<0.0001). Horses, that underwent a relaparotomy or were euthanized or died before discharge were significantly more likely to have an SSI than horses that survived to discharge (OR 11.7, 95% CI 3.81-36.10, p<0.001) (Figure 5).

Results of outcome and treatment costs are shown in table 4.

Figure 4, treatment costs (€) in relation to SSI occurrence.
Lines within box represent median treatment costs, crosses within box represent mean treatment costs. Upper and lower limit of box represent the 75th and 25th percentiles, upper and lower limit of the error bars represent the 90th and 10th percentiles. Dots represent outliers.
** Treatment costs were significantly higher for horses that developed SSI (p<0.0001).*

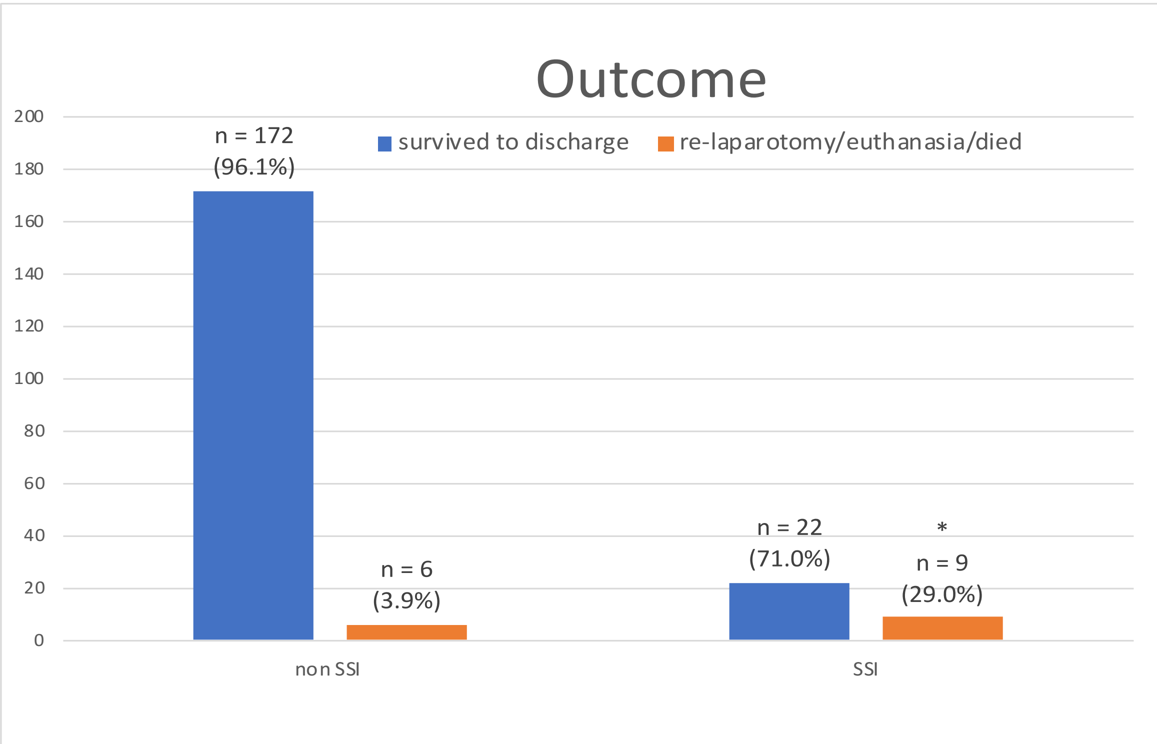

Figure 5, outcome in relation to SSI development.
** Horses with SSI were significantly less likely to survive to discharge (p<0.0001).*

Table 4, Univariable association between outcome and SSI occurrence in 209 horses undergoing emergency laparotomy at the University of Veterinary Medicine Vienna, 2015-2020, based on simple logistic regression models.

