Investigation into Factors Associated with Surgical Site Infections Following Tibial Plateau Leveling Osteotomy in Dogs

by

Alim Nazarali

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ABSTRACT

Investigation into Factors Associated with Surgical Site Infections Following Tibial Plateau Leveling Osteotomy in Dogs

Alim Nazarali
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Advisor:
Dr. Ameet Singh

Tibial plateau leveling osteotomy (TPLO) is one of the most common surgical techniques performed to stabilize a cranial cruciate insufficient stifle in dogs. Although it is classified as a clean surgical procedure, it is associated with a high surgical site infection (SSI) rate. Methicillin-resistant *Staphylococcus* pseudintermedius (MRSP) is the predominant pathogen causing TPLO SSI and is difficult to treat because of its multi-drug resistance. This thesis is an investigation into the use of perioperative antimicrobial prophylaxis and factors associated with SSI occurrence following TPLO in dogs, including MRSP carriage. We identified that perioperative antimicrobial prophylaxis protocols are not being administered appropriately, however, failure of adherence to these protocols was not associated with SSI. Furthermore, preoperative MRSP carriage was a risk factor and postoperative antimicrobial use was protective against the occurrence of TPLO SSI. Further study into the factors associated with TPLO SSI is required to understand this clinically important challenge.

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Declaration of Work Performed

I declare that, with the exception of the item below, all work within this thesis was performed by me.

Statistical analysis for *Chapter 2: Perioperative administration of antimicrobials during tibial plateau leveling osteotomy in dogs: 226 cases (2008 – 2010)* was performed by Dr. J. Scott Weese, Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.

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List of Abbreviations

ASA:	American Society of Anesthesiologists
CCLI:	cranial cruciate ligament insufficiency
CDC:	United States Centers for Disease Control and Prevention
dru:	direct repeat unit
ICU:	intensive care unit
LAMP:	loop-mediated isothermal amplification
MDR:	multi-drug resistant
MRSA:	methicillin-resistant *Staphylococcus aureus*
MRSP:	methicillin-resistant *Staphylococcus pseudintermedius*
NGD:	New Generation Devices
NNIS:	National Nosocomial Infections Surveillance System
OVCHSC:	Ontario Veterinary College Health Sciences Centre
PBP_{2a}:	penicillin-binding protein 2a
PCR:	polymerase chain reaction
SSI:	surgical site infection
TPLO:	tibial plateau leveling osteotomy

Chapter 1

Literature Review

1.1: Surgical Site Infections

Surgical site infections (SSIs) are infectious complications that manifest at the incision site of a surgical patient and are the result of a combination of host, pathogen and environmental factors that ultimately results in establishment of infection.[1] These types of infections are defined by the United States Centers for Disease Control and Prevention (CDC) using multiple different criteria and categories (Table 1.1).[2]

Table 1.1: CDC definitions for surgical site infections.[2]

Category	**Criteria**
Superficial SSI	Within 30 days Skin and/or subcutaneous tissues 1 or more of: - pus - bacteria - diagnosis by a surgeon -heat, redness, pain OR localized swelling AND incision reopened by surgeon UNLESS culture negative
Deep SSI	Within 30d, 1 year if implant Deep soft tissues of the incision 1 or more of: - pus - spontaneous dehiscence of deeper incision OR incision is deliberately opened when patient has fever, localized pain or tenderness UNLESS culture negative - Abscess or other evidence of infection on imaging or histology
Organ/Space SSI	Within 30 days, 1 year if implant Any area other than the incision that was encountered during surgery 1 or more of: - pus - bacteria - Abscess or other evidence of infection upon exam, re-operation, histology or imaging

It is important to note that by definition, a SSI does not have to have a proven positive culture. Most SSIs are caused by bacteria, although rare fungal infections may occur. The focus of this review, and this thesis, will be SSIs caused by bacteria, because they encompass the vast majority of infections. The dynamic relationship between the

size of the bacterial inoculum, the virulence of the bacteria and the resistance of the host is important to understand as it can help explain the inherent risk of developing a SSI for any given surgery.[3,4] This formula visually represents the relationship:

$$\text{Infection Risk} = \frac{\text{Contamination x Virulence}}{\text{Host Resistance}}$$

This equation can be useful to consider the factors that are involved in the pathophysiology of SSI, yet it is rather oversimplified, since many other related factors may be involved, and the three categories listed above encompass numerous components. For example, 'contamination' can involve various characteristics of the inoculated bacterium (species, virulence factors, antimicrobial resistance) and inoculation dose. However, this basic question is useful to revisit when considering pathophysiology or prevention. There are many factors that have been suggested to increase the risk of developing a SSI following surgery, but it must be understood that there will always be the potential for a SSI to develop following any surgical procedure.[3-5]

1.2: Surgical Site Infections in Human Medicine

1.2.1 - Incidence and Risk Factors

The incidence and risk factors for SSIs have been extensively investigated in human medicine and a wide range of SSI rates have been reported (Table 1.2). The type of surgery performed is a risk factor in itself as it affects other risk factors for SSI. For example, patients undergoing knee replacements or arthroplasty would be more likely to suffer from SSIs than patients undergoing hysterectomies because the former involves an increased duration of surgery, the placement of an implant as well as the location of the surgical site has minimal soft tissue coverage and vascularization.[4,6-8]

Numerous risk factors for the development of a SSI have been identified in the human literature (Table 1.3).[8] These factors include a wide range of patient and procedure factors, with some being very consistent across a wide range of studies and others more sporadically reported or more associated with selected procedures. Patient factors include gender, age, weight, status of *Staphylococcus aureus* carriage and comorbidities of the patient.[4,8-10] Comorbidities include concurrent endocrinopathy such

as diabetes and/or other illnesses or infections.[8] There are also treatment factors that can increase the risk of SSI such as duration of the surgical procedure, duration of anaesthesia time and the use of certain anaesthetic drugs such as propofol. [4,8,9,11]

Table 1.2: Surgical site infection rates in a variety of different surgery types in human medicine.

Author	**Procedure**	**SSI rate (%)**
Bakkum-Gamez *et al*, 2013[12]	Surgical management of endometrial cancer	9.9
Teija-Kaisa *et al*, 2013[9]	Breast operations (lumpectomy, mastectomy)	6.7
Lake *et al*, 2013[7]	Hysterectomy	2.71
Lopez-Contreras *et al*, 2012[13]	Total primary hip prosthesis Total primary knee prosthesis	3 3.3
Young *et al*, 2011[6]	Knee replacement, spinal surgery and arthroplasty	11.1
Huotari *et al*, 2006[14]	Hip arthroplasty Knee arthroplasty	3.9 2.3
Thomas *et al*, 2004[15]	Total hip replacement Total knee replacement	4.86 5.15
Chung *et al*, 1991[16]	Total hip replacement Other clean orthopedic surgeries	1.3 – 11 0.7 – 9

The nature of the surgical procedure can have a profound impact on SSI risk. Surgical procedures are categorized based on the level of contamination of the wound, which is one method used to assess the risk of developing a SSI. The wound types are stratified into four categories; clean, clean-contaminated, contaminated and dirty-infected (Table 1.4).[2,17] The more contaminated a wound is, the higher it is at risk for developing a SSI. [2,17]

Table 1.3: Risk factors for the development of surgical site infections in humans (Data from National Nosocomial Infections Surveillance System (NNIS) System Report: Data summary from January 1992–June 2004[8]; adapted from Barie *et al*, 2005[4]).

