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Systemic antimicrobial use in the prevention of surgical site infections: sense or nonsense?

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PREFACE

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1. ABSTRACT

Surgical site infections (SSI) are commonly seen in veterinary medicine. A prevalence of 0.8 to 18% is described in literature. Many risk factors, such as patient's, environmental and treatment factors can contribute to the development of SSI. Two cases of SSI in dogs are discussed in this master thesis. Both dogs had a front limb amputation, the first one because of an open intra-articular comminuted fracture of the right elbow and the second because of a soft tissue sarcoma at the right elbow. In both cases, cefazolin was administered pre-operatively. Postoperative administration of antimicrobials was continued in the first case for 9 days in total. The other dog did not receive any antimicrobials postoperatively. Both developed a SSI. The surgery in the first case could be classified as a contaminated surgery. The risk of SSI in this type of surgery varies from 6 to 29% according to the literature. In the second case, a clean-contaminated surgery was performed. Here, the reported risk for SSI varies from 4.5 to 10%. Prophylactic antimicrobials can be administered to prevent a SSI. The prophylactic and therapeutic use of antimicrobials in the prevention of SSI has been a subject of controversy in the literature in human and in veterinary medicine. Guidelines on whether or not to use prophylactic antimicrobials, the choice of antimicrobial, the concentrations that need to be administered, timing of administration and duration of the prophylaxis are recorded for human and veterinary surgeries. However, most of these guidelines originate from retrospective studies. Both cases will be subject to a comparative evaluation concerning antimicrobial use between what is reported in literature and in clinical situations.

2. SAMENVATTING

Postoperatieve wondinfecties zijn een veel voorkomend probleem in de diergeneeskunde. Een prevalentie van 0,8 tot 18% wordt beschreven. Factoren met betrekking tot de patiënt, omgeving en chirurgie hebben een invloed op het ontstaan van postoperatieve wondinfecties.
In deze masterproef worden 2 casussen besproken van postoperatieve wondinfecties bij de hond. Beide honden ondergingen een voorpootamputatie. In de eerste casus omwille van een open intra-articulaire fractuur van de rechter elleboog en in de tweede casus omwille van een tumoraal proces. In beide gevallen werd er preoperatief cefazoline toegediend. De postoperatieve toediening van antibiotica werd in 1 casus verdergezet voor 9 dagen in totaal. Echter, beide honden ontwikkelden een postoperatieve wondinfectie. De chirurgie in de eerste casus kan worden beschouwd als een gecontamineerde chirurgie. De kans op infectie voor gecontamineerde chirurgische procedures varieert van 6 tot 29%. In de tweede casus betreft het een schoon-gecontamineerde chirurgie waarbij het infectierisico 4,5 tot 10% bedraagt. Het profylactisch gebruik van antibiotica kan de kans op een wondinfectie verminderen. In de humane en diergeneeskundige literatuur is er redelijk veel beschreven over het profylactisch en therapeutisch gebruik van antibiotica. Er zijn richtlijnen die aangeven of het gebruik van profylactische antibiotica al dan niet is aangewezen. Zo wordt het afgeraden om antibiotica profylactisch toe te dienen in schone chirurgieën. Verder zijn er richtlijnen omtrent de keuze van het antibioticum, het tijdstip van toediening en de duur. Het intraveneus toedienen van cepfalosporines van de 1^{e} generatie, 30 tot 60 minuten voor de start van de chirurgie voor maximaal 24 uur postoperatief wordt aangeraden. Indien cefazoline wordt toegediend in een chirurgie die langer dan 4 uur duurt, is een herhaalde toediening slechts nodig 4 uur na de 1^{e} toediening. Echter, de meeste van deze aanbevelingen zijn afkomstig uit retrospectieve studies.
Aan de hand van beide casusbesprekingen zal een vergelijkende studie worden gemaakt over het gebruik van antibiotica in de praktijk en in de literatuur.

3. INTRODUCTION

Surgical site infections (SSI) are infections that occur at the level of a surgical wound. They usually occur within 14 to 30 days of the procedure (or within 1 year if an implant is left in place) (Salkind and Rao, 2008; Yap et al., 2015). The incidence of SSI in veterinary medicine ranges from 0.8 to 18% (Nelson, 2011; Boothe and Boothe, 2015; Walker et al., 2016; Hayes et al., 2017).

Three types of SSI are described in human medicine by the Center for Disease Control and Prevention (Verwilghen and Singh, 2015): superficial incisional SSI, deep incisional SSI and organ or space SSI (Table 1). A SSI is classified based on physical examination, laboratory data and medical imaging if necessary (Nelson, 2011; Turk et al., 2015; Verwilghen and Singh, 2015; Yap et al., 2015).

Table 1: Types of surgical site infection (SSI) in human medicine adapted from the Center for Disease Control and Prevention (Verwilghen and Singh, 2015)

Type of SSI	Definition	Criteria (≥1 needs to be present)
Superficial incisional SSI	A surgical wound infection which occurs within 30 days after surgery and only involves skin and subcutaneous tissue	- Purulent discharge of the superficial incision - Organisms isolated from fluid or tissue from the superficial incision - Clinical signs such as pain, localized swelling or redness
Deep incisional SSI	A surgical wound infection which occurs within 30 days after surgery or within 1 year if an implant is in place. It involves fascia and muscles	- Purulent discharge from the deep incision, but not coming from an organ of a space component - Dehiscence of the deep incision - An abscess or other evidence of infection involving the deep tissues
Organ or space infections	A surgical wound infection that affects an organ or space, other than the incised layer of the body wall, that was opened or manipulated during surgery	- Purulent discharge from an organ or space - Organisms isolated from fluid or tissue in the organ or space - An abscess or other evidence of infection is involving the organ or space

A reduced quality of life, increased morbidity and increased mortality are some of the consequences for patients with a SSI (Kurz et al., 1996; Bratzler and Houck, 2005; Nelson, 2011; Nicoll et al., 2014; Hayes et al., 2017; Gonzalez et al., 2017). Surgical site infections also result in significantly higher costs for the owner, because of prolonged hospitalization, more intensive postoperative care and frequent control visits. Several bandage changes, surgical interventions, and as a result, multiple sedations and/or anesthesia may be required (Nicoll et al., 2014).

3.1 ANTIMICROBIALS AND SURGICAL SITE INFECTIONS

3.1.1 Definition of an antimicrobial[1]

The terms 'antibiotics' and 'antimicrobials' are often used interchangeably. However, an antibiotic is produced by a microorganism and an antimicrobial is synthetic or from a natural origin. Both can be bacteriostatic, causing a delay of bacterial growth and replication, or bactericidal, which means that they kill the target organism. Antimicrobials and antibiotics can inhibit cell wand synthesis, cell membrane function, protein synthesis, nuclear acid synthesis or other molecular processes. The minimal inhibitory concentration (MIC) is often used to compare the antimicrobial activity of antimicrobials. The MIC_{90} is the lowest concentration of the antimicrobial whereby 90% of the bacteria are inhibited.

In literature, antimicrobials are often subdivided in a broad-spectrum and narrow-spectrum activity. Broad-spectrum antimicrobials are active against gram-positive and gram-negative bacteria. Antimicrobials with a narrow spectrum are only active against some microorganisms. Antimicrobials can be used systemically or topically.

3.1.2 Use of prophylactic antimicrobials in surgery

Prophylactic antimicrobial therapy is the administration of antimicrobials prior to surgery to prevent a non-established infection, without the intention of eradicating all bacteria in the tissues (Howe and Boothe, 2006; Bratzler et al., 2013; Boothe and Boothe, 2015). The ultimate goal is to reduce the amount of bacteria during surgery below a critical level needed to induce infection (less than 10^5 bacteria per gram of tissue), such that the host defense mechanism can eradicate them and thus prevent postoperative infection (Eugster et al., 2004; Nazarali et al., 2014; Aiken et al., 2015; Boothe and Boothe, 2015). It is believed to be a highly effective method to decrease the risk of SSI (Classen et al., 1992; Wong-Beringer and Schrock, 1995; Zelenitsky et al., 2013).

Recommendations for prophylactic and therapeutic antimicrobial use in veterinary medicine are based on a surgical classification system (Table 2). This classification system, defined by the National Research Council in 1964, was originally developed for use in human medicine but is currently also used in veterinary medicine (Vasseur et al., 1988; Mishriki et al., 1990; Nelson, 2011). Based on this classification system, the chance of developing a SSI can be estimated (Barie, 2002; Eugster et al., 2004; Willard and Schulz, 2012; Verwilghen and Singh, 2015). Administration of prophylactic antimicrobials are not always indicated, as several prospective studies reported no significant decrease in SSI when prophylactic antimicrobials were administered in clean and clean-contaminated surgeries (Vasseur et al., 1985 ; Brown et al., 1997; Daude-Lagrave et al., 2001).

[1] https://amrls.cvm.msu.edu/pharmacology/antimicrobials/tools/module-pdf-files/pharmacology: consulted on 15/04/2018

Table 2: Surgical classification system

Classification of surgery	Description	Infection rate	Use of prophylactic antimicrobials?	References
Clean	A surgery in which no inflammation is encountered and in which the respiratory, alimentary, genital or infected urinary tract are not entered	1.6- 6%	Controversial, depends on the individual situation. When the consequences of a surgical wound infection would be severe or when surgical implants are inserted, prophylactic antimicrobials should be given as well when surgery is expected to take longer than 90 minutes	Vasseur et al., 1988; Brown et al., 1997; Bellah and Williams, 1999; Howe and Boothe, 2006; Pavletic, 2010; Hayes et al., 2014; Aiken et al., 2015; Solano et al, 2015; Turk et al., 2015; Andrade et al., 2016
Clean-contaminated	Surgeries in which the respiratory, alimentary, genital, or infected urinary tract are entered under controlled conditions without major contamination. Clean surgeries with a minor break in sterile surgery technique are also included	4.5- 10%	Controversial, depends on the individual situation. The duration of anesthesia and surgery, and the immune status of the patient are factors that can help to determine whether or not antimicrobials should be given prior to surgery	Vasseur et al., 1988; Brown et al., 1997; Bellah and Williams, 1999; Howe and Boothe, 2006; Pavletic, 2010; Hayes et al., 2014; Aiken et al., 2015; Solano et al, 2015; Turk et al., 2015; Andrade et al., 2016
Contaminated	Surgeries with major breaks in sterile technique or gross spillage from the alimentary tract and incisions in which acute non-purulent inflammation is encountered	6-29%	Indicated. The antimicrobial should be selected based on the expected type of bacteria in the surgical field or based on the results of the culture and the susceptibility tests.	Vasseur et al., 1988; Brown et al., 1997; Bellah and Williams, 1999; Howe and Boothe, 2006; Pavletic, 2010; Hayes et al., 2014; Aiken et al., 2015; Solano et al, 2015; Turk et al., 2015; Andrade et al., 2016
Dirty	Surgeries in which viscera are perforated or surgeries with fecal contamination	10-20%	Therapeutic use of antimicrobials is indicated. The selected drugs should have a broad-spectrum and should be changed once bacterial culture and susceptibility test results are known to narrow the spectrum	Vasseur et al., 1988; Brown et al., 1997; Bellah and Williams, 1999; Howe and Boothe, 2006; Pavletic, 2010; Hayes et al., 2014; Aiken et al., 2015; Solano et al, 2015; Turk et al., 2015; Andrade et al., 2016

Concerning appropriate use of prophylactic antimicrobials, 4 important factors are associated with the risk of SSI: using the correct concentration of the correctly chosen antimicrobial appropriate to the surgical procedure and the expected pathogens, proper timing and a correct duration postoperatively (Burke, 1961; Rosenberg et al., 2008; Salkind and Rao, 2008).