Variable (unit) (n)	**Category**	**Surgical site infection**		**OR**	**95% CI**	**p-value**
		no	**yes**			
Outcome (209)	survived to discharge	88.7% (172)	11.3% (22)	referent		<0.0001 [a]
	re-laparotomy/euthanasia/died	40.0% (6)	60.0% (9)	11.72	3.81-36.10	<0.001
Duration of hospitalisation (days) (209)		12 ± 8	22 ± 15	1.07	1.03-1.12	<0.001
Cost (€) (203)		4360 ± 1431	6148 ± 2196	1.00	1.00-1.00	<0.001

p[a] – overall p-value for categorical data with more than two categories, based on univariable logistic regression analysis.
OR odds ratio, CI confidence interval, WBCC white blood cell count

Pre-, intra- and post-operative variables, that may have influenced SSI development were included in the multivariable analysis when reaching a significance level p<0.25. The final multivariable logistic regression model included five variables (Table 5). Horses operated in the first three months of the year (January-March) were significantly more likely to develop an SSI than horses operated in Spring (April-June) or in the last three months of the year (October-December).

Horses heavier than 570 kg were at a 4.22 times higher risk for developing SSI than lighter horses (OR 4.22, 95% CI 1.68-10.61, p=0.002).

The risk for SSI increased with increased rectal temperature on admission (OR 2.94, 95% CI 1.16-7.41, p=0.023) and longer duration of surgery (OR 1.01, 95% CI 1.00-1.02, p=0.028).

Horses with post-operative reflux were significantly more likely to develop SSI, than those having no reflux (OR 3.57, 95% CI 1.16-10.99, p=0.026).

Table 5, Results of the final multivariable logistic regression model evaluating the association between selected factors and SSI occurrence in 209 horses undergoing emergency laparotomy at the University of Veterinary Medicine Vienna, 2015-2020.

Variable	Category	OR	95% CI	p-value
Month	January-March	Referent		<0.0001 [a]
	April-June	0.25	0.06-0.95	0.042
	July-September	0.56	0.20-1.59	0.276
	October-December	0.16	0.30-0.82	0.028
Bodyweight	< 570 kg	Referent		<0.0001 [a]
	> 570 kg	4.22	1.68-10.61	0.002
Rectal temperature on admission (°C)		2.94	1.16-7.41	0.023
Duration of surgery (min)		1.01	1.00-1.02	0.028
Post-opertive reflux	no	Referent		<0.0001 [a]
	yes	3.57	1.16-10.99	0.026

p[a] – overall p-value for categorical data with more than two categories, based on multivariable logistic regression analysis.
OR odds ratio, CI confidence interval

4. Discussion

The prevalence of SSI of ventral midline celiotomies after emergency laparotomy at the equine hospital of the University of Veterinary Medicine Vienna was 14.8% and thereby comparable to other studies (9%-40%, Aitken et al. 2019, Costa-Farré et al. 2014, Darnaud et al. 2016, Isgren et al. 2019, Freeman et al 2012, Scharner et al. 2017).

In this study, we identified several factors that were associated with SSI development at our institution.

In our final model, time of the year (winter: January-March) in which the surgery was performed, increased bodyweight, increased rectal temperature on admission, longer duration of surgery and occurrence of post-operative reflux were associated with an increased risk to develop an SSI.

In case of SSI development, this resulted in a significant increase in treatment costs and duration of hospitalization. Further, horses that developed SSI were significantly less likely to survive to discharge.

The time of year was a significant factor, with SSI occurring more frequently during the months January-March compared to spring and autumn months. No difference was seen compared to summer months (July-September). Season has previously been described as a risk factor for SSI, with the highest SSI incidence occurring in summer, followed by winter and a significant reduction of SSI incidence in spring (Isgren et al. 2017).

This could be explained by the influence of a cold and dry climate on skin. Studies in humans and animals have shown an alteration in skin characteristics during wintertime (Engebretsen et al. 2016). During the colder season, the stratum corneum holds less lipids and is less hydrated, leaving the skin more rigid, more sensitive to irritation and more permeable. This may be a reason for increased SSI incidence during the colder months. During summer months, elevated temperatures may contribute to development a warm and humid ambience under the protective dressing, ideal for bacterial growth, which could explain the increased SSI incidence in summer.

Although the month in which an emergency surgery is performed is not a factor that can be changed, this knowledge enables clinicians to monitor horses at an increased risk for SSI more closely and to take extra measures to mitigate other risk factors.