Type of factor	Risk factors associated with the development of a SSI
Patient	Level of wound contamination Ascites Chronic inflammation Corticosteroid therapy (controversial) Obesity Diabetes Extremes of age Hypocholesterolemia Hypoxemia Peripheral vascular disease (especially for lower extremity surgery) Postoperative anemia Prior site irradiation Recent operation Remote infection Skin/nasal carriage of *Staphylococcus aureus* Skin disease in the area of infection (eg, psoriasis) Undernutrition
Treatment/procedure	Contaminated medications Inadequate disinfection/sterilization Inadequate skin antisepsis Inadequate ventilation Drains Emergency procedure Blood transfusion Procedure involving an implant Hypothermia Inadequate antibiotic prophylaxis Oxygenation (controversial) Prolonged preoperative hospitalization Prolonged operative time Prolonged anaesthesia time

Another more recent method of assessing risk of infection in the surgical patient is the National Nosocomial Infections Surveillance System (NNIS) surgical patient risk index.[18] There are three main components to this risk index: 1. A patient having an American Society of Anesthesiologists (ASA) preoperative assessment score of 3 or higher (maximum 5), 2. An operation classified as contaminated or dirty-infected and 3.

The duration of surgery being more than T hours, where T is dependent on the type of surgical procedure being performed.[17,19] It is believed that the latter SSI risk assessment is more accurate due to it taking multiple considerations into account.

Table 1.4: Definitions of the different surgical wound classes.[2,17]

Class I **Clean**	An uninfected surgical wound in which no inflammation is encountered and the respiratory, alimentary, genital, or uninfected urinary tracts are not entered. In addition, clean wounds are primarily closed and, if necessary, drained with closed drainage. Surgical wound incisions that are made after nonpenetrating (ie. blunt) trauma should be included in this category if they meet the criteria.
Class II **Clean-Contaminated**	A surgical wound in which the respiratory, alimentary, genital, or uninfected urinary tracts are entered under controlled conditions and without unusual contamination. Specifically surgeries involving the biliary tract, appendix, vagina, and oropharynx are included in this category, provided no evidence of infection is encountered and no major break in technique occurs.
Class III **Contaminated**	Open, fresh, accidental wounds. In addition, surgical procedures in which a major break in sterile technique occurs (eg. open cardiac massage) or there is gross spillage from the gastrointestinal tract and incisions in which acute, nonpurulent inflammation is encountered are included in this category.
Class IV **Dirty/Infected**	Old traumatic wounds with retained or devitalized tissue and those that involve existing clinical infection or perforated viscera. This definition suggests that the organisms causing postoperative infection were present in the wound before the surgical procedure.

Methicillin-resistant *Staphylococcus aureus* (MRSA) carriage in humans has been well documented and is seen to be carried anywhere from 0% to 6.8% of the human population being studied (Table 1.5). [10,20-24] It has been identified that the risk of developing a SSI is increased in patients that are colonized with MRSA and up to 6.8% of the population are carrying this bacterium (Table 1.6).[10,20,22]

Table 1.5: Prevalence of methicillin-resistant *Staphylococcus aureus* carriage in human populations.

Author	**Prevalence of methicillin-resistant *Staphylococcus aureus* carriage (%)**
Kalra *et al*, 2013[20]	4.3
Gomez-Sanz *et al*, 2013[21]	1.5
Gupta *et al*, 2011[22]	6.6
Bode *et al*, 2010[23]	0 (18.8% methicillin-susceptible *Staphylococcus aureus*)
Pofahl *et al*, 2009[24]	6.8
Yano *et al*, 2009[10]	2.6

A recent study assessed the risk of developing a MRSA SSI when colonized with MRSA in 9006 patients.[20] They reported that patients that were positive for MRSA carriage at least 30 days prior to surgery were 9 times more likely to develop a MRSA SSI.[20] Another study assessing the same association in 4238 patients documented a 12-fold increase in the risk of developing a MRSA SSI when the patient carried MRSA preoperatively colonized.[22] Yano *et al* assessed the association between preoperative carriage of MRSA and development of MRSA SSI in all patients undergoing orthopaedic surgery.[10] In this study, 2423 patients were screened for MRSA carriage preoperatively and monitored for SSIs caused by MRSA.[10] It was identified that a preoperative nasal culture positive for MRSA carriage independently increased the likelihood of developing a MRSA SSI by 11 times.[10] These studies provide excellent data to prove that preoperative colonization of MRSA substantially increases the likelihood of a patient developing a MRSA SSI.[10,20,22]

Table 1.6: Incidence of surgical site infections in patients colonized with methicillin-resistant *Staphylococcus aureus* versus non- carriers in the human population.

Incidence of surgical site infections in patients colonized with methicillin-resistant *Staphylococcus aureus* versus non- carriers (%)		
Author	**MRSA colonized SSI**	**Non-carrier SSI**
Kalra *et al*, 2013[20]	1.86	0.2
Gupta *et al*, 2011[22]	1.2	0.16
Yano *et al*, 2009[10]	6.3	0.5

1.2.2 - Impact

Surgical site infections can be a devastating complication and associated with increased patient morbidity[1], increased hospital stay[1,25,26], economic costs[25-27], and even mortality.[25] The frustration and grief of families and medical caregivers must also be considered. In general, the greater the severity of the SSI (superficial vs deep vs organ space), the greater the complications however, even apparently minor SSIs results in significant complications in some patients.[25,26,28]

It is estimated that over 500 000 SSIs occur in the Unites States and the cost associated with SSI treatment can be as high as $10 billion.[1,25,26] In one study assessing 41 SSIs following thoracic surgery it was found that patients that developed a SSI stayed an average of 20 extra days in hospital compared to patients that did not develop a SSI.[25] The Pennsylvania Health Care Cost Containment Council released a report in 2005 where they collected data on 1,569,164 patients statewide.[26] They identified that patients diagnosed with a SSI were hospitalized for 16.1 extra days, compared to patients that recovered from the same procedure without complication.[26] An analysis conducted in 1992 reported an average of 7.3 extra days of hospitalization for patients that developed a SSI.[1] If a patient is not healthy enough to defend themself from a severe infection and develops a deep or organ space SSI there is a much greater risk of the patient dying.[25] Hollenbeak et al. found that 22% of patients that developed a deep chest SSI died within a year.[25] Treatment of SSIs can be prolonged, leading to protracted morbidity and economic impacts.

Economic costs associated with diagnoses and treatment of SSI can be astounding. In a group of patients from a study by Hollenbeak *et al* in 2000 that developed a deep chest SSI and died, economic costs for SSI management averaged $81, 474 per patient.[25] Total treatment cost was ~ 8 times greater in these compared to a patient that did not develop a SSI.[25] In the state of Pennsylvania, it was reported in 2005 that average increased treatment costs were $153 871 per patient that was suffering from any kind of hospital acquired infection.[26] In 2004, a prominent insurance company in Pennsylvania was billed an additional $2.3 billion for all hospital acquired infections.[26] These are staggering extra costs for just a single state. This cost can be compared to the average economic cost for managing a SSI in 1992, where average

extra cost per patient was $3152.[1] Surgical site infections are devastating to both patient health and financial standing and costs are only increasing.

1.2.3 - Pathogens

In human medicine, the most common pathogens isolated from SSI include *Staphylococcus sp., Enterococcus sp*. and *Escherichia coli* (Table 1.7).[8,29-34] Other common bacteria that cause SSI after gastrointestinal surgery include gram-negative bacilli.[4]

Table 1.7: Prevalence of bacteria isolated from surgical site infections following various types of surgery in humans (Data from Emori *et al*, 1993[34]; adapted from Barie *et al*, 2005[4]).