The chosen antimicrobial should be effective against at least 80% of the most likely pathogens (Willard and Schulz, 2012). In veterinary medicine, first generation cephalosporins such as cefazolin are preferred because of their relatively broad spectrum. They are active against the most common pathogens that cause SSI. When injected intravenously (IV), high concentrations of cefazolin are quickly obtained in soft tissues. Cefazolin is as effective as second, third or fourth generation cephalosporins and is cheaper compared with them. Lastly, it is the most widely studied antimicrobial so much is known about its efficacy and safety; side effects are not commonly reported (Whittem et al., 1999; Boothe and Boothe, 2015; Gonzalez et al., 2017). Table 3 lists common surgeries in small animals, their associated pathogen(s) and recommended antimicrobial(s). Results of susceptibility tests, based on historical data of other patients in a similar situation and individual variation between patients always have to be kept in mind when using this table to determine the ideal antimicrobial.

Table 3: Summary of recommendations of prophylactic antimicrobial use

Surgical site	**Pathogen(s)**	**Recommended antimicrobial(s)**
Skin	*S. aureus, S. pseudintermedius*	Cefazolin
Head and neck	*Staphylococcus* spp, *Streptococcus* spp, anaerobes	Cefazolin or clindamycin
Closed fractures and spinal decompression	*Staphylococcus* spp	Cefazolin
Open fractures	*Staphylococcus* spp, *Streptococcus* spp, anaerobes	Cefazolin
Cardiothoracic	*Staphylococcus* spp	Cefazolin
Hepatobiliary	*Clostridia* sp, gram-negative bacilli, anaerobes	Cefoxitin
Gastric and small intestines	Gram-positive cocci, enteric gram-negative bacilli	Cefazolin
Large intestines	*Enterococci* spp, gram-negative bacilli, anaerobes	Cefoxitin
Ruptured bowel	Enteric gram-negative bacilli, *Enterococci* spp, anaerobes, gram-positive cocci	Ampicillin combined with fluoroquinolone
Abdominal surgery	*Staphylococcus* spp	Cefazolin
Urogenital	*Escherichia coli, Streptococcus* spp, anaerobes	Cefazolin or ampicillin

Adapted from Willard and Schulz, 2012 and Verwilghen and Singh, 2015.

Re-administration of antimicrobials depends on the type of bacteria, dosage, half-life and pharmacokinetics of the antimicrobial (Howe and Boothe, 2006). Intraoperatively, it was recommended to re-administer time-dependent antimicrobials such as cephalosporins and penicillins after 2 half-lives of the drug to obtain adequate tissue concentrations (Polk and Christmas, 2000; Golembiewski, 2009; Nazarali et al., 2014; Verwilghen and Singh, 2015; Yap et al., 2015; WHO

Guidelines, 2016). A recent prospective study in 12 dogs, however, reported that administering a single IV injection of cefazolin (22 mg/kg) preoperatively provides protection during 4 hours or less against the most common skin bacteria of dogs (Gonzalez et al., 2017).
Several studies investigated the ideal timing of administration. The first dose of prophylactic antimicrobials should be administered 30 to 60 minutes prior to the start of surgery (Dellinger, 2007; Verwilghen and Singh, 2015; Yap et al., 2015; Gonzalez et al., 2017; Weber et al., 2017). Antimicrobial concentrations should also exceed the MICs for the expected pathogens in the surgical field (Bratzler and Houck, 2005; Tourmousoglou et al., 2008; Aiken et al., 2015).
Postoperatively, there is no need to continue antimicrobial therapy for longer than 24 hours (Aiken et al., 2015; Verwilghen and Singh, 2015, Yap et al., 2015). In cardiac surgery, however, prophylactic antimicrobial can be continued up to 48 hours postoperatively due to effects of cardiopulmonary bypass on immune function and pharmacokinetics (Bratlzer et al., 2003; Salkind and Rao, 2011; Goede et al., 2013).

Inappropriate use of antimicrobials, such as choosing the wrong type, administration of prophylactic antimicrobials in clean or clean-contaminated procedures or postoperative administration longer than 24 hours, not only can cause SSI but can also result in increased costs, increased incidence of complications, antimicrobial resistance and an altered bacterial flora leading to changes in colonization (Prospero et al., 2011; Bratzler et al., 2013; Aiken et al., 2015; Boothe and Boothe, 2015; Pratesi et al., 2015).

Besides the prophylactic use of antimicrobials, antimicrobials can also be used therapeutically. Therapeutic use of antimicrobials is indicated in patients undergoing contaminated or dirty surgical procedures and when an infection is already present at the surgical site or in a body cavity (Waddell and Rotstein, 1994; Barie, 2002; Howe and Boothe 2006; Salkind and Rao, 2011; Nelson, 2011; Bratzler et al., 2013). Before starting antimicrobial therapy, representative samples for cytology, Gram staining, culture and susceptibility testing should be obtained (Willard and Schulz, 2012). Broad-spectrum empiric antimicrobial therapy, appropriate for the most likely microorganisms, can be administered prior to surgery (Howe and Boothe, 2006; Nelson, 2011; Willard and Schulz, 2012). This broad-spectrum therapy should be continued in the postoperative period. Once culture results and the results of the susceptibility test are available, antimicrobial therapy should be altered to narrow the spectrum (Howe and Boothe, 2006; Nelson, 2011). When several antimicrobials are effective to treat infection, it is important to select the one that reaches the target tissue and has the least toxic effects on the patient's normal microbiota. Furthermore, it may not negatively affect the host immune system and it should be easy to administer. Choosing broad-spectrum antimicrobials when bacteria are susceptible to narrow-spectrum antimicrobials, can result in antimicrobial resistance and development of multi-resistant bacteria (Howe and Boothe, 2006; Willard and Schulz, 2012). After clinical improvement postoperatively (normalization of the amount of leukocytes and resolution of fever), therapy should be continued for at least another 2 to 3 days (Howe and Boothe, 2006; Willard and Schulz, 2012). In absence of clinical improvement, a re-evaluation of the patient is advised as well as a change in antimicrobial therapy (Willard and Schulz, 2012).
Reasons for not responding to antimicrobial treatment might be diverse: development of a secondary bacterial complication, persistent inflammation or a wrong diagnosis such as non-infectious disease (Garrod, 1972; Bassetti et al., 2018). Choosing an antimicrobial when resistance

towards this antimicrobial is reported, should be avoided (Bassetti et al., 2018). Tetracyclines are known to inhibit the effect of penicillin, and as a consequence this antagonistic combination should not be administered (Garrod, 1972). Lastly, choosing the wrong route or timing of administration can also cause antimicrobial therapy failure (Garrod, 1972).

3.1.3 Antimicrobial resistance

Antimicrobial resistance is the ability of bacteria to survive in the presence of an antimicrobial which would normally inhibit the growth or kill the bacteria. Antimicrobial resistance can be subdivided into 2 groups; intrinsic and acquired resistance. Intrinsic resistance, also known as natural resistance, is caused by genes that are naturally found in the DNA of bacteria. The organism will lack target sites of the antimicrobial or the organism's transport mechanisms that allow the antimicrobial to be effective, might be absent or ineffective. Acquired resistance is caused by changes in the bacterial genome through mutation, transfer of genetic material between organisms or both. This results in a change in function and structure of the bacteria (Umber and Bender, 2009; Boothe and Boothe, 2015).

Several mechanisms can lead to acquired antimicrobial resistance of bacteria (Umber and Bender, 2009; Boothe and Boothe, 2015). Firstly, overuse of antimicrobials, the use of broad-spectrum antimicrobials or the use of antimicrobials close to or below the MIC, leading to selective pressure of bacteria and development of resistant microorganisms. Secondly, physical contact, which will allow the transfer of resistance between organisms or when the environment allows bacteria to grow, such as biofilms, will increase the emergence of resistant bacteria (Umber and Bender, 2009). Thirdly, the size of the inoculum is important in the development of resistant microorganisms; an increased risk of antimicrobial resistance is associated with a larger inoculum size. When more bacteria are present, more drug molecules need to be present to inhibit or kill the bacteria. Besides, the more bacteria present, the more spontaneous mutations will occur in a resistant colony forming unit (Boothe and Boothe, 2015). Lastly, the first dose of an antimicrobial therapy is important to decrease the risk of antimicrobial resistance. The higher the first dose, the less likely suboptimal antimicrobial concentrations will be present in the tissues and serum. For this reason, a short duration with high drug concentrations will lower the emergence of antimicrobial resistance compared to long term antimicrobial therapy (Boothe and Boothe, 2015).

A prospective study in dogs, observed a very low resistance of all bacterial species, except for *Pseudomonas aeruginosa,* to cephalosporins and amoxicillin clavulanic acid . Almost no resistance of all species was detected to trimethoprim-sulfamethoxazole. *S. pseudintermedius* was reported to be resistant to penicillin, fusidic acid and macrolides. High levels of antimicrobial resistance of *E.coli* were detected for ampicillin, sulfonamides, trimethoprim, tetracyclines and streptomycin (Pedersen et al., 2007).

S. pseudintermedius is the most commonly found bacteria in canine SSI (Verwilghen and Sing, 2015). The emergence of methicillin resistant *S. pseudintermedius* (MRSP) is reported with an increased frequency (Walker et al., 2016). Different rates of colonization with MRSP are reported going from 1.5 to 30% in dogs (Weese and van Duijkeren, 2010). One prospective study in 549 dogs undergoing a tibial plateau level osteotomy reported that 37 dogs developed SSI and MRSP was isolated in 11 (34%) of them (Nazarali et al., 2015).

These bacteria are not only resistant to β–lactam antimicrobials but also to other antimicrobial classes. Infections with MRSP occur more commonly in dogs compared with cats (Van Duijkeren et al., 2011). In a study from Kjellman et al. (2015), 2.6% of the healthy dogs were carrier of MRSP.
Another challenge in the treatment of *S. pseudintermedius* is their ability of producing biofilms (Verwilghen and Singh, 2015; Walker et al., 2016). A biofilm is a sessile layer or community of bacteria in which bacteria adhere to each other and/or to a surface. Once attached, the bacteria will grow and produce an extracellular matrix which will protect them from antimicrobials (Boothe and Boothe, 2015; Verwilghen and Singh, 2015; Walker et al., 2016). A prospective study reported that the MIC of biofilm-producing *S. pseudintermedius* was significantly higher for all tested antimicrobials compared with bacteria that do not produce a biofilm. No differences were reported between the MIC for MRSP and methicillin-susceptible *S. pseudintermedius* (Walker et al., 2016).

3.2 RISK FACTORS OF SURGICAL SITE INFECTIONS

The risk of SSI correlates directly with pre-operative factors, perioperative factors and postoperative factors (Barie, 2002; Cheadle, 2006; Nelson, 2011). Reported risk factors in veterinary medicine are listed in Table 4.