We found a significant association between horse weight and SSI occurrence. Hence, horses weighing more than 570 kg were at a 4.7 times higher risk of developing SSI than lighter horses, which stands in contrast to the findings of other studies that found no association between bodyweight and SSI (Coomer et al. 2007, Tnibar et al. 2013, Andersen et al. 2015, Darnaud et al. 2016, Isgren et al. 2019).
It is difficult to interpret this fact, since it is impossible to retrospectively determine whether the horses, that were heavier, were also taller, more muscular or had more bodyfat, or as previously described (Isgren et al. 2019), the extra weight was attributed to a heavier abdominal content.
There was a tendency for a lower SSI rate in pony breeds, compared to Icelandic horses and Warmbloods. This is consistent with two other studies, where Warmbloods had a higher SSI incidence (Darnaud et al. 2016) and ponies tended to have less SSI (Scharner et al. 2018). This may be due to the smaller size and weight of abdominal organs and therefore smaller tension posed on the wound, or to the better wound healing capacities described in ponies (Wilmink et al. 2005).
Possibly, the increased risk for SSI of heavier horses can be attributed to anesthesia-associated hypotension and reduced tissue perfusion and oxygenation (Ahern and Richardson 2012).
The important role of intra-operative, but also early post-operative tissue oxygenation in the development of SSI has been examined in previous studies (Ragheb and Buggy 2004, Hopf and Rollins 2007,Costa-Farré et al. 2014). We were not able to find an association between intraoperative arterial oxygen partial pressure and SSI occurrence, but in the present study, only the lowest point of PaO_2 was recorded. However, the duration and severity of hypoxemia intraoperatively and during the recovery phase might be a better indicator. Beside arterial oxygenation, tissue perfusion plays a crucial role in tissue oxygenation, which is traditionally monitored by measuring arterial blood pressure. Here as well, no significant association with

SSI was found, possibly because only the lowest point of mean arterial blood pressure was recorded in this study. In this way, an increase of blood pressure as a response to possible anti-hypotensive treatment was disregarded. Future research should either record arterial blood pressure in more detail (duration and severity of hypotension, anti-hypotensive treatment), or measure tissue oxygenation directly, since in tissues with a constant oxygen consumption, this parameter reflects tissue perfusion more precisely than arterial blood pressure (Ragheb and Buggy 2004). Possibly, the increased SSI rate in heavier horses could be attributed to an increased proportion of body fat, since obesity is commonly associated with increased risk for SSI in human patients (Waisbren et al. 2010, Takeuchi et al. 2016). However, our study found no association between body condition score and weight. Body condition score was only recorded for 78 horses though and of these, 58 were classified as fat or obese, leaving a small number of horses in the other categories. More focused research is necessary, to investigate the effect of this factor and also the possibility to measure related parameters, like percent body fat or the amount of visceral fat should be evaluated.

Interestingly, rectal temperature on arrival was a significant factor in the final multivariable model, with an OR of 2.94, meaning that with every degree °C increase in rectal temperature, the odds of developing SSI increased 2.94-fold.
Elevated rectal temperatures are consistent with infection and endotoxemia on admission has been identified as a predisposing factor for SSI (Smith et al. 2007). However, in the present study, other factors marking endotoxemia, like increased heart and respiratory rate, hyperemic mucous membranes, prolonged CRT and increased PCV were not associated with increased risk for SSI.
Interestingly, average temperature values were within normal limits in both the SSI and the non-SSI group (37.9 ± 0.5 °C vs. 37.7 ± 0.6 °C) in this study. Possibly, an anti-pyretic pre-treatment (e.g., non-steroidal anti-inflammatory drugs) administered by the referring veterinarian decreased elevated rectal temperatures into normal limits. However, pre-treatment was not recorded in this study, so no definite conclusions can be drawn regarding this aspect.

Thus, this factor warrants further research.

In accordance with the findings of others (Haridas et al. 2008, Freeman et al. 2012, Li et al. 2013, Costa-Farré et al. 2014, Anderson et al. 2015), the duration of surgery was a significant factor in the final multivariable model of this study.
The influence of surgical duration on SSI occurrence is complex and might result from the combined effect of several factors (Ahern and Richardson 2012).
Longer surgical duration may lead to more desiccation of the tissue of the wound margins, which may predispose for infection (Haridas et al. 2008).
The tissue concentrations of AMD decrease with increased surgery time and might drop below effective concentrations if not re-dosed properly, which might lead to an increased SSI rate (Cheng et al. 2017). This study has not recorded intra-operative re-dosing of AMDs. Possibly, this could be an area of further research and improvement of treatment standards.
Also, surgeon and team fatigue and hence proneness to technical errors have been addressed as potentially co-founding factors (Li et al. 2013).
Prolonged surgical duration has been associated with more complicated surgical interventions (Cheng et al. 2017). In our study procedure type was not significantly associated with increased SSI rate. However, we identified a non-significant trend for reduced SSI rate in horses, that underwent only an exploratory laparotomy or solely necessitated a reposition or decompression via suction by needle, compared to horses undergoing enterotomies of any kind. Also, the presence of a strangulating lesion resulted in a significantly longer surgical duration. Strangulating lesions often necessitate a resection and anastomosis of the affected bowel, which can be a complex and lengthy procedure.
Hence, measures should be taken to keep surgical time as short as possible, without rushing the procedure itself. This could be achieved by optimizing workflow through a thorough preparation of the operating room, intensive training of the support staff and optimized intra-operative teaching. Further, care should be taken to protect incision edges from desiccation and to comply with recommendations on re-dosing of intra-operative AMDs.