Bacteria	**Prevalence (%)**
Staphylococcus spp.	19
Coagulase-negative *Staphylococcus* spp.	14
Enterococcus spp.	12
Escherichia coli	8
Pseudomonas aeruginosa	8
Miscellaneous aerobic gram-negative *bacilli*	8
Enterobacter spp.	7
Streptococcus spp.	6
Klebsiella spp.	4
Miscellaneous anaerobic bacteria	3
Miscellaneous aerobic gram-positive bacteria	2

Some bacteria are common causes of infection because they are opportunistic pathogens that are commonly found in or on the body as a part of the commensal microbiota.[30] When the host immune system is compromised or other components of the body's natural barrier systems are compromised (e.g. surgical incision), these bacteria have an increased opportunity to proliferate and cause disease.

Staphylococcus aureus is a coagulase positive, facultative anaerobic, gram-positive coccus and a commensal bacterium that can cause a wide range of infections when circumstances permit.[35] It is an opportunistic pathogen that can cause skin and soft tissue infections, hospital-acquired and ventilator-acquired pneumonia, vascular catheter

infections as well as SSIs when its host is compromised. Another reason that makes *Staphylococcus aureus* a common pathogen is its ability to become methicillin-resistant,[30] as this property confers protection against most of the commonly used perioperative antimicrobials. Methicillin resistance, specifically, is associated with the presence of the *mecA* gene. This gene encodes for the production of an altered penicillin-binding protein 2a (PBP_{2a}) which confers resistance to methicillin and virtually all β-lactams by drastically reducing its affinity for β-lactam antimicrobials.[36-38] Detection of methicillin-resistance is important for both clinical management and disease surveillance.[35] Confirmation of methicillin-resistance can be achieved by detection of PBP_{2a} by latex agglutination test[39] or *mecA* by DNA amplified polymerase chain reaction (PCR).[39]

1.2.4 - Preventive Measures

Methods of preventing, or at least minimizing, the development of SSI following surgery is a well-researched topic in human medicine because of the imact associated with SSI discussed previously. There are many routine preventative measures commonly performed for all surgical procedures with the goal of decreasing bacterial contamination of the surgical wound and limiting the compromise of the patients' immune response[1,40-42]

One of the most common preventive measures to reduce the risk of developing a SSI is administration of perioperative antimicrobials.[42] Perioperative antimicrobial prophylaxis is commonly used with surgeries that are at a higher than normal risk for SSI.[42,43] The purpose of administering antimicrobials at the time of surgery is to reduce intraoperative contamination by bacteria to a level in which the host can prevent infection.[1] It has been considered as a method to minimize infection, although globally accepted standards regarding their use have not been developed.[42] The potential efficacy of antimicrobial prophylaxis is affected by multiple different factors and an area that receives major emphasis in human surgery is timing of antimicrobial administration.[42,44,45] The primary goal of antimicrobial prophylaxis is to have therapeutic antimicrobial levels present at the surgical site prior to incision and throughout the surgical procedure. Standard recommendations from human medicine, when using time dependant drugs, are to administer an appropriately selected antimicrobial at a

maximum of 1 hour prior to first incision and then to discontinue the use of antimicrobials within 24 hours following completion of the procedure.[42] Exact timing for optimal preoperative administration of antimicrobials has not yet been determined in human or veterinary surgical practice. However, general guidelines recommend administration to be within two half-lives of the antimicrobial prior to surgery in order to ensure peak serum and tissue concentrations of the antimicrobial are present at the time of incision.[42] The half-life of the drug must then be considered when determining whether further dosing is required. The short half-life of most beta-lactams, the most commonly used drugs for perioperative prophylaxis, means that adequate drug levels may not be maintained during most surgical procedures after a single preoperative dose.[46,47] It is therefore widely recommended that administration be repeated every 2 half-lives until the procedure is complete.[46,47]

Some reports from the human medical literature show disappointing results when considering adherence to timing of antimicrobial administration. For example, one report considered administering preoperative doses within 120 minutes before incision and yet only 60% of patients had been given adequately timed doses in a study of 2847 individuals.[42] Similarly, Braztler *et al* showed that only 55.7% of 34133 surgical patients received antibiotics within 60 minutes prior to incision.[43] One potential method to improve antimicrobial timing is the use of a preoperative checklist, which is becoming increasingly common in human medicine.[48,49] Preparing such a checklist can help ensure that prophylactic treatment is initiated prior to the start of the procedure and therefor adequate concentration of the antimicrobial will be present in the tissues at the time of incision.[46,47]

Although reports have shown inadequacies in timing of antimicrobial prophylaxis, its effect on occurrence of SSI may be limited. There are studies that have assessed the association of timely administration of prophylactic antimicrobials during surgery with SSI using matched data from the Surgical Care Improvement Program and National Surgical Quality Improvement Program. Some studies observed no decrease in SSI occurrence when perioperative antimicrobials were administered according to protocol.[50-52] In fact, one study noted a statistically significant increase in the likelihood of SSI occurring when patients undergoing colorectal surgery were administered antimicrobial prophylaxis as per recommended guidelines.[52] Compliance to guidelines for antimicrobial prophylaxis was as high as 99% in some of these studies, yet the risk of developing a SSI was not

decreased. [50-52] There are many reports providing evidence against the effectiveness perioperative antimicrobial prophylaxis guidelines in minimizing SSI.

Another aspect of perioperative antimicrobial prophylaxis is the use of postoperative antimicrobials. This subject is more controversial as there is increasing concern about excessive or inappropriate antimicrobial therapy. It is not typically recommended to administer postoperative antimicrobial treatment beyond 24h in humans undergoing surgical procedures that are not considered contaminated.[2,42,51,53,54] Extending the duration of postoperative antimicrobial administration has not been shown to reduce SSI rates and may contribute to the development of antimicrobial resistance and additional morbidity, along with additional treatment costs.[54-56]

A preventive measure that is gaining popularity in human surgery is the practice of decolonizing preoperative MRSA positive patients prior to the time of surgical procedure.[57,58] The most common methods of decolonization include either mupirocin nasal ointment, clorhexidine soap or wash cloths, or both treatments given simultaneously.[57,58] Mupirocin is an antimicrobial that is administered via an intranasal spray to preoperatively colonized patients prior to surgery.[58] Clorhexidine is an antiseptic or disinfectant that is available as a body wash or impregnated cloth and is used for the decolonization of skin prior to surgery.[57] Optimal timing for these decolonization treatments have not yet been solidified and have been reported to be administered anywhere from 24 hours to 7 days prior to surgery.[57,58] van Rijen *et al* conducted a meta-analysis using four studies that treated preoperatively colonized MRSA patients with mupirocin ointment (range of duration 24hours – 7days) and reported that patients who were not preoperatively decolonized of MRSA were 1.8 times as likely to develop a SSI caused by MRSA.[58] Thompson *et al* conducted a preoperative MRSA decolonization trial using a 5-day treatment of mupirocin ointment and clorhexidine impregnated wash cloths.[57] The study was conducted over a three year period and only included four types of surgeries; cardiac, neurosurgery, orthopaedic and vascular. A decrease in MRSA SSI development of 72% over the three year period was reported.[57] The change in MRSA SSI rate was compared to the MRSA rate of surgeries that were not included in the study over the duration of the study period and therefore not treated for decolonization of MRSA carriage. Over the three-year period the MRSA SSI rate in the excluded surgeries increased by 200%, further emphasizing the success and importance of this intervention on the reduction of SSI development.[57] One study assessed the effectiveness of

mupirocin spray and clorhexidine soap treatment on patients preoperatively colonized with methicillin-susceptible *Staphylococcus aureus* and found that patients that were not decolonized prior to surgery were 2.4 times as likely to suffer from a methicillin-susceptible *Staphylococcus aureus* SSI.[23] It should be noted that without the ability to rapidly detect MRSA via real time PCR, preoperative decolonization treatments would not have been possible.[59]