Table 4 : Risk factors of surgical site infections

Risk factors	Clarification	References
1. Pre-operative factors		
Age	Dogs < 1 year and > 10 years are at higher risk	Nicholson et al., 2002; Howe and Boothe, 2006; Nelson 2011; Willard and Schulz, 2012
Gender	Male intact dogs have a higher risk	Weichmann et al., 1996; Nicholson et al., 2002; Solano et al., 2015; Verwilghen and Singh, 2015; Yap et al., 2015
Breed	Labrador Retrievers have a lower risk	Solano et al., 2015; Yap et al., 2015
Nutritional status	Malnutrition as well as obesitas increases the risk of SSI	Nicholson et al., 2002; Eugster et al., 2004; Howe and Boothe, 2006; Nelson, 2011; Hayes et al., 2014; Solano et al., 2015; Yap et al., 2015
Endocrinopathy	Animals suffering from diabetes mellitus, hyperadrenocorticism and hyperthyroidism have 8.2 times increased risk	Nicholson et al., 2002; Howe and Boothe, 2006; Nelson 2011; Hayes et al., 2014; Verwilghen and Singh, 2015
Intake of immunosuppressive drugs	These drugs inhibit the normal function of the host defense and increase the risk of SSI	Howe and Boothe, 2006
Presence of an infection at distant site	The remote infection causes immune suppression and increases the risk of a SSI	Brown et al., 1997
Hypoalbuminemia	Impairs the immune status	Turk et al., 2015
Aortic stenosis	Dogs with moderate to severe subaortic stenosis are at higher risk of developing a SSI	Kienle et al., 1994
ASA physical status	The higher the ASA score, the higher the risk of developing a SSI	Eugster et al., 2004; Nelson, 2011
Inappropriate use of prophylactic antimicrobials	Administration of prophylactic antimicrobials in clean procedures, choosing the wrong type, wrong dosage or given at the wrong time increase the risk of SSI	Nicholson et al., 2002; Eugster et al., 2004; Howe and Boothe , 2006; Verwilghen and Singh, 2015
2. Perioperative factors		
Blood pressure	Perioperative hypotension increases the risk of SSI.	Turk et al., 2015
Hypoxia	Oxygen is important during wound healing; systemic hypoxia causes a decreased oxygenation of the surgical wound leading to a higher risk of SSI	Nelson, 2011; Hayes et al., 2014
Hypothermia	Patients with a mild to severe perioperative hypothermia are 3 times more likely to develop a SSI	Beal et al., 2000; Nelson, 2011; Hayes et al., 2014
Disinfection/sterilization of the medical device	When medical devices are not properly sterilized or disinfected, the risk of SSI increases	Nelson, 2011
Time and method of hair removal	If hair removal occurs before induction and when shavers are used instead of clippers, the risk of SSI is increased	Brown et al., 1997; Nelson, 2011
Skin antisepsis	Inadequate skin antisepsis of the surgical team and/or patient increases the risk of SSI	Howe and Boothe, 2006; Nelson, 2011; Hayes et al., 2014; Hardy et al., 2017
Gloves	Wearing double gloves or changing gloves during long surgeries decrease the risk of SSI	Eugster et al., 2004; Nelson, 2011; Hayes et al., 2014; Verwilghen and Singh, 2015; Andrade et al., 2016; Meakin et al., 2016; Hayes et al., 2017
Number of persons in the operation room	For each person present in the operating room, the risk of developing a SSI increases with a factor 1.3	Eugster et al., 2004; Turk et al., 2015; Verwilghen and Singh, 2015; Andrade et al., 2016
Surgeon	Surgeries performed by less experienced surgeons have an increased risk of SSI	Vasseur et al., 1988; Nelson, 2011; Mayhew et al., 2012

Table 4 : Risk factors for surgical site infections (continued)

Classification of surgery	Dirty surgeries are at higher risk compared with clean surgeries	Brown et al., 1997; Bellah and Williams, 1999; Howe and Boothe, 2006; Pavletic, 2010; Hayes et al., 2014; Solano et al, 2015; Turk et al., 2015
Surgical technique	Strict application of Halsted's principles, avoiding the use of surgical implants and using less invasive surgical techniques decreases the risk of SSI	Nicholson et al., 2000; Nelson, 2011; Mayhew et al., 2012; Turk et al., 2015; Meakin et al., 2016
Duration of anesthesia	Volatile anesthetics cause immunosuppression. For each additional hour of anesthesia, the risk of SSI increases with 30%	Beal et al., 2000; Nicholson et al., 2002; Eugster et al., 2004; Howe and Boothe, 2006; Pratesi et al., 2015; Solano et al., 2015; Turk et al., 2015; Verwilghen and Singh, 2015; Yap et al., 2015
Duration of surgery	During longer surgeries, there is an increased chance of bacterial colonization and tissue dehydration, which decreases the host resistance to wound infection. For each additional minute of surgical intervention, the SSI risk increases with a factor 1.01	Brown et al., 1997; Vasseur et al., 1988; Nicholson et al., 2002; Eugster et al., 2004; Turk et al., 2015; Verwilghen and Singh, 2015; Yap et al., 2015
Use of propofol	Due to its composition, propofol can support the bacterial growth. Animals induced with propofol, have 3.8 times increased risk of SSI	Heldmann et al., 1999; Nicholson et al., 2002; Howe and Boothe, 2006; Nelson, 2011; Franci et al., 2015; Solano et al., 2015; Verwilghen and Singh, 2015; Yap et al., 2015
3. Postoperative factors		
Duration of pre- and postoperative hospitalization	The longer the pre- and postoperative hospitalization period, the higher the SSI risk	Nicholson et al., 2000; Eugster et al., 2004
Bandage	Placing a bandage decreases seroma formation and thus decrease the risk of SSI	Pitt and Stanley, 2014; Howe, 2015
Drains	Drains reduce the number of bacteria necessary to cause wound infection and are ideal routes for bacteria to migrate into the surgical wound and thus increase the SSI risk. Active drains are preferred over passive drain since the retrograde contamination is lower	Eugster et al., 2004; Nelson, 2011 ; Hayes et al., 2014
Inappropriate use of antimicrobials	The administration of prophylactic antimicrobials postoperatively longer than 24 hours increases the risk of SSI	Nicholson et al., 2002; Eugster et al., 2004; Howe and Boothe , 2006; Verwilghen and Singh, 2015

ASA = American Society of Anesthesiologists

4. CASES

4.1 CASE 1

A 14-year-old male intact Fox terrier was presented on emergency at the Small Animal Department (Faculty of Veterinary Medicine, Ghent University) within 6 hours after being hit by car. At initial presentation to the referring veterinarian, immediately after the incident, general physical examination was within normal limits. A wound was noticed in the right axillary region and the dog was very painful. The dog was sedated intramuscularly (IM) with a combination of medetomidine and ketamine and radiographs were taken. A thoracic radiograph reveled no abnormalities. However, a complicated open intra-articular fracture of the right elbow was present. The wound was flushed with saline and a protective bandage was applied. An IV catheter was placed and infusion therapy was started; an IV injection of amoxicillin, meloxicam and methadone was administered, prior to referral.

Approximately 1.5 hours later, the dog was presented at the Small Animal Clinic. The body weight was 10 kg and body condition score (BCS) was 5/9. On physical examination, the dog was mildly sedated, hypothermic (35.8°C), and mild increased lung sounds were present on auscultation. The oral mucosa was sticky, capillary refill time was 2 seconds and heart rate was 100 beats per minute. In the right axillary region, a wound was present. Hair was bloody and entangled in the wound. Damaged muscles were visualized in the depth, no active bleeding was present. Pain could be evoked upon touching or manipulating the right front leg. A temporary bandage with a lubricating jelly (KY gel, Johnson & Johnson), non-adhesive compresses (Melolin®, Smith & Nephew), a synthetic padding (Orthoband®, Millpledge) and a cohesive bandage (Vet Wrapz®, Millpledge) was applied to prevent further contamination of the wound during further work-up and stabilization.

Routine blood analysis was normal besides significantly increased alanine aminotransaminase (ALT, 763 U/L; upper reference limit 125 U/L) and hyperglycemia (16.2 mmol/L; upper reference limit 7.95 mmol/L).

Fluid therapy was started to correct for 5% dehydration (Ringer lactate, Hartmann®, Baxter), as well as methadone (0.1 mg/kg every 4 hours) and cefazolin (Cefazoline®, Sandoz; 20 mg/kg IV 3 times daily). Additional radiographs of the thorax and right elbow were obtained. Left-right lateral and ventrodorsal thoracic radiographs revealed mild atelectasis on the left side and severe subcutaneous emphysema in the right axillary region (Fig 1). A mediolateral radiograph of the elbow revealed an open intra-articular comminuted fracture of the right elbow with multiple ulnar and radial fragments of different size and shape (until +/- 8mm). A fracture of the humeral trochlea was suspected as well (Fig 2).

Because of the complexity of the fracture, fracture reduction and osteosynthesis was deemed impossible. Therefore, amputation of the right front leg was advised.

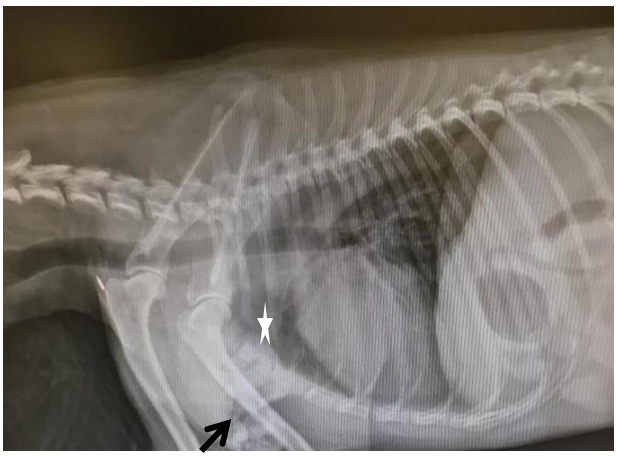

Figure 1. Left-right lateral thoracic radiograph of a 14-year-old male intact Fox terrier. The left cranial lung lobe is mildly increased in opacity (asterisk). At the level of the right humerus, gas opacities within the subcutaneous tissues are present (arrow).

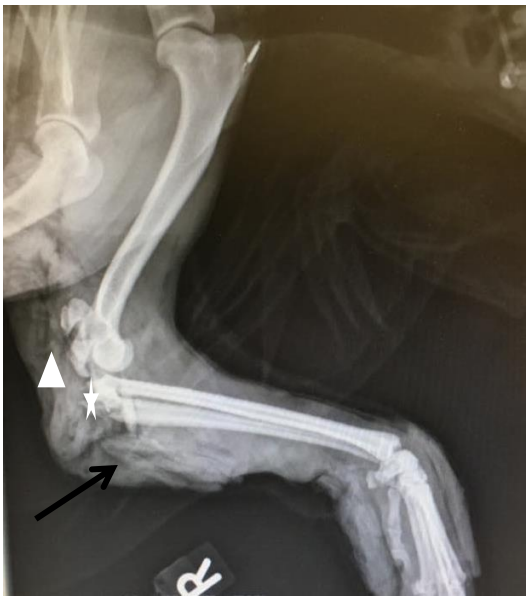

Figure 2. Mediolateral radiograph of the right front leg. Presence of severe soft tissue swelling around the elbow joint with gas opacities within the subcutaneous tissue on the entire right front (arrow). An open comminuted intra-articular fracture of the proximal radius and ulna is present (asterisk). A fracture of the humeral trochlea is suspected (arrow head).

Ten hours after presentation, the dog was anesthetized for leg amputation. The dog was premedicated with a combination of dexmedetomidine (Dexdomitor®, Orion Corp; 5 µg/kg IV) and methadone (Comfortan®, Dechra; 0.2 mg/kg IV), induced with propofol (Propovet®, Zoetis; 6 mg/kg IV to effect) and anesthesia was maintained with isoflurane vaporized in oxygen. Cefazolin (20 mg/kg IV) was given 2 hours prior to surgery. During anesthesia, Ringer lactate (5 mL/kg/h IV) was administered as well as a constant rate infusion (CRI) of fentanyl (Fentanyl®, Janssen Cilag; 5 µg/kg/h IV) for intraoperative analgesia.