To the authors knowledge, this is the first study, that identified post-operative reflux as a risk factor for SSI. Horses, that developed reflux, were at a 3.6-fold higher risk of developing an SSI, than horses that developed no reflux.
The reason for this might be fluid, electrolyte and acid-base imbalances caused by the post-operative ileus, which may affect tissue perfusion and immune response negatively. This may predispose to the development of a pronounced SIRS. Patients with SIRS have been described to be more prone to infections at an increased risk for organ system dysfunction (Taylor 2015).
However, in this study, not enough post-operative parameters indicating SIRS were recorded, so an association between these parameters cannot be made definitely. Future research could focus on investigating the relation between SIRS-related parameters, like post-operative heart rate, respiratory rate, capillary refill time and color of mucous membranes and SSI occurrence.
Measures should be taken to treat reflux as best possible and to monitor fluid, electrolyte and acid-base status of the patient and balance the negative influence of reflux by intensive treatment in order to minimize this risk factor for SSI.

Intradermal and subcuticular skin sutures were identified as factors decreasing SSI rate in previous studies (Colbath et al. 2014). Also, in human patients, subcuticular sutures are known to lead to less SSI development than transdermal sutures and clips (Gwilym et al. 2021). In the current study, none of the 26 horses, that had intradermal or subcuticular skin sutures developed an SSI. In the univariable analysis skin suture pattern was a significant factor ($p=0.037$) but did not remain as a significant variable in the final multivariable model.
The beneficial effect of intradermal and subcuticular patterns might result from the suture material not penetrating the epidermis. Transdermal sutures may act as a path for bacteria to traverse the skin barrier, ascend into deeper layers and cause SSI. Since suture technique is an easily controllable factor, our institution could benefit from a prospective study with a large enough sample size to determine the effect of this factor on SSI occurrence.

The quality of recovery has been identified as a factor influencing SSI occurrence (Freeman et al. 2012), possibly because of its impact on early bacterial wound contamination. In the present study, no such association was found. However, since this study was performed retrospectively, little specific information could be obtained from the medical records, such as how much time was spent in sternal position or whether the wound was still completely covered by the protective dressing after the recovery. Also, the design of the recovery sheets used at our institution contains little reference points for discrimination between good and difficult recoveries. This may have led to more subjective judgement and underestimated the difficulty of some recoveries. Future research should document these details and possibly apply two separate scales, one for the general quality of recovery, with well formulated criteria and another one, for the quality of wound protection.

The use of stent and abdominal bandages did not prove significant in this study. This stands in contrast to the findings of others, where horses wearing abdominal bandages (Smith et al. 2007) and stent bandages (Tnibar et al. 2013) had less incisional complications than those without. However, it should be noted that in the course of the better part of the study period, stent and abdominal bandages were not used in a standardized manner and that standards of operation changed during these five years. It is difficult to discern, whether horses received an abdominal bandage prophylactically, or because they already showed incisional complications, like insufficient apposition of the wound margins, drainage or swelling. This could have influenced the impact of abdominal bandages on SSI rate. In future research, abdominal bandages should be applied in a standardized manner and also the different kinds of abdominal bandages should be recorded, since there might be differences in physical properties, like insulation, breathability and pressure posed on the surgical wound, that may play a role. If not applied properly, hook-and-loop bandages can lead to an accumulation of urine at the incision site in male horses and, like hernia belts, they tend to shift more than bandages consisting a sterile layer of cotton covered by adhesive tape. It is also worth investigating, whether the use of abdominal bandages in summer leads to more sweating, thus contributing to conditions that facilitate bacterial growth.