1.3: Surgical Site Infections in Veterinary Medicine

1.3.1 - Incidence and Risk Factors

While less intensively studied compared to human medicine, SSIs occur in small animal patients at rates similar to those reported in humans (Table 1.8).[5,28,60-73]

As would be expected, SSI rates are influenced by both patient and procedure factors. Risk factor studies have been reported for canine and feline patients. While many were of limited by sample size or studying broad or ill-defined patient populations, numerous risk factors have been reported.[62,70,74]

There are minimal studies in the veterinary literature addressing the epidemiology and risk factors for the development of SSI in small animals. Many risk factors for small animals are similar to those found in human medicine, which is unsurprising since the majority of basic principles of medicine and surgery are shared across disciplines. Risk factors that have been associated with increased rates of SSI in small animals include factors specific to the patient as well as factors regarding variations in treatments (Table 1.9).[5,28,69,71,72,78]

It has been documented that the obese surgical patient is at a higher risk for the development of a SSI, where the risk of SSI is increased as the weight of the patient is increased.[5] This weight association is likely due to an inadequate tissue concentration of prophylactic antimicrobials at the time of surgery, although a controlled study assessing this risk is needed.[79] There is also evidence that intact males have a higher likelihood of developing a SSI when compared to other sexes.[5] It is suggested that this may be due to immunoregulatory effects of androgenic hormones that alter the balance of pro-inflammatory and anti-inflammatory mediators.[69] Another factor relating to patient health

and the development of a SSI includes the presence of endocrinopathy such as hyperadrenocorticism or hypothyroidism within a patient. These diseases have shown to increase a patient's likelihood of developing a SSI by as much as 8.2 times.[80] If species differentiation in adrenal gland activity between dogs and humans is minimal, hyperadrenocorticism may cause a decrease in the production of natural killer cells and T lymphocytes.[81] The increased risk of SSI in hypothyroid dogs needs to be further evaluated as hypothyroidism is not a risk factor in humans.[82] One study has demonstrated that the ASA score of a patient is associated with the development of a SSI. It was seen that the higher the ASA score given to a patient prior to surgery, the higher was the patient's likelihood to develop a SSI.[5] The risk factors identified above were from two studies assessing a wide range of potential factors associated with SSI and controlled studies should be performed to assess these risk factors in detail.[5,69] Another study identified that patients were also most likely to develop a SSI if their wounds were contaminated prior to surgery.[71] Based on the surgical wound classification system, it was noted that the risk of SSI increased as the contamination of the wound increased.[71]

Other risk factors for SSI have been identified as preoperative treatments or procedures. [5,28,70,71] Two studies have shown that patients that received antimicrobials prior to surgery (not including their initial perioperative dose prior to incision) were at a higher risk of developing SSI than patients that received perioperative antimicrobial prophylaxis alone, as per protocol.[5,71] When preparing the patient for surgery, the risk of SSI is increased by up to 3 times when patients are clipped prior to induction.[28,70,71] It is suggested that bacterial colonization of the skin is increased after clipping due to the irritation and damage done to the skin, therefore increasing the risk of developing a SSI.[71]

Some perioperative risk factors for SSI have also been documented in the veterinary literature.[5,28,62,69,71,78] The use of propofol as an anaesthetic during clean surgeries has been associated with a high rate of SSI.[78] Propofol is delivered through lipid based emulsion and is a reservoir for bacterial and fungal growth.[11] This delivery method is a likely reason it is associated with high SSI rates.[11,78] The retrospective nature of this study may limit the usefulness of the data, but since it is a well identified risk factor in human medicine the finding is most likely accurate.[4] Another study identified the number of personnel in the operating room as being a risk factor for

developing SSI, where the likelihood of developing a SSI was increased as the number of personnel in the room increased.[5] This finding was identified in a large, but generalized SSI study and has not been identified in a controlled setting. The duration of both surgery and anaesthesia have an effect on the risk of developing SSI.[28,69-71] The risk of SSI developing in a patient is increased as the duration of surgery is increased and this is likely because the wound is exposed to contaminants and is immune compromised for a longer period of time.[69-71] Prolonged duration of anaesthesia increases chances of developing a SSI by many different factors that cause the host to become immune compromised such as the use of certain anaesthetics and hypothermia.[28,69,78] Method of skin closure during surgery has also been documented to play a role in the development of SSI, where using staples rather than suture to close an incision has shown to be a risk factor.[62]

Risk factors have also been identified to emerge during the postoperative period.[5,71] Two studies reported that the administration of postoperative antimicrobials was a risk factor for developing a SSI[5,71], although contradictory evidence can also be found in the veterinary literature.[60,62,77] One study reported that patients who had a drain placed at the surgical site were more likely to develop SSI.[5] The same study identified type of postoperative stay in hospital is another risk factor for SSI.[5] Results showed that patients were twice as likely to develop a SSI if recovered in an intensive care unit (ICU) when compared to the average patient.[5] The study was not specifically designed to assess these observed risk factors for SSI and therefore more evidence is needed.

It is difficult to identify and assess risk factors associated with SSI in veterinary medicine as there have been minimal studies conducted. The majority of reports that identify risk factors did not design their study to specifically assess them. Most studies collected information on a large number of factors potentially associated with SSI in a retrospective or observational manner. Many of the risk factors observed in veterinary medicine require further investigation using controlled prospective observational studies or trials.

Table 1.8: Surgical site infection rates in a variety of veterinary surgical procedures in small animals.

Author	Procedure	SSI rate (%)
Savicky *et al*, 2013[63]	TPLO	14.3
Etter *et al*, 2013[64]	TPLO	9.6
Gallagher *et al*, 2012[68]	TPLO	7.4
Singh *et al*, 2012[75]	All surgical procedures	3.0
Mayhew *et al*, 2012[70]	Minimally invasive surgeries into pleural and peritoneal cavities	1.7
Thompson *et al*, 2011[76]	TPLO	4.8
Gatineau *et al*, 2011[77]	TPLO	2.9
Fitzpatrick *et al*, 2010[60]	TPLO	6.6
Frey *et al*, 2010[62]	Extracapsular lateral suture and TPLO	6.1
Corr *et al*, 2007[67]	TPLO	15.8
Weese *et al*, 2006[73]	Cranial cruciate rupture surgery	3.6
Eugster *et al*, 2004[5]	All surgeries, excluding dental and ophthalmologic	3.0
Priddy *et al*, 2003[61]	TPLO	12
Nicholson et al, 2002[69]	All clean contaminated surgeries	5.9
Beal *et al*, 2000[28]	All clean surgeries	4.8
Whittem *et al*, 1999[72]	Clean orthopedic surgeries	7.1
Brown *et al*, 1997[71]	All surgeries	5.5
Vasseur *et al*, 1988[66]	All clean surgeries All dirty surgeries	2.5 18.1
Vasseur *et al*, 1985[65]	Various surgeries	0.8

Table 1.9: Risk factors for the development of surgical site infections in small animals.