The patient was placed in lateral recumbency with the affected limb uppermost covered in sterile wrapz distant to the surgical site. After aseptic preparation of the surgical field, an incision was made from the dorsal border of the scapula over the scapular spine, to the proximal third of the humerus. The incision was continued laterally and medially in a circular pattern from the shoulder towards the region of the triceps. Hemostasis was obtained with bipolar electrocoagulation. The rhomboideus, serratus ventralis and latissimus dorsi muscles were carefully detached from the dorsal border of the scapula. The caudal border of the scapula was retracted laterally to expose the axillary artery and vein and the brachial plexus. All structures were identified and the axillary artery and vein were isolated and were respectively double and single ligated with 3/0 polyglecaprone (Monocryl®, Johnson & Johnson Medical) and transected. Bupivacaine hydrochloride (Marcaine® 0,5%, Aspen Pharma; 0.1 mg/kg) was used to infiltrate the brachial plexus and individual nerves were sharply transected. Subsequently, the brachiocephalic and pectoral muscles were transected at their insertions on the proximal humerus and the limb was removed. The deep and superficial muscular layers were closed separately with continuous suture pattern with 2/0 polydioxanone (PDSII®, Johnson & Johnson Medical). An active drain (Multipurpose drain 10 French, Mila) connected to a

200 mL redon (Medicoplast) was placed and attached with a Chinese fingertrap suture in 2/0 nylon (Ethilon®, Johnson & Johnson Medical). The subcutaneous tissue was apposed and anchored to the underlying muscle fascia with a continuous suture pattern with 3/0 polyglecaprone. Skin was closed with an intradermal suture pattern using polyglecaprone 3/0. A supportive bandage was placed around the thorax to prevent edema and seroma formation. This bandage was made with a synthetic padding and cohesive bandage material. Total duration of anesthesia and surgery was 2 hours and 25 minutes and 1 hour and 45 minutes, respectively.
Postoperatively, the dog was hospitalized for 2 days. Analgesia consisted initially of IV methadone (0.2 mg/kg every 4 hours) and was changed the next day to tramadol hydrochloride (Tramadol®, Eurogenerics; 3 mg/kg orally 3 times daily) combined with meloxicam (Metacam®, Boehringer Ingelheim; 0.1 mg/mL orally once daily). Cefazolin (20 mg/kg IV) was continued until the day after surgery when it was changed to oral administration of cephalexin (Rilexine® 300mg, Virbac; 15 mg/kg twice daily). One day after surgery, the dog was able to walk with limited support. The drain was minimally productive and was removed 2 days after surgery. The incision remained dry and clean. At home, antimicrobial therapy was continued for another 7 days and analgesia for another 5 days. Automutilation was prevented with the use of a protective shirt (Medical Pet Shirt, Millpledge). It was advised to walk on a leash for short distances only during the first two weeks, and no swimming, bite or pulling games were allowed.

Five days after surgery, the owners noticed discharge from the wound and wound dehiscence occurred (Fig 3). The dog developed total anorexia and was presented to the Small Animal Clinic. The last administration of cephalexin was the day before presentation. On physical examination, no abnormalities were detected except of wound dehiscence and necrotic skin edges.

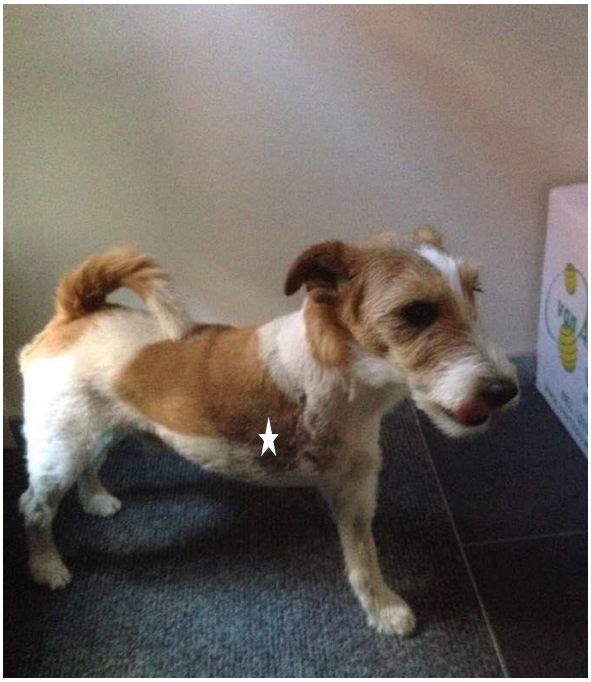

Figure 3. Mild wound dehiscence (asterisk). Images taken by the owners, 5 days after surgery

The dog was sedated with a combination of dexmedetomidine (5 µg/kg IV) and methadone (0.2 mg/kg IV). Once the dog was sedated, midazolam IV (Dormicum®, Roche; 0.16 mg/kg IV) and carprofen (Rimadyl 5%®, Zoetis; 4 mg/kg IV) were added. The wound was debrided and flushed with saline (Vetivex®, Dechra) and a swab was taken for bacteriology. A silicone sheet impregnated with honey (Tulle, L-Mesitran®), covered by non-adhesive compresses, was applied and the wound was covered with a soft padded bandage, similar to the one postoperatively. After wound care, atipamezole (Antisedan®, Orion; 2.5 µg/kg IM) was given to speed recovery. Total duration of sedation was 50 minutes, total duration of wound care was 30 minutes.

The patient was hospitalized and carprofen (2 mg/kg orally twice daily) was continued for 5 days. After respectively 2 days and 3 days of hospitalization, bandage changes were performed. On both occasions, a similar bandage as the first time was placed. After 3 days of hospitalization, the laboratory results of the bacteriology and the susceptibility test were known. Multiple colonies of *Escherichia coli* (*E. coli*) were cultured. The susceptibility test showed that, at least *in vitro*, *E. coli* was only resistant to non-potentiated ampicillin (Table 5). Antimicrobial therapy using amoxicillin clavulanic acid (Kesium®, Ceva 12.5 mg/kg orally twice daily) was started.

Table 5: Results of the susceptibility test

Antimicrobial	**Susceptibility**
Ampicillin	R
Amoxicillin clavulanic acid	S
Cephalexin	S
Ceftiofur	S
Gentamycin	S
Tetracycline	S
Trimethoprim-sulfamethoxazole	S
Enrofloxacin	S
Marbofloxacin	S
Doxycycline	S
Minocycline	S

R= resistant; S= susceptible

The fourth day of hospitalization, the bandage was changed again. Wound edges were cleaned and a new bandage was placed using a hydrocellular polyurethane bandage (Allevyn®, Smith & Nephew), a synthetic padding and cohesive bandage material.

Primary wound closure was performed 6 days after first debridement of the wound. The dog was sedated with a combination of dexmedetomidine (2.5 µg/kg IV) and methadone (0.2 mg/kg IV). Induction was obtained with propofol (6 mg/kg IV to effect) and anesthesia was maintained with isoflurane vaporized in oxygen.
After aseptic preparation of the surgical field, resection of the wound edges and curettage of the granulation tissue was performed. The subcutaneous tissue was closed using continuous suture pattern with 3/0 polyglecaprone. The skin was closed with single interrupted sutures with 3/0 nylon. Total duration of anesthesia was 55 minutes; total duration of surgery was 30 minutes.
One day after surgery, the dog was discharged with the same activity guidelines as previously. The stitches could be removed after 14 days by the referring veterinarian (Fig 4). Antimicrobial therapy was continued for another 10 days and carprofen for another 5 days.

The owners were contacted approximately 2 years after the accident. The dog was in a perfect health and no problems were noticed after the second surgery (Fig 5).

Figure 4. State of the wound after the last surgery. Single sutures with 3/0 nylon are visible.

Figure 5. Current state of the dog, 2 years after the accident. The wound healed completely.

4.2 CASE 2

A 13-year-old male castrated crossbreed was presented at the Small Animal Clinic with an eroded mass at the level of the right elbow. The mass was already present for 3 or 4 years. Three years ago, the referring veterinarian did an ultrasound; a cavernous mass was visualized and a tentative diagnosis of a synovial cell carcinoma was made. Carprofen was administered. Nine days prior to presentation at the Small Animal Department, fine needle aspirate of the mass were performed, results were unknown. Because of continued hemorrhagic oozing from the puncture site, a protective bandage was placed. This bandage was changed daily by the owners. One day prior to presentation, the mass opened at a different site. At first, a large amount of bloody discharge came out and later, it started to drip. The dog was not lame, but its endurance was decreased. Besides carprofen, the dog was on tramadol hydrochloride since 4 weeks.
Upon arrival at the Small Animal Clinic, the dog was alert but calm. The body weight was 22.2 kg and BCS was 6/9. On general physical examination, no abnormalities were detected, besides an enlarged prescapular lymph node at the right side. A large soft tissue mass with central necrosis was present caudal of the right elbow (Fig 6) and several nodules were palpated on the right thoracic wall.

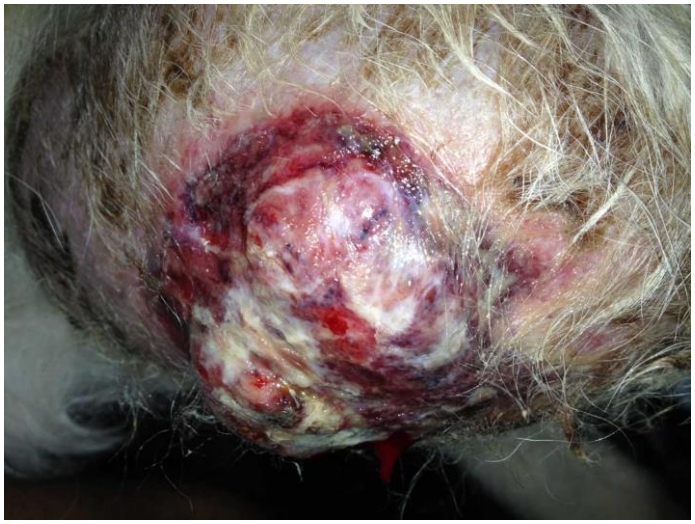

Figure 6. Mass at the right elbow of a 13-year-old male castrated crossbreed at first presentation at the Small Animal Clinic.

General blood examination revealed a mild leukocytosis (18.53 x 10^9/L; upper reference limit 5.05 x 10^9/L), monocytosis (1.78 x 10^9/L; upper reference limit 1.12 x 10^9/L), neutrophilia (13.82 x 10^9/L; upper reference limit 11.64 x 10^9/L) and basophilia (0.11 x 10^9/L; upper reference limit 0.10 x 10^9/L), as well as a very mild hypoalbuminemia 21 g/L; lower reference limit 22 g/L). Fine needle aspirates of the mass were repeated and were not diagnostic because of blood contamination; the masses on the thorax were all lipomas.

Right-left lateral, left-right lateral and ventrodorsal radiographs of the thorax revealed no signs of metastasis. A large semicircular area of extra-thoracic fat opacity was seen on the right side of the thorax and a smaller one ventral of the thorax; both represented subcutaneous lipomas. A mediolateral radiograph of the right elbow revealed a large soft tissue swelling caudal to the elbow,

from the distal part of the humerus to the mid diaphyseal region of the radius/ulna. Small mineralizations were present within the mass, no signs of bone involvement were present (Fig 7).

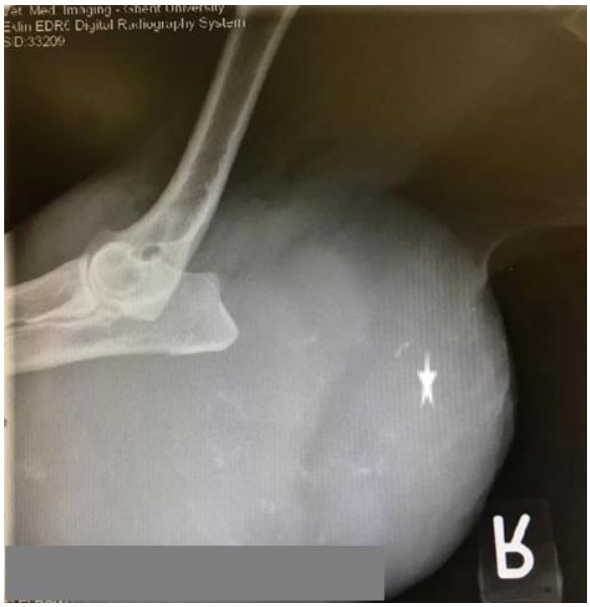

Figure 7. Right-left lateral projection of the right elbow. A large soft tissue swelling is seen caudal to the elbow from the distal part of the humerus to the mid diaphyseal region of the radius. Small mineralizations are present within the mass (asterisk).

Analgesia was continued as previously until amputation of the right front leg.

Premedication consisted of methadone (0.2 mg/kg IV). Midazolam (0.2mg/kg IV) was administered just prior to induction with propofol (6 mg/kg IV to effect). Isoflurane vaporized in oxygen was used to maintain anesthesia. Thirty minutes prior to surgery, cefazolin (20 mg/kg IV) was administered as well as 2 hours after the previous administration. During surgery, Ringer lactate was given at 5 mL/kg/h as well as a CRI of fentanyl (5 µg/kg/h) was administered to obtain perioperative analgesia.