Since the mean duration of post-operative AMD use did not differ between the SSI and non-SSI group, these results suggest that prolonged AMD use does not effectively prevent the development of SSI, which is consistent with the findings of others (Durward-Akhurst et al. 2013, Darnaud et al. 2016, Aitken et al. 2019, Isgren et al. 2019, Dziubinski et al. 2020).
The average duration of post-operative AMD use in our study was notably longer than recommended in international guidelines for this kind of surgery. Previously, no difference in SSI rate was found in horses that were receiving AMD prophylaxis for <36h or >36h (Freeman et al. 2012).
Hence, the administration of post-operative AMD prophylaxis should be kept as short as recommended in international guidelines (BEVA 2021, AVPG 2021), which recommend an administration as short as 24h after clean-contaminated surgery and up to 72h if ischemic damage to the GI was observed. Pre-operative AMDs should be administered less than 60min prior to surgery (AVPG 2021). Unfortunately, this study did not record the time pre-operative AMDs were used. Further research is necessary, to determine whether there is room for improvement in this area at our institution.
Strict and responsible standards of operation regarding the use of AMDs are vital in the light of increasing occurrence of drug resistant bacteria (Dziubinski et al. 2019).

Elevated white blood cell count due to neutrophilia was found in horses that developed SSI. The concentration of circulating neutrophils in horses is a marker for systemic inflammation (Anderson and Singh 2018) and could be consistent with SSI. For horses that developed SSI, CBC was performed significantly later than for horses that showed no signs of SSI (64 ± 52 hours vs. 35 ± 29 hours after surgery). Since post-operative CBC was not performed routinely for every horse at our institution, this indicates that blood for CBC was drawn after horses showed elevated rectal temperatures or signs of wound infection, which could have acted as a bias.

Hence, the results of our study regarding this factor should be interpreted carefully.

The results of this study show, that SSI is a costly complication, associated with an average cost increase of 1.800€ (41.0%) (6148 ± 2196 € vs. 4360 ± 1431 €, p<0.001).

In human patients undergoing pancreaticoduodenectomy SSI was found to increase treatment costs by 23.8% (Javed et al. 2013), in canine patients undergoing soft tissue surgery SSI led to a total cost increase of 74.4% (Espinel-Rupérez et al. 2019). Several authors hypothesized that SSI would increase treatment costs in equine surgical colic patients (Bischofberger et al. 2010, Colbath et al. 2014, Durward-Akhurst et al. 2013, Freeman et al. 2012, French et al. 2002), but to our knowledge none have investigated this factor before.

SSI has been found to prolong hospital stay (Freeman et al. 2012) and to be negatively associated with patient survival (Ingle-Fehr et al. 1997, Gazzero et al. 2015) before.

Our study results show that SSI results in an average extension of hospital stay of 10 days, while also worsening the prognosis, since horses that had to undergo a re-laparotomy or were euthanized/died were 11.7 times more likely to have an SSI than horses that survived to discharge.

This could be a result of severe concurrent co-morbidities, that necessitate prolonged treatment or re-laparotomy. The association between SSI occurrence and the occurrence of other post-operative morbidities (e.g., thrombophlebitis, peritonitis, pneumonia) should be object of further research.

This emphasizes the severe impact of SSI on equine wellbeing and owner’s finances. Hence, all measures should be taken to mitigate potential risk factors that could increase the risk for SSI development.

Due to its retrospective nature, this study faced some limitations that are connected to the design. One of them is the fact, that information on the patients was only available for the time they were present at the hospital. In some cases, the owners called after discharge to ask questions or give information on the horse’s recovery, which was then noted in the medical records. However, systematic long-term follow-up was not available to us. This might have underestimated the prevalence of SSI, since some horses might have developed one after discharge. Also, some potential risk factors like number of people in the operating room or cleanliness of the horse on admission and incision length could not be evaluated, since this information was not recorded routinely. Some other procedures, like the use of protective dressings or the performance of CBC post-operatively were not standardized and hence their interpretability is limited.

This study identified several factors influencing the incidence of SSI which can help identify and monitor horses at a higher risk of SSI development. Horses, that are heavier than 570kg, show increased rectal temperatures on admission, those operated between January and March and during summer months, as well as horses that develop post-operative reflux should be treated with special care and monitored closely for signs of SSI and surgery duration should be kept as short as possible.
We also identified a marked cost increase, an extension of hospital stay and a decreased chance to survive to discharge as a result of SSI.
Further, this study may also help with the design and execution of future randomized prospective studies, since several areas that need more investigation have been discovered, which could help identify controllable risk factors for SSI.