Author	Risk Factors
Singh *et al*, 2012[75]	Hypotension, class of surgical wound, placement of implant
Mayhew *et al*, 2012[70]	Increase in time between clipping of surgical site and start of surgery, duration of surgery
Frey *et al*, 2010[62]	Use of stainless steel skin staples for skin closure
Eugster *et al*, 2004[5]	Obesity, increase in ASA score, level of wound contamination, number of personnel in surgery, pre or postoperative antimicrobial administration, recovery in ICU, presence of drain
Nicholson *et al*, 2002[69]	Intact males, endocrinopathy (hyperadrenocorticism, hypothyroidism), duration of surgery, duration of anaesthesia
Beal *et al*, 2000[28]	Clipping of surgical site prior to patient induction, duration of anaesthesia
Brown *et al*, 1997[71]	Clipping of surgical site prior to patient induction, duration of surgery, pre or postoperative antimicrobial administration

1.3.1 - Impact

The impact of SSI in small animal surgery is currently not well documented. Surgical site infections can cause many detrimental circumstances such as poor cosmesis[74], delayed wound healing[5], increased treatment and medication costs[83], revision surgery[76], increased economic costs[84] and even patient death[5], but the overall impacts have not been adequately quantified.

There are several considerations when assessing the negative impact that SSIs cause including patient health, economic impact and zoonotic risk.[5] There have been studies that showed a delay in wound healing, extended hospital stay and the need for additional evaluation hospital visits due to the development of a SSI.[5,84] Eugster *et al* documented that average hospital stay for patients that developed a SSI was twice as long compared with patients who recovered without any complication.[5] Another recent study has strengthened these results by reporting that patients that developed SSI spent an average of 4 extra days in hospital and had an average of 4 more postoperative recheck visits due to SSI management.[84] For surgeries that involve the placement of an implant and subsequent implant associated SSI, the likelihood of added costs, hospitalization and surgeries is likely heightened because of the common need for implant removal.[68,76,83-85] This has been supported by a study following TPLO SSI cases conducted by Savicky *et al* where removal of the implant resolved infections even in the absence of antimicrobial treatment.[83]

When interventions as extreme as additional surgeries are required, economic costs can be very substantial.[84] There has only been one study in the veterinary literature that reports the economic impact caused by developing a SSI.[84] Nicoll *et al* assessed postoperative management of SSIs following TPLO in dogs where the average postoperative cost for patients affected by a SSI was $1559 compared to the average cost of $212 for a patient that did not develop a SSI.[84] These increased costs were a result of more postoperative recheck visits, necessary medication such as antibiotics and for most cases, a follow up surgery for removal of the implant.[84]

1.3.2 - Pathogens

There are limited reports of pathogens isolated from SSI in veterinary medicine, though some common bacteria have been identified.[68,74,83,84,86] Common pathogens associated with SSI development in small animal surgery include *Staphylococcus pseudintermedius, Staphylococcus aureus*, coagulase negative *Staphylococcus* spp*., Enterococcus spp. and Pseudomonas spp.*[68,83,84,86] Staphylococci are of particular concern with development of SSIs because of their commensal nature and ability to become resistant to antimicrobials.[36] When considering procedures where patients are administered antimicrobials prophylactically, there is no benefit to the host if they are colonized by methicillin-resistant staphylococci as β-lactam antimicrobials have no effect on those organisms.[36] While S. aureus is the leading cause of SSIs in humans, S. pseudintermedius dominates in dogs. Issues pertaining to this bacterium in dogs are very similar to those with S. aureus in humans including concerns about methicillin-resistance..[36,68,83,84]

Despite its importance in dogs, *S. pseudintermedius* is a relatively recently described organism. In 1976, Hajek discovered a new species of *Staphylococcus* that was thought to be carried by a wide variety of species including dogs, pigeons, horses and mink. It was named *Staphylococcus intermedius*.[87] It was later discovered that there are multiple species of staphylococci that are similar to *S. intermedius*, and one was co-evolving with Canoidea family (dog, skunk, raccoon, weasel, red panda and bear family).[88] Devriese *et al* then realized that a species being labelled *Staphylococcus intermedius* that was being isolated from dogs was not actually correct and proposed to name it *Staphylococcus pseudintermedius sp. nov*.[89] Since its discovery, many previously identified *S. intermedius* strains have been reclassified as this novel staphylococcal strain.[90] *S. pseudintermedius* is a coagulase positive, facultative anaerobic, gram-positive coccus.[36,89] It is a resident flora commonly isolated from dogs and most commonly acts as an opportunistic pathogen, causing secondary pyoderma, bacterial otitis, wounds and abscesses.[36,85,90] This also led to the conclusion that *S. pseudintermedius* was the leading cause of pyoderma in dogs, not *S. intermedius*.[89,90] *S. pseudintermedius* was also isolated from human infections as well as their dogs and confirms that human infections are due to zoonotic transfer from dogs.[85,91] It is now understood that *S. pseudintermedius* is the leading canine opportunistic pathogen.[36,92,93]

Considering the commonness of *Staphylococcus pseudintermedius* in dogs, another potential factor in the development of SSI is antimicrobial resistance. There is evidence to show that the administration of antimicrobials prior to surgery increases the risk of SSI by multi-drug resistant (MDR) bacteria.[36] Due to the rapid emergence of MDR pathogens, perioperative antimicrobial prophylaxis is coming under scrutiny as to whether it is a risk factor or protective effect for the development of SSI.[36,93] Antimicrobial resistance of *Staphylococcus pseudintermedius* can develop based on the bacteria`s genetic makeup and previous or current exposure to antimicrobials[36] In a recent article by Frank & Loeffler, they showed that the average prevalence of MRSP was 13.8%, which is alarmingly high compared to other studies reporting MRSP prevalence being between 2-7.4%.[92,94-96] Sasaki *et al* also identified an extremely high MRSP prevalence of 29.8% in a hospital in Japan.[97] MRSP is of significant concern as there may be few viable treatment options.[63,68,76,92] The inherent resistance of MRSP to beta-lactams raises another concern, since typical perioperative prophylaxis practices that use cephalosporins will have no effect on this leading SSI pathogen. Although there are several reports of MRSA increasing the likelihood of SSI in human medicine, parallel studies are lacking in the veterinary literature when considering preoperative methicillin-resistant bacterial colonization. Similarly, studies for preoperative decolonization of methicillin-resistant bacteria in small animals are also currently non-existent in the veterinary literature.

1.3.4 - Preventive Measures and Protective effects

Assessment of SSI prevention measures are limited in the veterinary literature (Table 1.10),[60,66,70,74,86,98] yet a wide range of pre, peri and postoperative steps are routinely taken to reduce the risk of SSI. Typically these are adapted from human medicine protocols.[74]