Amputation of the right front leg and type of postoperative bandage was performed as described before. In contrary to the first case, no drain was placed before skin closure. Total duration of the anesthesia was 3 hours and 25 minutes, total duration of surgery was 2 hours and 40 minutes. The entire right foreleg, the axillary and prescapular lymph nodes were sent for histology. The mass was a soft tissue sarcoma grade 2; examination of the lymph nodes showed no evidence of metastasis.
Postoperatively, the dog was hospitalized for 2 days. Analgesia was obtained with methadone (0.2 mg/kg IV every 4 hours) and carprofen (2 mg/kg orally, twice daily). Because the dog was painful, a bolus of ketamine (Nimatek®, Dechra; 0.5 mg/kg IV) was administered twice after surgery. One day postoperatively, methadone was increased to 0.3 mg/kg every 4 hours.

Although the dog did not want to walk, he was discharged 2 days after surgery. Small dehiscence of the surgical incision as well as inflammation of the skin around the wound were present. An ointment consisting of chlorhexidine acetate and vitamin A (Neocutigenol®, Takeda) was applied to the wound and a new bandage was placed using a synthetic padding and cohesive bandage material.

Postoperative instructions were similar as those described above. Carprofen (2 mg/kg orally twice daily) was continued for another 7 days and tramadol hydrochloride (3 mg/kg orally 3 times daily) was administered for 5 days.

Two days later (4 days postoperatively), the bandage was soaked by fluid coming from the wound (Fig 8 and Fig 9). The owners went to the referring veterinarian who diagnosed wound dehiscence and took a swab for bacteriology. A new bandage with honey was placed and therapy with amoxicillin-clavulanic acid and enrofloxacin was installed. One day later, the dog was presented to the Small Animal Clinic. On presentation, the dog was calm and still reluctant to walk. No abnormalities were detected on general physical examination.

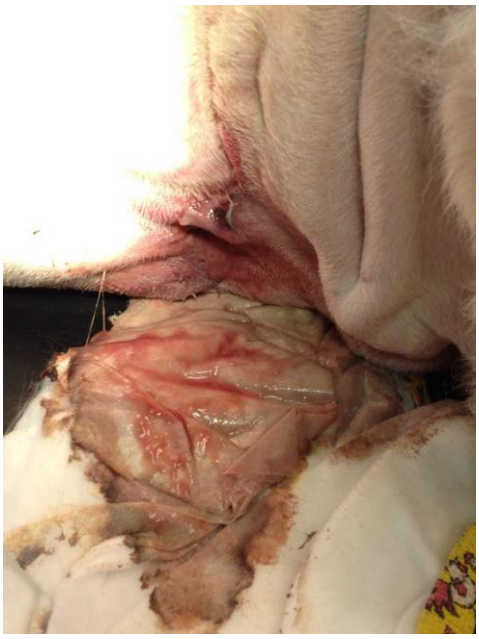

Figure 8. Serohemorrhagic to purulent discharge from the incision 4 days postoperatively

Figure 9. Wound dehiscence 4 days postoperatively

Bacteriology showed the presence of numerous colonies of *Enterococcus faecalis* and *Enterobacter cloacae* complex. The susceptibility results for both bacteria are listed in Table 6.

Table 6: Results of the susceptibility test

Antimicrobial	**Susceptibility results for *Enterococcus faecalis***	**Susceptibility results for *Enterobacter cloacae* complex**
Ampicillin	S	/
Cephalexin	/	R
Ceftiofur	/	R
Cefpodoxime	/	R
Gentamycin	/	R
Tetracycline	/	R
Trimethoprim-sulfamethoxazole	/	S
Enrofloxacin	R	R
Marbofloxacin	R	R

R= resistant; S= susceptible

The dog was anesthetized for wound debridement. The dog was sedated with methadone (0.2 mg/kg IV). Anesthesia was induced with propofol (6 mg/kg IV to effect) and maintained with isoflurane vaporized in oxygen. During anesthesia, Ringer lactate solution (5 mL/kg/h) was administered. Amoxicillin-clavulanic acid (Augmentin®, GlaxoSmithKline; 20 mg/kg IV) was administered 7 hours before wound care.
The wound was debrided and rinsed with saline. A silicone sheet impregnated with honey was placed into the wound and the wound was provisionally closed with a cruciate suture pattern in 3/0 nylon. A bandage with non-adhesive compresses, a synthetic padding and cohesive bandage material was applied. Total duration of the anesthesia was 50 minutes, total duration of surgery was 30 minutes. Antibiotic therapy was continued the day of surgery and the day after with amoxicillin clavulanic acid (8.75 mg/kg SC, Synulox®, Zoetis) and analgesia consisted of methadone (0.2 mg/kg IV every 4 hours) and gabapentine (Neurontin® 100mg, Pfizer; 10 mg/kg 3 orally times daily).

Two days later the dog was anesthetized using the same protocol as before. One hour and 25 minutes prior to surgery, amoxicillin clavulanic acid (12.5 mg/kg orally twice daily) was administered. Local anesthesia of the wound was obtained by using a splash block of lidocaine hydrochloride (Xylocaïne® 2%, Astra Zeneca; 2 mg/kg). Sutures and the tulle were removed and samples for cytology were taken. They showed persistence of several intracellular bacteria. An antimicrobial compress (Acticoat®, Smith & Nephew) was placed within the wound and the wound was closed with 2/0 nylon. Additionally, bandage with a non-adhesive compresses, synthetic padding and cohesive bandage material was applied. Total duration of anesthesia was 50 minutes; total duration of surgery was 30 minutes. Amoxicillin clavulanic acid and analgesia were continued as before.

Three days later, sutures and the antimicrobial bandage were removed. Cytology was repeated after rinsing the wound. Neutrophils and a small amount of macrophages were detected. No bacteria were detected. It was decided to close the wound surgically. Anesthesia and perioperative analgesia were similar as during the initial surgery. Amoxicillin clavulanic acid (20 mg/kg IV) was administered 30 minutes prior to surgery.
Wound edges were resected and curettage of the wound bed occurred. A cruciate suture pattern with 3/0 polyglecaprone was used to close the deep subcutaneous tissue. Superficial subcutaneous tissue was closed with a continuous suture pattern using 3/0 polyglecaprone. Skin was closed with cruciate sutures in 3/0 nylon. A soft padded bandage was placed. Total duration of anesthesia was 1 hour and 10 minutes; total duration of surgery was 50 minutes.
Postoperatively, analgesia consisted initially of methadone (0.2 mg/kg IV every 4 hours) and was changed to tramadol hydrochloride (3 mg/kg orally 3 times daily) the day after surgery, combined with gabapentine (10 mg/kg orally 3 times daily) and carprofen (2 mg/kg orally twice daily). Amoxicillin clavulanic acid was given once more after surgery and then stopped.

The incision site remained dry and clean after surgery. The dog was discharged 3 days after wound closure. The owners had to support the dog while walking by using a towel or a harness.
After 18 days, the dog was presented for a control visit. The surgical wound was healed well and the skin sutures were removed. The owners still had to stimulate the dog to walk. To avoid pressure wounds when the dog was laying, the owners had to protect the left elbow with a Vaseline ointment (Qualiphar) for hydration of the skin.

The owners were called approximately 7 months after the surgery. The dog was in perfect health and no problems were noticed after the last visit (Fig 10). The dog was happy to walk on his 3 legs without any support approximately 7 days after the last control visit.

Figure 10. Current aspect of the surgical site of the dog, 7 months after the surgery at the Small Animal Clinic.

5. DISCUSSION

A SSI is a common complication after a surgical procedure. It develops within 14 to 30 days (Salkind and Rao, 2008; Yap et al., 2015). Both dogs described above developed a SSI. In the first case, discharge from the wound and wound dehiscence occurred 5 days postoperatively. In the second case, fluid discharge was noticed 4 days postoperatively.
The source of bacterial contamination can be endogenous, which are bacteria coming from the patient's flora, or exogenous, which are originating from the environment. In the first case, *E. coli* was isolated. *E. coli* are gram-negative bacteria, commonly found in the large intestines of mammals (Erb et al., 2007). Howe and Boothe (2006) reported that *E. coli* was commonly found in SSI. In the second case, *Enterococcus faecalis* and *Enterobacter cloacae* complex were present in the wound. These are gram-positive and gram-negative bacteria respectively, that are both part of the gastrointestinal flora in mammals (Harbarth et al.,1999). Bacteria causing SSI in both cases were most likely endogenous, from fecal origin. Lack of hygiene by clinical staff or owners increases the risk of spreading bacteria by inappropriate hand hygiene when handling catheters, dressings and/or the wound itself. As both dogs underwent a forelimb amputation and were less mobile postoperatively, there was an increased risk of soiling the bench and subsequently an increased risk of SSI. Licking/washing themselves and automutilation are other possible causes of this type of SSI, although this is less likely in these cases as both dogs had a bandage postoperatively.

5.1 RISK FACTORS OF SURGICAL SITE INFECTIONS

The first dog was presented with an open fracture, but did receive IV antimicrobials within the golden period after the accident. According to MacPhail (2012) "The golden period" is the first 6 to 8 hours between wound contamination at injury and bacterial multiplication to greater than 10^5 CFU per gram of tissue. Wound lavage and debridement first needs to be performed in this period before administering antimicrobials (MacPhail, 2012). In the first case, wound lavage was performed by the referring veterinarian. The second dog had a tumor with central necrosis and erosion. In both patients, the affected limb was covered in sterile wrapz distant to the surgical site throughout the whole surgery. In the first case, a higher risk of sepsis was present due to the open fracture. In both dogs, primary closure of the forelimb amputation was performed and the wounds were considered contaminated and clean-contaminated, respectively. Besides surgical classification, the risk of SSI was moderate in both cases since risk factors described in the literature were present in both cases.

The signalment in both cases matched partially with described risk factors of SSI. Both patients were older than 10 years, 14 and 13 years, respectively. It has been described that older dogs are at higher risk of developing SSI because of impairment of their immune system or because of the presence of concurrent diseases (Nicholson et al., 2002; Howe and Boothe, 2006; Nelson, 2011; Willard and Schulz, 2012). Furthermore, it has been described that male intact dogs are at higher risk to develop SSI (Nicholson et al., 2002; Solano et al., 2015; Verwilghen and Singh, 2015; Yap et al., 2015). Both dogs described were male, but only the dog of the first was intact. A study in mice proved that the release of interleukin-2 (IL-2), IL-3 and interferon-γ by peripheral T cells was significantly decreased in intact male mice compared to castrated mice (Wichmann et al., 1996). The presence of androgenic

hormones, which are higher in male intact dogs, could explain these differences (Wichmann et al., 1996). Very little is investigated in veterinary literature about the association of the breed and the SSI risk. Solano et al. (2015) and Yap et al. (2015) both stated that Labrador Retrievers are at a lower risk to develop SSI after osteotomy technique to repair cruciate ligament rupture, but an explanation for this finding has not been given.

Hypoalbuminemia causes an impairment of the immune status (Cheadle, 2006; Turk et al., 2015). It can be associated with liver insufficiency, malnutrition or an active acute-phase response (Haridas and Malangoni, 2008). Even though the dog of the second case had a very mild hypoalbuminemia, it is unlikely that it was low enough to cause an impaired immune system.

The ASA (American Society of Anesthesiologists) physical status of a dog is developed for determining the status of the surgical patient before undergoing general anesthesia. It also is a good indicator of the SSI risk (Nelson, 2011). A study of Garibaldi (1991) showed a direct association between the occurrence of wound infection and a high ASA score. An ASA score of ≥3 increases the risk of developing a SSI (Barie, 2002; Eugster et al., 2004). Although the dogs described had different ASA scores (ASA II for case 1 and ASA III for case 2 due to the presence of the neoplasia), they both developed a SSI.