5. Summary

Surgical site infections continue to be a common complication in horses undergoing emergency laparotomy for signs of acute colic. This complication increases treatment costs, prolongs hospital stay and negatively influences horse survival.

A number of risk factors that influence the occurrence of SSI has been addressed in the past decades, however there are still discrepancies regarding various factors. This retrospective study sought to determine prevalence of SSI and identify risk factors specific to the population of horses presented to the equine clinic of the University of Veterinary Medicine Vienna. Medical records of horses older than 8 weeks that underwent emergency laparotomy for colic signs between August 2015 and August 2020 at our institution and survived at least 10 days were reviewed. Associations between various pre- intra- and post-operative factors and SSI occurrence were examined.

The prevalence of SSI in 209 horses that met inclusion criteria was 14.8% (31/209). The season in which the surgery was performed (winter and summer months), increased weight of the horse and increased rectal temperature on admission, longer duration of surgery and the development of post-operative reflux significantly increased the risk of SSI occurrence. SSI negatively influenced horse survival, prolonged duration of hospitalization and led to a significant increase in treatment costs.

Results of this study suggest, that surgical duration should be kept as short as possible and that horses to whom the abovementioned factors apply should be paid special attention to and monitored closely for signs of SSI development. Further, several areas that warrant more research were addressed, like some aspects of AMD use and the possible beneficial effect of the use of intradermal suture techniques, which could be controllable factors in the mitigation of SSI occurrence.

6. Zusammenfassung

Wundinfektionen nach Operationen sind weiterhin eine häufige Komplikation bei Pferden, die einer Notfall-Laparotomie wegen Anzeichen einer akuten Kolik unterzogen wurden. Diese Komplikation erhöht die Behandlungskosten, verlängert den Krankenhausaufenthalt und beeinflusst das Überleben des Pferdes negativ. Eine Reihe von Risikofaktoren, die das Auftreten von Wundinfektionen beeinflussen, wurden in den letzten Jahrzehnten an verschiedenen Kliniken untersucht, wobei Diskrepanzen in Bezug auf verschiedene Faktoren vorhanden sind.

Ziel dieser retrospektiven Studie war es, die Prävalenz von Wundinfektionen zu bestimmen und Risikofaktoren zu identifizieren, die für die Pferdeklinik der Veterinärmedizinischen Universität Wien gültig sind. Die Krankengeschichten von Pferden, die älter als 8 Wochen waren und zwischen August 2015 und August 2020 in unserer Einrichtung einer Not-Laparotomie wegen Koliksymptomen unterzogen wurden und mindestens 10 Tage überlebten, wurden analysiert. Verbindungen zwischen verschiedenen prä-, intra- und postoperativen Faktoren und dem Auftreten von SSI wurden untersucht.

Die Prävalenz von SSI bei 209 Pferden, die die Einschlusskriterien erfüllten, betrug 14,8% (31/209). Die Jahreszeit in der die Operation durchgeführt wurde, erhöhtes Gewicht des Pferdes und erhöhte rektale Körpertemperatur bei Aufnahme, die längere Dauer der Operation und die Entwicklung von postoperativem Reflux waren mit dem Auftreten von Wundinfektionen assoziiert. Die Entwicklung einer Wundinfektion verringerte die Überlebenswahrscheinlichkeit, verlängerte die Dauer des Krankenhausaufenthaltes und führte zu einem signifikanten Anstieg der Behandlungskosten.

Die Ergebnisse dieser Studie legen nahe, dass die Operationsdauer so kurz wie möglich gehalten werden sollte und dass Pferden, für die die oben genannten Faktoren gelten, besondere Aufmerksamkeit geschenkt und diese engmaschig auf Anzeichen einer Wundinfektion überwacht werden sollten. Darüber hinaus wurden einige Bereiche angesprochen, die weiterer Forschung bedürfen, wie einige Aspekte der antibiotischen Prophylaxe und der mögliche vorteilhafte Effekt der Verwendung

intradermaler Nahttechniken, welches beeinflussbare Faktoren bei der Verringerung des Auftretens von Wundinfektionen sein könnten.

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8. List of figures and tables