An important preventive measure for decreasing the risk of SSI is the use of perioperative antimicrobial prophylaxis.[60,62,72,73,98] Similar to human medicine, the use of antimicrobials is a controversial subject and there are significant gaps in knowledge pertaining to when and how to use antimicrobial perioperatively. While objective criteria are currently lacking, antimicrobials are most widely recommended for contaminated and dirty procedures, some clean-contaminated procedures, procedures involving an implant

and clean procedures lasting longer than 90 minutes.[60,62,66,72,73] Timing may be one of the most important factors when looking at perioperative antimicrobial prophylaxis. Similar guidelines to ones found in human medicine are not present in veterinary surgery, although the concepts of antimicrobial prophylaxis should apply equally across species. There has been limited scrutiny of current perioperative antimicrobial prophylaxis practices performed in small animal surgery within the veterinary literature.[73,98] Timing of administration varies depending on the animal that is having surgery, as half-lives of antimicrobials vary for different species. Considering the 48 minute half-life of cefazolin in dogs, these guidelines correspond to administration within 60 minutes of incision and approximately every 90 minutes (two half-lives) thereafter.[46,47] A study assessing perioperative antimicrobial prophylaxis in clean surgical procedures in dogs and cats provided evidence to support these guidelines. When compared to patients that did not receive antimicrobials for surgical procedures surpassing 90 minutes, patients that were administered perioperative antimicrobials were less likely to develop a SSI .[66] In surgeries with an extended duration where antimicrobials are required, it is recommended that an intraoperative dose be given 90 minutes after the initial dose.[66,73] Another study assessing perioperative antimicrobial prophylaxis in elective orthopaedic surgeries in dogs recorded an increased number of SSI in dogs that were not administered any perioperative antimicrobials, further solidifying the need for perioperative antimicrobial prophylaxis when an implant is involved.[72] Weese *et al* demonstrated that less than 60% of dogs received appropriately timed antimicrobials when undergoing cranial cruciate rupture repair.[73] This displays that there is much needed improvement in perioperative antimicrobial prophylaxis practices in some veterinary institutions.[73]

The use of postoperative antimicrobials is an especially controversial subject as the potential for clinical efficacy needs to be balanced with concerns about selecting for antimicrobial resistance.[5,71,73] Recent evidence has emerged to suggest that postoperative antimicrobials may be indicated for certain procedures, particularly TPLO.[60,62,77] It was first reported in 2010 that postoperative antimicrobial administration of 3 – 14 days following TPLO demonstrated a protective effect against the development of SSI.[62] Two other studies showing that 10 and 14 day postoperative antimicrobial administration decreased the likelihood of SSI were later published as well.[60,68] Although all of these studies were retrospective in nature and therefore limited inference can be

from the findings, it is unlikely all three studies reported the same effect from postoperative antimicrobial administration by chance. Due to a multitude of differences in patients factors between dogs and humans it is recommended that this finding be further evaluated in a controlled clinical trial, even though it is not recommended to administer antimicrobials after 24 hours of surgery in human medicine.[42] Another protective effect identified in dogs undergoing surgery pertains to the breed of the dog.[60] It was observed that Labrador Retrievers were less likely to develop a SSI, although the reason behind this finding requires further investigation.[60]

Table 1.10: Preventive measures and protective effects for development of a surgical site infection in small animal veterinary medicine.

Author	**Preventive Measure/Protective Effect**
Fitzpatrick *et al*, 2010[60]	Postoperative administration of antimicrobials, Labrador Retrievers
Whittem *et al*, 1999[72]	Administration of perioperative antimicrobials in elective orthopaedic surgeries
Vasseur *et al*, 1988[66]	Administration of perioperative antimicrobial prophylaxis in surgeries exceeding 90 minutes in duration

1.4: Cranial Cruciate Ligament Insufficiency in Dogs

Cranial cruciate ligament insufficiency (CCLI) is one of the most common causes of pelvic limb lameness in dogs.[99,100] Trauma is rarely a cause of CCLI, as most dogs suffer from progressive pathological fatigue and failure of the ligament.[101] Small dogs (<15kg) can regain limb function through conservative management with success rates exceeding 80% in one study.[102] However, in larger breeds (>15kg) many studies state that complete limb function will not return without surgical intervention.[103] While there are several different surgical techniques used to stabilize CCLI and there is no consensus about the optimal method, tibial plateau leveling osteotomy is one of the most commonly performed surgical techniques.[103]

1.5: Treatment of Cranial Cruciate Ligament Insufficiency with Tibial Plateau Leveling Osteotomy

The TPLO was first proposed by Slocum and Slocum in 1993 and involves a radial osteotomy of the proximal tibia with subsequent rotation of the proximal segment to reduce tibial plateau slope negating cranial tibial thrust.[104] Briefly, following inspection of intra-articular structures via arthrotomy or arthroscopy, the proximal tibia is approached, and a semi-circular osteotomy is made based on preoperative planning. The distal portion of the tibia is then rotated so that the tibial plateau angle is ~ 5^{o} and a TPLO specific plate is placed to secure the angle of rotation (Figure 1).[105]

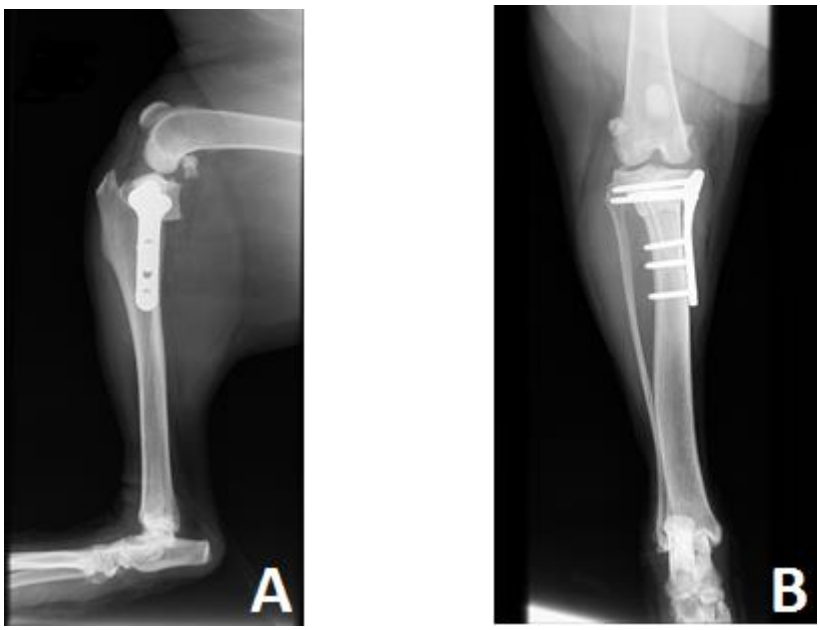

Figure 1.1: A. Lateral stifle radiograph of a dog following TPLO.

B. Craniocaudal stifle radiograph of a dog following TPLO.

1.6: Surgical Site Infections Following Tibial Plateau Leveling Osteotomy

1.6.1 - Incidence and Risk Factors

TPLO is considered to be an elective, clean orthopedic surgical procedure but suffers from a high incidence of SSI when compared to other clean surgical procedures. (Table 1.11).[60-64,67,68,76,77,106]

The reason for this high SSI rate is complex and cannot be easily identified. It is likely due to multiple factors including duration of surgery and anaesthesia[69], aggressive periosteal dissection of the tibia[103], reduced soft tissue coverage over the proximal tibia, thermal damage from the saw and presence of an impant.[68]

Table 1.11: Surgical site infection rates following tibial plateau leveling osteotomy procedures in dogs.

Author	**Procedure**	**SSI rate (%)**
Savicky *et al*, 2013[63]	TPLO	14.3
Etter *et al*, 2013[64]	TPLO	9.6
Gallagher *et al*, 2012[68]	TPLO	7.4
Thompson *et al*, 2011[76]	TPLO	4.8
Gatineau *et al*, 2011[77]	TPLO	2.9
Frey *et al*, 2010[62]	TPLO	8.4
Fitzpatrick *et al*, 2010[60]	TPLO	6.6
Corr *et al*, 2007[67]	TPLO	15.8
Pacchania *et al*, 2003[106]	TPLO	2.5
Priddy *et al*, 2003[61]	TPLO	12

While numerous studies have reported TPLO SSI rates, these have almost exclusively been retrospective in design and relied on medical records for data. A common concern is the potential for underreporting of SSI rates as some patients may be diagnosed and treated with SSI at their local veterinarian and the surgical facility not being informed of it. Therefore the incidence of SSI development may be underreported. Some of the studies had a very small sample size, which could result in a reported SSI rate that may not extrapolate to the larger surrounding population.[67,83] Older studies that may not have been using current definitions for SSI may have reported an inaccurate and possibly overestimated SSI rate.[61,106] Although the study design may not be optimal

to retrieve the most accurate SSI rates possible, some studies were large in scale and still allows one to appreciate the impact of SSI following TPLO.