Oxygen (O_2) is important during wound healing and in preventing SSI. Depending on the production of bactericidal superoxide radicals by molecular oxygen, neutrophils kill pathogenic bacteria. This is the most important immune defense against pathogens (Sessler, 2006; Nelson, 2011; Hayes et al., 2014). Therefore, perioperative systemic hypoxia increases the risk of SSI because it results in decreased oxygenation of the surgical wound. Studies showed that perioperative oxygen supplementation by endotracheal intubation decreased the infection risk with 25 to 50% (Greif et al., 2000; Myles et al., 2005). A prospective study in humans reported a decreased SSI risk when administering perioperative oxygen supplementation up to 6 hours postoperatively (Belda et al., 2005). However, the effect of postoperative oxygen supplementation on its own in reducing SSI rate is not studied yet. General anesthesia can cause hypoxia if no oxygen is supplemented due to vasoconstriction and a decreased oxygen tension. Additionally, when tissues are not gently handled and when blood loss is severe during surgery, tissue hypoxemia can occur (Chang et al., 2010).
In both cases, the dogs were pre-oxygenated for a few minutes and received oxygen during the entire surgery. In case 1, 3 L O_2/min was administered for 30 minutes. Afterwards, the amount of O_2 was decreased to 1.5 L O_2/min. In case 2, 2 L O_2/min was administered throughout the surgery. However, no oxygen supplementation was given to the dogs after extubation. This is not generally performed in veterinary medicine. It is unclear if the differences in oxygen supplementation had any influence on the development of SSI in both cases and if the risk could have been reduced by supplementation postoperatively.

Halsted's principles are important when performing surgery . These principles include gentle tissue handling, meticulous hemostasis, preservation of blood supply to tissues, strict asepsis, accurately apposing tissues with minimal tension and elimination of dead space (Barbieri, 2018). Although hemostasis was obtained by ligating the axillary artery and vein and by bipolar electrocoagulation if other blood vessels were cut, moderate to major blood loss occurs during limb amputating as muscles bellies are cut, which causes diffuse bleeding. Electrocoagulation of the muscles is no option

since coagulation causes necrosis which gives a higher risk of developing a SSI. The creation of dead space also is inevitably when detaching the muscles from its insertion. To avoid dead space as much as possible, the deep and superficial muscle layers were closed separately. In case 1, an active drain was placed to avoid any fluid build-up in dead space and thus avoiding the formation of a seroma . No drain was placed in the second case.

A trial of Kurz et al. (1996) in humans showed that patients with even a mild perioperative hypothermia were 3 times more likely to develop SSI and their postoperative hospitalization duration was one week longer compared with normothermic patients. Hypothermia during surgery is commonly seen, mainly induced by anesthesia, because it inhibits the normal response to heat, or due to exposure to cold surfaces and a change in distribution of heat inside the body (Kurz et al., 1996). Hypothermia generates many adverse effects. First, it triggers vasoconstriction, which decreases the blood flow to the extremities and the skin in an attempt to reduce heat loss. This causes a low partial oxygen pressure, which impairs neutrophil function and impairs wound healing, thus leading to a higher risk of SSI (Kurz et al., 1996; Polk and Christmas, 2000). Secondly, hypothermia reduces the release of leukocytes of the bone marrow, which gives rise to impaired phagocytosis. Lastly, increased blood loss, myocardial problems, a negative nitrogen balance, prolonged anesthesia recovery, increased duration of hospitalization and discomfort are all observed more commonly when patients are hypothermic intraoperatively (Sessler, 2006). In both cases, a large surgical wound was created to amputate the forelimb. As a consequence, heat loss was present. Although the patients were placed on a heat pad during the entire surgery, a mild perioperative hypothermia was present in case 1 and case 2; a mean perioperative temperature of respectively 36.3°C and 36.6°C was measured. Placing latex gloves filled with warm water next to the patient, forced air devices or warming up the IV infusion are some options that could have been used during surgery to maintain a normal body temperature.

If medical devices are not sterile before use, it can cause SSI (Barie, 2002; Nelson, 2011; Bratzler et al., 2013). All medical devices, however, were disinfected and sterilized by using strict protocols before usage and indicators are used to check the sterility of the devices. Both surgeries were performed in a university setting where students and surgical assistants are helping to pass the medical device in a sterile way. However, it should be kept in mind that something can go wrong in this stage. If a student or assistant is touching the sterile instrument when opening the package or if an instrument touches the outside, not sterile part, of the package, the instrument itself is not sterile anymore.

Similarly, inadequate antisepsis of the place of the surgical incision can be the origin of the SSI. The surgical incision place was scrubbed in the operation room, using chlorhexidine gluconate, followed by spraying alcohol (70%) twice over the surgical field, as recommended in several guidelines (Nelson, 2011; Boothe and Boothe, 2015; Berrios-Torres et al., 2017). However, if this step is not performed properly, it can have attributed to the risk of developing a SSI. The affected limb was covered in sterile wrapz during the entire surgery. A prospective study on 40 canine cadaveric pelvic limbs reported that a distal leg wrap was not effective to prevent bacterial contamination of the surgical field. It was advised to incorporate a sterile impermeable barrier into the wrap to prevent bacteria entering the surgical field (Vince et al., 2008). However, a sterile impermeable barrier into a wrap is never used in our clinic.

Surgical hand preparation, which consists of a pre-surgical hand rub will reduce the bacterial count on the surgeon's hands but will not sterilize the skin (Hayes et al., 2017). In case of a glove puncture, a low amount of skin bacteria will colonize the wound (Barie, 2002). The pre-surgical rub must be performed according to certain guidelines for 1.5 minutes (Verwilghen and Singh, 2015; Boothe and Boothe, 2015). Further, despite correctly performed surgical rubbing, fingernails and the subungual regions will still have a higher bacterial load. A study of Hardy et al. (2017) identified risk factors for an increased bacterial load under fingernails or in the subungual region. No higher bacterial concentrations were reported in the (sub)ungular region between students or experienced surgeons. The only factor that increased the bacterial load under the fingernails and in the subungual region in this study was the length of the nails (Hardy et al.,2017). Several studies have shown that wearing double gloves decreases the risk of SSI (Nelson, 2011; Hayes et al., 2017). When glove puncture takes place during surgery when wearing single gloves , bacterial migration of the surgeon's skin and the outer surface of the gloves, can occur resulting in a higher risk of SSI (Hayes et al., 2017). Glove punctures are present in 26 to 43% of the cases during long surgeries (Meakin et al., 2016). Of all intraoperative glove punctures, only 34% are detected by the surgeon (Andrade et al., 2016; Hayes et al., 2014; Meakin et al., 2016). No differences were described in perforation risk in the dominant or non-dominant hand (Meakin et al., 2016). A study of Hayes et al. (2017) showed that, when wearing double gloves, perforations occurred more in the outer glove than in the inner glove. No differences were reported in the amount of perforations in the outer glove compared with the amount of perforations when wearing single gloves. Double gloves thus provide an extra barrier to the patient and, therefore, decreases the risk of SSI. However, double gloving is not that commonly used by veterinary surgeons. Surgeons can be reluctant to wearing double gloves due to discomfort, loss of dexterity and numbness (Hayes et al., 2014). In none of the described cases here, double gloves were worn by the surgeons, neither were the gloves changed after a while, even though both surgeries were relatively long in duration (respectively 1 hour and 45 minutes in case 1 and 2 hours and 40 minutes in case 2).

In a university setting, many persons like students, anesthesiologists and surgical assistants attend the surgeries. Students follow the surgery to learn, assistants help to pass medical devices in order to accelerate the duration of surgery (which decreases the risk of SSI) and anesthesiologists are necessary to ensure a safe anesthesia. However, this causes an increased the risk of developing a SSI in both cases. A study from Eugster et al. (2004) described that for each person present in the operation room, the risk to develop SSIs increase with a factor 1.3. The more persons present in the operating room, the more bacteria are present in the air (Eugster et al., 2004; Verwilghen and Singh, 2015; Andrade et al., 2016). However, a study of Turk et al. (2015) did not report a significant association between the risk of SSI and the number of persons present in the operating room. To decrease the risk of SSI in presence of many people attending the surgery, it can be recommended to enter and leave the operation room as little as possible and only if necessary.

The duration of anesthesia is correlated to the risk of SSI (Beal et al., 2000; Eugster et al. 2004). In both cases, anesthesia was maintained with isoflurane vaporized in oxygen. Volatile anesthetics cause immunosuppression due to impairment of neutrophils, alveolar macrophages, dendritic cells and natural killer cells immune functions (Beal et al., 2000). For each additional hour of anesthesia, the risk of SSI in dogs increases with 30% (Beal et al., 2000; Eugster et al., 2004). A study from Yap et al. (2015) and of Pratesi et al. (2015) showed that for each additional minute of anesthesia, the

likelihood for developing an SSI increased with respectively 4% and 2%. The long duration of anesthesia, caused by clipping and scrubbing the patients, increased the risk of SSI in both dogs. Nevertheless, if clipping and scrubbing of the patients would not be performed carefully or before the start of anesthesia, the risk of SSI will increase. A good communication between surgeons and anaesthetists is still necessary to minimalize the non-surgical duration of anesthesia which will decrease the SSI risk. However, a study from Turk et al (2015) reported no significant association between the duration of anesthesia and the risk of SSI in dogs.
The products used during anesthesia might also influence the risk of SSI. Propofol is a lipid based emulsion and is widely used for induction anesthesia at our clinic, and thus in both patients, because it is fast acting, non-cumulative and safe in patients with liver insufficiency or kidney insufficiency. (Howe and Boothe, 2006). Two different formulations of propofol are used in our clinic; Propovet® (Zoetis) and Diprivan® (Aspen Pharma). The main difference between both products is the absence of a preservative, benzylalcohol, in Diprivan®. As a result, the latter type of propofol can promote bacterial growth, especially gram-positive and gram-negative bacteria. The administration of bacterially contaminated propofol may lead to a SSI. For this reason, it is very important to use aseptic techniques when using this anesthetic and it is recommended not to store it at room temperature. Contamination of propofol can occur in many different ways; even when opening a vial whose surface has not been disinfected or when multiple withdrawals made from the vial are performed (Heldmann et al., 1999; Franci et al., 2015). A study of Heldmann et al. (1999) showed an increased risk with a factor of 3.8 when animals were given propofol compared with those not induced with propofol. It is thus advised to avoid contamination of propofol by transfusing the rest of the propofol into different syringes after opening the vial, together with a correct aspect technique and a proper storage of the remnants (Franci et al., 2015). A study of Yap et al. (2015) reported no significant association between the induction with propofol and the risk of SSI. However, both patients were induced with Propovet®, containing the preservative benzylalcohol which results in less bacterial contamination. This formulation also can be stored for 28 days at room temperature. As an alternative, alfaxalone also can be used for induction of the anesthesia.

Long surgeries result in a higher risk of SSI due to a higher chance of bacterial colonization of the wound and tissue dehydration (Vasseur et al., 1988; Brown et al., 1997; Bratzler et al., 2013; Verwilghen and Singh, 2015; Yap et al., 2015). The longer the surgical duration, the higher the chance of a suppression of the immune system will take place (Nicholson et al., 2002). A retrospective study in cats and dogs undergoing clean surgeries reported that surgeries that required more than 90 minutes were at a higher risk of SSI compared with a surgical duration of 60 minutes. (Brown et al., 1997). More specifically, studies from Eugster et al. (2004) and Yap et al. (2015) reported that the likelihood of a SSI in dogs increases with respectively a factor 1.01 and 7% for each additional minute in surgical time. Both forelimb amputations were long surgical procedures and thus an increased risk of SSIs was present in both dogs.
When performing a surgery, a balance should be found between operating as quick as possible to decrease the risk of SSI on the one hand and, on the other hand, to take time to correctly apply the principles of Halsted which also will reduce the risk of SSI.