It is unclear as to why TPLO is plagued by such a high SSI rate and what risk factors are involved, but it is likely multifactorial. Potential risk factors include thermal damage by the saw blade used to perform the osteotomy, minimal soft-tissue coverage of the proximal tibia, excessive soft tissue dissection at time of surgery, aggressive periosteal dissection, presence of an implant, periosteal compression by the implant, prolonged surgery and anaesthesia times, and increasing prevalence of opportunistic pathogens (particularly staphylococci) that are resistant to antimicrobials used for perioperative prophylaxis.[62,63,67,75,76] Current risk factors for TPLO documented in the literature include weight, gender, breed and severity of CCLI of the patient, the use of staples when closing the skin incision, the performance of an arthrotomy, undergoing simultaneous bilateral TPLO and the brand of implant used for the procedure (Table 1.12).[62,63,67,75,76,107,108]

Patient health and traits are important considerations when determining the risk of developing a SSI following TPLO. There is evidence to show that the weight of a patient can alter the risk of developing a SSI.[60] The relationship documented in TPLO procedures is the more obese a patient, the higher the risk of suffering from a SSI.[60] It has also been reported that the risk of SSI occurring is increased by 1.85 times when the patient is an intact male.[60] Another study reported that the breed of the dog can affect the risk of SSI, where Rottweilers were more likely to develop a SSI following TPLO than other breeds involved in the study.[106] A final patient health factor that has been discovered to increase the risk of SSI development following TPLO is the severity of the CCLI that the patient presents with.[60] Dogs that presented with a complete cranial cruciate ligament tear were 1.7 times more likely to acquire a SSI than patients that presented with only a partial tear.[60]

There have been some risk factors identified when considering the equipment or hardware used when performing a TPLO.[62,76,83] One study has demonstrated that the method of closing the skin after the TPLO has been completed alters the likelihood of whether the patient will develop a SSI during the recovery period.[62] It was shown that the use of stainless steel skin staples to close the skin resulted in a 1.9 times increase in the likelihood of the patient developing a SSI.[62] When considering which brand of implant to

use for TPLO, there is conflicting information in the literature.[76,83] Results from one study reported that significantly more Slocum TPLO plates were removed from dogs following the development of an infection, when compared to New Generation Devices (NGD) TPLO plates. This could suggest that there is a higher likelihood of developing SSI when using Slocum TPLO plates.[76] Contradicting results were documented in another study where more NGD plates were removed than Slocum plates following diagnosis of SSI in patients.[83] This evidence helps to suggest that using NGD TPLO plates could increase the likelihood of developing SSI.

Other risk factors have been identified when considering variation in procedures from one patient to another.[61,106] The performance of an arthrotomy on the stifle, which is done prior to performing a TPLO, has been suggested to increase the risk of developing a SSI following TPLO. An arthrotomy is a procedure where the joint space is entered in order to assess damage to the meniscus. If there is damage evident, then the meniscus can be removed by a procedure called a meniscectomy. Patients that are diagnosed with bilateral cruciate rupture can undergo TPLO procedures by different methods. It can be completed in one of two ways; both legs can be operated on simultaneously or a staged intervention can be planned, where the second hind leg has a TPLO performed after the first hind leg has recovered from its TPLO.[61] It has been reported that performing simultaneous bilateral TPLO procedures, in comparison to either unilateral TPLO or bilateral staged TPLO, significantly increases the likelihood of developing a SSI.[61]

Although interesting data has been reported for risk factors associated with TPLO SSI, none of these studies were developed to specifically address any one risk factor. All studies were retrospective in nature and were designed to identify risk factors for TPLO SSI using a large set of parameters. In order to use this information in a clinical setting, further assessment of these risk factors is permitted.

Table 1.12: Risk factors for the development of surgical site infections following tibial plateau leveling osteotomy in dogs.

Author	Risk Factors
Savicky *et al*, 2013[83]	Brand of implant used for surgery (New Generation Devices > Synthes > Slocum)
Thompson *et al*, 2011[76]	Brand of implant used for surgery (Slocum > New Generation Devices)
Frey *et al*, 2010[62]	Use of stainless steel skin staples for skin closure
Fitzpatrick *et al*, 2010[60]	Obesity, intact males, having a complete cranial cruciate ligament tear (versus partial)
Priddy *et al*, 2003[61]	Undergoing bilateral TPLO surgeries simultaneously
Pacchania *et al*, 2003[106]	Rottweilers, performance of an arthrotomy

1.6.2 - Impact of TPLO SSI

Surgical site infections in TPLO can have detrimental consequences on patient recovery, limb function, treatment costs and frustration for the client and clinician alike.[61,67,68,76,77,83] Surgical site infections can cause mild problems such as delayed healing of the incision, to very serious issues such as osteomyelitis where the healing of the tibial osteotomy is greatly delayed or where treatment is futile and amputation is required.[61] A mild incisional SSI, if caused by an antimicrobial susceptible pathogen, can usually be treated by administering antimicrobials and allowing the wound to properly heal. On the other extreme, severe osteomyelitis can cause delayed bone healing and extended lameness which is solved through antimicrobial administration, surgical wound flush procedures and eventually a plate removal after the tibia has fully healed.[68,76,77,83]

The most common clinical signs for SSI in a patient are lameness, the presence of an open wound, the presence of a draining tract and pain on palpation of the surgical site.[61,67,83] It has been shown that some dogs that are lame during the period of postoperative infection do not fully recover limb function on the operated leg, even following an implant removal.[83] These dogs were identified with intermittent lameness even after a full year of recovery from the time of implant removal.[83] Treatment costs would be extremely high in patients such as this since they would require much more postoperative care compared to a patient that suffered from a superficial SSI on the skin of the incision.

There is currently only one study that investigates the economic impact on clients due to their pets developing TPLO SSI in the veterinary literature. Nicoll *et al* reported an average postoperative cost of $1559 for dogs that had developed SSI, compared to an average cost of $212 for dogs that recovered without complication.[84] Depending on the severity of the infection and treatments required to resolve them, postoperative costs varied between $145 and $5022.[84] The more complicated and severe SSIs that required additional hospitalization and surgery are more likely to fall in the latter half of the reported economic cost. There is supporting evidence, where patients that had MRSP isolated from their SSI had increased postoperative visits, hospitalization, and experienced an average economic cost of $2294 compared to the overall average complication cost of $1559.[84]

Infections that occur at the site of implants can result in bacterial colonization of the plate and subsequent biofilm production. This greatly hampers elimination of the bacterium by antimicrobials and the immune system, and often leads to a need to remove the implant to successfully resolve the infection.[68,83,109,110] The importance of implant removal is highlighted by a study that reported implant removal alone was just as efficient as implant removal in conjunction with antimicrobial use at eliminating infection from the patient and more effective than antimicrobial administration without implant removal.[83] This evidence supports that the best mode of action for a contaminated implant showing clinical signs of infection is to remove the implant as soon as possible in order to effectively resolve the current infection as well as any future sequelae of SSI,[83] but early implant removal is not always possible since the osteotomy site must be adequate healed before the implant can be safety removed.