A seroma is an accumulation of sterile fluid within the subcutaneous tissues and is a common complication after a forelimb amputation. Few clinical signs are associated with a seroma. However, wound healing will be delayed and seromas also will provide a medium for bacterial growth

(Remedies, 1999). For this reason, seroma formation needs to be avoided. Practicing Halsted's principles, placing drains and bandages can decrease the risk of seroma formation (Remedies, 1999).
In both cases, a supportive circumferential bandage was placed around the thorax to prevent edema and seroma formation by applying pressure to the wound. This bandage was made up of synthetic padding. Negative wound pressure therapy (NPWT) also can be used in limb amputations in dogs to prevent seroma formation. This includes a wound dressing placed onto the wound, an adhesive, an airtight seal, a vacuum pump or suction and a canister in which the fluid is captured (Pitt and Stanley, 2014; Howe, 2015). The pump's pressure is -125 mmHg. In addition, NPWT can improve wound healing by removing exudate, reducing the amount of edema, increasing perfusion, enhancing formation of granulation tissue and reducing bacterial levels (Howe, 2015). However, it is not reported yet that NPWT would be a better option compared with a traditional bandage in decreasing seroma formation.

Drains are foreign objects, reducing the number of organisms needed to create wound infection which enhances sepsis (Magee et al., 1976; Cheadle, 2006; Eugster et al., 2004). Further, drains are ideal routes for bacteria to migrate into the surgical wound (Magee et al., 1976; Nelson, 2011). Therefore, a drain should only be placed when fluid has to be removed out of the wound (Magee et al., 1976). Different types of drains can be used in surgical wounds but active drains are preferred compared with passive drains, as the chance of retrograde contamination is lower. In the first case, an active closed suction drain was placed. A prospective study in humans compared the rate of SSI in wounds without drains and wounds subjected to drainage (Magee et al., 1976). The infection rate in the wounds without drain was lower. Also, the position of the drain in the wound played a role. When a drain was completely inserted in the wound, a higher infection rate was measured (Magee et al., 1976).
When a drain is placed, the volume of drainage needs to be recorded daily to objectively measure the amount of fluid that is removed. If the amount is increasing, this indicates that the therapy has not the desired effect. An abrupt decrease in the volume of fluid is an indicator for drain removal (Bristow et al., 2015). As long as a drain is present, cytology of the fluid should be performed on daily basis to help decide the optimal time of drain removal. With the aid of cytology, the inflammatory and microbial status of the wound can be assessed. In one study, degenerative neutrophils were constantly present in the drained fluid. This can be explained by the fact that the drain, which is a foreign object by itself, was present in the wound and thus causes a reaction of the immune system (Szabo et al., 2011). As a consequence, the presence of degenerative neutrophils in itself is not a reason to leave the drain on site.

5.2 PROPHYLACTIC ANTIMICROBIALS AND SURGICAL SITE INFECTIONS

Even though, both dogs had an amputation of the forelimb; the indications and timing of first antimicrobial administration were different . The dog in the first case already received an IV injection of amoxicillin by the referring veterinarian just prior to referral. In both cases, the dogs received the same type of preoperative antimicrobial, cefazolin (20 mg/kg IV). In case 1, cefazolin was administered already 120 minutes prior to the start of surgery; it was not repeated intra-operatively (surgery time of 1 hour and 45 minutes) but only 8 hours after the previous dosage. In case 2, cefazolin was injected 30 minutes prior to surgery and re-administered intraoperatively 2 hours later (surgery time 2 hours and 40 minutes); no further antimicrobials were administered. The first dog, on the other end, received cephalosporins during 9 days postoperatively. Nevertheless, both of them developed a SSI; fluid discharge, pain, redness and delayed healing were observed in both dogs.

5.2.1 Choice of the antimicrobial in case of prophylaxis

Antimicrobials should be safe, cheap and based on the location of the surgery, the type of surgery and the expected pathogens (Polk and Christmas, 2000; Barie, 2002; Tourmousoglou et al., 2008; Weber et al., 2008; Willard and Schulz, 2012). However, antimicrobials do not need to be active against every type of bacteria that will be present in the surgical wound since not every organism will result in SSI (Waddell and Rotstein, 1994; Weed et al., 2003; Salkind and Rao, 2011).

Depending on the site of surgery, the most likely type of bacteria to cause a SSI will differ. *Staphylococcus* spp. are the most commonly found bacteria on the skin. Superficial SSI are most commonly colonized by *S. aureus* and *S. epidermis.* In deep SSI, *E. coli* and *Klebsiella* spp. are often present (Polk and Christmas, 2000).

In companion animals, many bacteria found in SSI are opportunistic bacteria that are part of the normal flora of the patient (Table 7). They normally do not harm the patient, except in cases when the host defense is impaired (Stull and Weese, 2015). Howe and Boothe (2006) reported that *S. aureus*, other *Staphylococcus* spp. and *E. coli* were most often cultured from the surgical site. Turk et al. (2015) and Verwilghen and Singh (2015) reported that *S. pseudintermedius* was the most important pathogen in canine SSI.

Table 7: Common pathogens in surgical site infections in small animals

Acinetobacter spp.
Bordetella bronchiseptica
Chlamydophila felis
Enterococcus spp.
Escherichia coli
Pseudomonas aeruginosa
Salmonella spp.
Staphylococcus spp.

Adapted from Stull and Weese, 2015.

In the first case, *E. coli* was isolated. In the second dog, *Enterococcus faecalis* and *Enterobacter cloacae* complex were present in the wound. All 3 bacteria are part of the gastrointestinal flora in mammals. Poor hygiene can have caused SSI in both patients.

It is recommended to choose antimicrobials with a relatively broad spectrum to avoid the emergence of antimicrobial resistance but antimicrobial selection should also be based on published data on microbiology of common small animal infections (Dellinger et al., 1994; Willard and Schulz, 2012). The antimicrobial associated with the lowest grade of toxicity and side effects must be chosen if more than one antimicrobial can be administered for prophylactic use (Polk and Christmas, 2000). For these reasons, cephalosporins are often used as a prophylactic antimicrobial (Salkind and Rao, 2011). It is beneficial to use this group of antimicrobials because of their low toxicity, few side effects, high safety level and high efficiency (Whittem et al., 1999; Polk and Christmas, 2000; Golembiewsky, 2009; Bratzler et al., 2013; Gonzalez et al., 2017). First generation cephalosporins such as cefazolin are preferred because they are highly effective against gram-positive organisms and they have a moderate activity against gram-negative pathogens. The pathogens that cause SSI such as *S. aureus* and *Streptococcus* spp., which are the most likely infecting microorganisms from the skin and soft tissues, are thus mainly susceptible to cefazolin (Ohge, 1999; Barie, 2002; Weed, 2003; Gonzalez et al., 2017). Cefazolin is also as effective as second, third or fourth generation cephalosporins if no anaerobic bacteria are expected to be present in the wound. Lastly, cefazolin is cheaper compared with second, third or fourth generation cephalosporins (Boothe and Boothe, 2015). In both dogs described, IV cefazolin was administered preoperatively.

A study from Whittem et al. (1999) compared the efficacy of potassium penicillin G with cefazolin in 126 dogs undergoing clean orthopedic surgeries. Potassium penicillin G is cheap, as well as cefazolin, and has a low incidence of side effects although allergic reactions are described in potassium penicillin G. However, potassium penicillin G is more rapidly eliminated. Further, it is only active against gram-positive aerobes and anaerobes. Both drugs were, however, equally effective in decreasing the SSI risk in dogs undergoing an elective orthopedic surgery (Whittem et al. 1999). In both patients, the administration of potassium penicillin G would not have been a good choice because it would not have been active against gram-negative bacteria such as *E. coli* in case 1 and *Enterobacter cloacae* in case 2.

Second generation cephalosporins like cefmetazole and cefoxitin are used in surgeries affecting the large intestines because they are also active against anaerobes (Wong-Beringer and Schrock, 1995; Weed, 2003; Howe and Boothe, 2006). They are not commonly used in the prevention of SSI. Often, clindamycin or metronidazole are supplemented to gain a higher activity against anaerobic bacteria (Mujagic et al., 2011; Boothe and Boothe, 2015; Weber et al., 2017).

Third and fourth generation cephalosporins are not commonly used as prophylactic antimicrobials against SSI. First, they are active against pathogens that are not commonly detected in postoperative wounds (Weed, 2003; Howe and Boothe, 2006). Secondly, their use will increase the risk of antimicrobial resistance (Silver et al., 1996; Weed, 2003; Bratzler and Houck, 2005; Howe and Boothe, 2006). Third, there is no to little evidence that those antimicrobials result in lower infection rates compared with the older cephalosporins (Bratzler and Houck, 2005; Bratzler et al., 2013). Lastly, the second, third and fourth generation cephalosporins are far more expensive than cefazolin (Weed, 2003).

Amoxicillin clavulanic acid is a broad-spectrum, time-dependent antimicrobial that is effective against most gram-positive, gram-negative and anaerobic bacteria. Due to the presence of clavulanic acid, it has an increased activity against gram-negative bacteria compared with cefazolin (Unterer et al,

2011). It also can be used as a prophylactic antimicrobial[2], but mostly in gynecological and intestinal surgeries (Van Kasteren et al., 2013). However, a higher resistance towards amoxicillin clavulanic acid, compared with cefazolin, is reported (Boothe and Boothe, 2015).

5.2.2 Concentrations of prophylactic antimicrobials to be administered

It is advised to administer prophylactic antimicrobials IV to quickly obtain high concentrations of the antimicrobial at the surgical wound (Waddell and Rotstein, 1994; Rosenberg et al., 2008; Boothe and Boothe, 2015). For most antimicrobials, high tissue concentrations are achieved within 30 to 60 minutes after IV injection (Boothe and Boothe, 2015). When antimicrobials are given orally it will take longer to obtain the same peak concentrations. Similarly, when the patient is in shock, peak concentrations will occur slower, due to decreased distribution (Boothe and Boothe, 2015). In case 1, the antimicrobial was given IV 120 minutes prior to surgery. In the second case, cefazolin was injected at the correct time to achieve adequate tissue concentrations, namely 30 minutes prior to surgery.

When determining the optimal dose of prophylactic antimicrobials, it is important to use a dose that is on the high side of the usual therapeutic range dose (Dellinger et al., 1994). Adequate antimicrobial levels in tissue and serum at the beginning of the surgery and throughout surgery should be achieved (Polk and Christmas, 2000; Gonzalez et al., 2017). It is important to consider that tissue concentrations of an antimicrobial may underestimate the true surgical site concentrations because the interstitium is filled and thus diluted with intracellular fluid (Gonzalez et al., 2017). The antimicrobial concentrations should exceed the MICs for the expected pathogens in the surgical field (Bratzler and Houck, 2005; Tourmousoglou et al., 2008; Aiken et al., 2015; Yap et al, 2015). An *in vitro* study from Wong-Beringer and Schock (1995) reported that administering antimicrobials with a concentration below the MIC_{90} would result in a decreased virulence due to morphological changes in the bacteria. Cephalosporins are time-dependent drugs, which means that their efficacy depends on duration the antimicrobial concentration is above the MIC_{90} (Gonzalez et al., 2017). When using cefazolin as the prophylactic antimicrobial, a dose of 22 mg/kg is recommended. A prospective study in 12 dogs reported that a dose of cefazolin of 22 mg/kg resulted in effective tissue concentrations for at least 4 hours (Gonzalez et al., 2017). In both cases and actually in all cases at our clinic, a dose of 20 mg/kg of cefazolin IV is administered prior to surgery. Since there are no biodistribution data in the literature on the dose of 20 mg/kg it seems wise to adapt the dose to 22 mg/kg. When the dose is too low, antimicrobial resistance can develop and the efficiency of the antimicrobial will decrease; the antimicrobial concentrations will not exceed the MIC_{90} for the pathogens.

The duration of the antimicrobial's activity should always be kept in mind when administering a single dose of the antimicrobial (DiPiro et al., 1986). Timing of administering a second dose of antimicrobials depends on the type of bacteria, the dose, the half-life and the pharmacokinetics of the antimicrobial (Howe and Boothe, 2006).
Determining the re-administration of prophylactic antimicrobials is a subject of controversy in human and veterinary medicine. It has been recommended by human guidelines that time-dependent antimicrobials such as cephalosporins should be re-administered every 2 half-lives to achieve

[2] https://www.bsava.com/Resources/Veterinary-resources/PROTECT/Reducing-Prophylaxis: consulted on 12/05/2018

adequate tissue concentrations (Polk and Christmas, 2000; Golembiewski, 2009; Nazarali et al., 2014; Verwilghen and Singh, 2015; Yap et al., 2015; WHO Guidelines, 2016). It has been suggested for a long time that the half-life of cefazolin was approximately 1 hour in companion animals (Verwilghen and Singh, 2015). Therefore, the traditional recommendation was a re-administration every 2 hours (Golembiewsky, 2009; Verwilghen and Singh, 2015). A more recent prospective study in 12 dogs reported that cefazolin concentrations were maintained above 4 µg/ml for 4 hours after a single injection of cefazolin (22 mg/kg IV). This dose should provide protection against the most common SSI pathogens in dogs; *S. pseudintermedius* (MIC_{90} is 4 µg/ml) and *Streptococcus spp.* (MIC_{90} is 2 µg/ml) (Westermeyer et al., 2010). Intra-operative re-dosing of cefazolin is thus not necessary if the surgery lasts 4 hours or less and if *Staphylococcus pseudintermedius* and/or *Streptococcus* spp are expected to be the pathogens (Gonzalez et al., 2017). If *E. coli* (MIC_{90} is 128 µg/ml) or other gram-negative bacteria are suspected as pathogen, another antimicrobial and dosing regimen must be considered (Thungrat et al., 2015; Gonzalez et al., 2017).
In case 1, no re-administration of cefazolin was performed throughout the whole surgery. In case 2, however, cefazolin was re-administered 2 hours after the first IV injection. A re-administration of cefazolin every 2 hours is outdated and it is only recently changed to every 4 hours at our clinic. The duration of surgery in case 2 was 2 hours and 40 min; a re-administration of cefazolin was not necessary.

Besides route of administration and the dose of the antimicrobial, other factors such as renal function and protein binding are also important to achieve bactericidal concentrations at the surgical site by the time of surgical incision (Boothe and Boothe, 2015; Gonzalez et al., 2017). An association is seen between the concentrations of free drugs in the serum and the tissue concentration; weakly bound antimicrobials such as cefazolin will achieve high tissue concentrations (Boothe and Boothe, 2015). Further, blood loss during surgery, duration of surgery, lipid solubility and pH also will affect the antimicrobial concentration in the tissue and serum of the patient (Golembiewski, 2009; Boothe and Boothe, 2015). In both patients, blood loss was clearly present. Major blood loss can be considered an important variable to decide to re-dose short half-life antimicrobials (Barie, 2002). The recommendations of Gonzalez et al. (2017), which includes the re-administration of cefazolin after 4 hours in surgeries lasting 4 hours or less, will not be sufficient to decrease the infection rate, especially in the first dog, in which the injection was given too early to obtain optimal intra-operative levels and in which it was not repeated intra-operatively. In such cases, a second dose of cefazolin should be administered earlier to maintain adequate tissue and serum levels intra-operatively and thus to decrease the infection rate (Marita et al., 2005; Dellinger, 2007). An amputation is a surgery in which major blood loss is inevitable. Muscles bellies are being cut, which causes a lot of bleeding. However, electrocoagulation of the muscles is contraindicated as coagulation causes necrosis which gives rise to a higher risk of SSI. The single use of long acting antimicrobials in long surgeries or in surgeries where major blood loss occurred is also accepted (Salkind and Rao, 2011).

5.2.3 Timing of administration

Determining the ideal timing of administering prophylactic antimicrobials is essential concerning the appropriate use of prophylactic antimicrobial therapy and the timing is also associated with the SSI risk (Burke, 1961; Bratzler and Houck, 2005). A prospective study in cats and dogs showed that the SSI rate after clean surgeries varied depending on the timing of prophylactic antimicrobial administration. When the antimicrobials were administered more than 2 hours prior to surgery or postoperatively, a higher infection rate was reported compared to cats and dogs that did not receive any antimicrobial prophylactically (Brown et al., 1997). However, this study is actually outdated because nowadays it is generally accepted that prophylactic antimicrobial administration is contraindicated in clean surgeries. It would be unnecessary to administer them in clean surgeries due to its low grade of bacterial contamination (Howe and Boothe, 2006; Aiken et al., 2015). Also, because administering antimicrobials is not without risk, the advantages of giving antimicrobials do not outweigh the possible disadvantages in clean surgeries (Weed, 2003).
In general, it is recommended to administer the first dose of prophylactic antimicrobials 30 to 60 minutes prior to the start of surgery (Howe and Boothe, 2006; Verwilghen and Singh, 2015; Yap et al., 2015; Gonzalez et al., 2017).

Many studies have reported the ideal timing of administering prophylactic antimicrobials and conflicting results have been described. A prospective study from Wong-Beringer and Schrock (1995) showed that the most optimal timing of administration of the antimicrobials was at induction of the anesthesia. This timing resulted in adequate serum and tissue concentrations at the time of incision. However, due to the long preoperative preparation of both patients, this timing would not have been optimal.
A prospective study in humans investigated the ideal timing of administration on the SSI risk in more detail. No differences in the wound infection rates were reported when prophylactic antimicrobials were given in the interval of 30 to 60 minutes or 0 to 30 minutes prior to incision (Van Kasteren et al., 2007). In contrast, a prospective study in humans reported that when antimicrobials were administered within 30 minutes before surgery, the infection rate was 0.8% lower compared with the patients who received the drugs between 31 minutes and 60 minutes prior to surgery (Steinberg et al., 2009). Dellinger (2007) stated that the longer the surgery takes, the more benefit is achieved when administering the antimicrobial as close as possible to the start of surgical incision. A more recent study in humans reported that the risk of SSI was the lowest when the antimicrobials were administered between 74 minutes and 30 minutes prior to surgery compared with the patients who received the antimicrobials in the last 30 minutes prior to incision (Weber et al., 2008). A possible explanation is that the serum and tissue levels have not reached yet the MIC at the time of incision to prevent infection when giving the antimicrobials in the final 30 minutes before surgery (Weber et al., 2008; Mujagic et al., 2011). According to the WHO (World Health Organization) guidelines (2016), no differences in infection rate were reported when administering prophylactic antimicrobials within 0 to 30 minutes, 30 to 60 minutes or 60-120 minutes prior to incision. This makes sense as long an intra-operative re-administration is considered. It is recommended to take into account the half-life of the antimicrobial in order to decide the optimal time of administration (WHO Guidelines, 2016).
A recent study in dogs reported that administering cefazolin (22 mg/kg IV) 30 to 60 minutes prior to short-term surgeries (lasting 3 hours or less) would be ideal. The interstitial fluid concentrations of the antimicrobial were above the MIC_{90} at the start of surgery and remained as such throughout the procedure (Gonzalez et al., 2017). The surgeries of both dogs described in this thesis lasted less than

3 hours (1 hour and 45 minutes in case 1 and 2 hours and 40 minutes in case 2). One-time cefazolin should thus have been sufficient as antimicrobial prophylaxis, provided that it was administered 30 to 60 minutes prior to the start of surgical incision. However, this was not the case in the first patient. In case 1, cefazolin (20 mg/kg) was given IV 120 minutes prior to surgery. Adequate serum and tissue levels of the antimicrobial above the MIC_{90} could not be guaranteed after 1 hour of surgery. In the second case, on the other hand, cefazolin was injected 30 minutes prior to surgery, which is a correct time to achieve adequate tissue concentrations and re-administration was already performed after 2 hours.

5.2.4 Duration of systemic prophylactic antimicrobials

Prophylactic antimicrobial use should be limited in time. A short duration, less than 24 hours after the end of surgery or when closing the surgical wound, is recommended (Waddell an Rotstein, 1994; Barie, 2002; Bratzler et al., 2003; Salkind and Rao, 2011; Verwilghen and Singh, 2015). Prolonging the prophylactic antimicrobial use after clean-contaminated surgeries beyond 24 hours has no advantages (Dellinger et al., 1994; Nelson, 2011; Salkind and Rao, 2011). Different studies in veterinary medicine reported no benefits between administering postoperative antimicrobials for longer than 24 hours and a decreased risk of SSI (Aiken et al., 2015; Yap et al., 2015). Indeed, an increased risk of nosocomial infections, increased costs and the development of antimicrobial resistance are reported (Barie, 2002; Bratzler and Houck, 2005; Howe and Boothe, 2006; Hedrick et al., 2006; Aiken et al., 2015; Pratesi et al., 2015). Even in the presence of drains and catheters, no evidence is present to justify the continued use of prophylactic antimicrobials (Polk and Christmas, 2000; Salkind and Rao, 2011). In the second dog, no antimicrobials were given postoperatively. This is correct according to different guidelines and studies; no therapeutic antimicrobial therapy should be started after a clean-contaminated surgery

After contaminated surgeries, dirty surgeries or when preoperative infection is present, antimicrobials need to be continued postoperatively. However, this antimicrobial treatment is therapeutic rather than prophylactic (Zanetti et al., 2001; Howe and Booth, 2006; Nelson, 2011).

In case 1, the dog received antimicrobials postoperatively for 9 days in total. The day of surgery, cefazolin (20 mg/kg IV 3 times daily) was continued followed by cephalexin (15 mg/kg orally twice daily) for another 8 days. The continuation of the antimicrobial therapy for therapeutic use is acceptable; the surgery in this dog was a contaminated surgery. Cephalexin was a good choice in this patient. This antimicrobial is, as well as cefazolin, a first generation cephalosporin and thus it is cheap and has few side effects. It is also active against the most common expected skin pathogens.

6. CONCLUSION

The risk of SSI could have been decreased in both patients by paying attention to some risk factors. First, both patients were hypothermic during surgery which can contribute to impaired wound healing and thus an increased chance of a SSI. It is important to maintain a normal body temperature by using heat pads during surgery, forced air devices or gloves filled with hot water. Because the standardly used distal leg wraps are not effective in preventing bacterial contamination of the surgical field, a sterile impermeable barrier should be incorporated in the wrap to prevent bacteria entering the surgical field. Further, extra attention can be paid in future to the amount of people attending the surgery. It is important to communicate to the students and surgical assistants to enter (and leave) the operation room only if necessary. Wearing double gloves and/or changing gloves in long-term surgeries can also help to decrease the risk of SSI. A good communication between surgeons and anaesthetists is necessary to minimalize the non-surgical duration of anesthesia which will decrease the SSI risk. During surgery, it is important to pay attention to the principles of Halsted. Lastly, placement of a drain or bandage should be used to prevent seroma formation. Drains are ideal routes for bacteria to migrate into the surgical wound. To decrease this risk of bacterial migration, an active drain can be placed or a bandage is covering the distal end of the drain. The amount of fluid in the drain as well as cytology of the fluid should be evaluated daily.

Concerning the use of prophylactic antimicrobials, it is important to choose the correct type of antimicrobial, if indicated. Cefazolin is mostly the drug of choice due to its spectrum, low toxicity and low cost. Secondly, determining the correct dose and timing of administration is important. A recent study in veterinary medicine reported that a dose of cefazolin of 22 mg/kg resulted in effective tissue concentrations for at least 4 hours. It is recommended to inject the first dose 30 to 60 minutes prior to the start of surgery. The preferred route is IV since high concentrations of the antimicrobial at the surgical site are then quickly obtained. A re-administration of cefazolin is only necessary after 4 hours if the surgery is still ongoing. A short duration of prophylactic treatment, less than 24 hours after the end of surgery, is recommended.

In conclusion, the use of prophylactic antimicrobials can decrease the risk of SSI but it does not replace aseptic techniques, meticulous tissue handling and appropriate wound care. Prudent use of antimicrobials is advised.

7. REFERENCES

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