1.6.3 - Pathogens

The most common bacteria isolated from TPLO SSI are coagulase positive *Staphylococcus spp.* (Table 1.13),[60,64,68,76,83] particularly *Staphylococcus*.[36,38,92,93] A variety of other pathogens are less commonly involved, including a range of Enterobacteriaceae and *Enterococcus* spp.[64,68] There is increasing concern about antimicrobial resistance in veterinary medicine in general, and TPLO infections in particular. Methicillin-resistant staphylococci have been reported as leading causes of

TPLO SSI in recent studies[60,83] and these infections may be difficult to manage because of the limited antimicrobial options.

Table 1.13: Bacteria isolated from surgical site infections following tibial plateau leveling osteotomy in dogs.

Author	Bacteria	N (%)
Savicky *et al*, 2013[83]	*Staphylococcus pseudintermedius*	26 (32.9)
	Methicillin-resistant *Staphylococcus pseudintermedius*	20 (25.3)
	Methicillin-resistant *Staphylococcus aureus*	15 (19)
	Coagulase negative staphylococci spp.	10 (12.7)
	Pseudomonas aeruginosa	8 (10.1)
Etter *et al*, 2013[64]	*Staphylococcus (pseud)intermedius*	5 (21.8)
	Methicillin-resistant *Staphylococcus (pseud)intermedius*	1 (4.3)
	Staphylococcus aureus	7 (30.5)
	Methicillin-resistant *Staphylococcus aureus*	1 (4.3)
	Pseudomonas aeruginosa and Enterococus sp.	1 (4.3)
	Enterococcus sp.	3 (13.1)
	Corynebacterium sp.	2 (8.8)
	Seratia marcescens	1 (4.3)
	Klebsiella pneumonia	1 (4.3)
	Escherichia coli	1 (4.3)
Gallagher *et al*, 2012[68]	*Staphylococcus* spp.	7 (33.3)
	Methicillin–resistant *Staphylococcus*	2 (9.5)
	Non-hemolytic coagulase negative *Staphylococcus*	3 (14.3)
	Hemolytic coagulase negative *Staphylococcus*	2 (9.5)
	Enterococcus spp.	3 (14.3)
	Actinomyces spp.	1 (4.8)
	Corynebacterium spp.	1 (4.8)
	Serratia marcesens	2 (9.5)
Thompson *et al*, 2011[76]	*Staphylococcus* spp.	64 (63.4)
	Pseudomonas spp.	16 (15.8)
	Coagulase negative Staphylococcus spp.	9 (8.9)
	Beta Haemolytic Streptococcus spp.	5 (4.9)
	Corynebacterium spp.	2 (2)
	Escherichia coli	1 (1)
	Enterococcus spp.	1 (1)
	Acinetobacter spp.	1 (1)
	Stenotrophomonas spp.	1 (1)
	Bacillus spp.	1 (1)
Fitzpatrick *et al*, 2010[60]	*Staphylococcus aureus*	17 (38.6)
	Methicillin-resistant *Staphylococcus aureus*	4 (9.2)
	Staphylococcus (pseud)intermedius	6 (13.6)
	Coagulase negative *Streptococcus* spp.	6 (13.6)
	Pseudomonas aeruginosa	7 (15.9)
	Actinobacter spp.	3 (6.8)
	Escherichia coli	1 (2.3)

1.6.4 - Protective Effects

Little is known regarding preventive measures or protective effects that are directly related to TPLO. Many of the guidelines for preventive measures taken for orthopaedic surgeries involving an implant in human and veterinary medicine are also followed when performing a TPLO.[73] There have been a small number of protective effects for reducing the likelihood of developing SSI following TPLO in the veterinary literature. They consist of the administration of postoperative antimicrobials following surgery and if the dog is a specific breed (Table 1.14).[60,62,77,106]

Three recently published canine studies indicated a protective effect from the administration of postoperative antimicrobials against the development of SSI following TPLO, regardless of which class of antimicrobial was used. These findings are contradictory to most general recommendations that antimicrobial therapy should be discontinued within 24 hours of surgery.[44,60,62,77] These three studies were not designed to assess the protective effect of administration of postoperative antimicrobials and therefor an appropriately designed study should be developed in order to assess the role of postoperative antimicrobials in reducing the likelihood of developing a SSI following TPLO.

A protective effect related to the breed of dog having surgery has been reported to reduce the likelihood of developing SSI following TPLO.[60,106] Two studies have documented that Labrador Retrievers are at a reduced risk of developing SSI following TPLO when compared to all other breeds of dog. [60,106] The relationship behind this finding is still unclear, and further investigation is warranted.

Table 1.14: Protective factors to reduce the likelihood of development of surgical site infections following tibial plateau leveling osteotomy.

Author	**Protective Effect**
Gatineau *et al*, 2011[77]	Postoperative administration of antimicrobials
Frey *et al*, 2010[62]	Postoperative administration of antimicrobials for 3 – 14 days (any class)
Fitzpatrick *et al*, 2010[60]	Postoperative administration of antimicrobials, Labrador Retrievers
Pacchania *et al*, 2003[106]	Labrador Retrievers

1.7: Thesis Objectives and Hypotheses

The purpose of this research is to collect information on current antimicrobial prophylaxis practices and the prevalence of preoperative MRSP carriage in dogs, while identifying potential factors associated with the development of SSI following TPLO.

Objectives:

- Retrospectively evaluate perioperative antimicrobial administration in dogs undergoing TPLO
- Determine the surgical site infection rate following TPLO at the OVCHSC
- Identify factors associated with SSI development following TPLO
- Prospectively evaluate the SSI rate in a heterogeneous and geographically diverse population of dogs undergoing TPLO
- Determine overall and site-specific prevalence of per-operative carriage of MRSP in dogs undergoing TPLO in multiple veterinary referral centres
- Determine MRSP carriage following TPLO at multiple veterinary referral centres
- Evaluate the impact of MRSP carriage on the SSI rate following TPLO at multiple veterinary referral centres

Hypotheses:

- Current antimicrobial prophylaxis practices in dogs undergoing TPLO can be improved
- The SSI rate following TPLO at the OVCHSC will be between 2.5-15.8%
- Factors associated with the development of SSI following TPLO such as duration of surgery and anaesthesia will be identified
- The SSI rate following TPLO in the prospective multicentric study will be between 2.5-15.8%
- Methicillin-resistant *Staphylococcus pseudintermedius* carriage with be identified in 1-7% of dogs undergoing TPLO
- Preoperative MRSP carriage will be a risk factor for the development of SSIs following TPLO
- Methicillin-resistant *Staphylococcus pseudintermedius* carriage will be apparent in dogs that were not preoperatively colonized with MRSP

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Chapter 2

Perioperative administration of antimicrobials during tibial plateau leveling osteotomy

2.1: Perioperative Administration of Antimicrobials during TPLO

Perioperative Administration of Antimicrobials during Tibial Plateau Leveling Osteotomy

Alim Nazarali[1] BSc, Ameet Singh[1] DVM, DVSc, Diplomate ACVS, and J Scott Weese[2] DVM, DVSc, Diplomate ACVIM

[1]Department of Clinical Studies and [2]Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Canada.

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2.2: Abstract

Objective: To evaluate perioperative antimicrobial administration during tibial plateau leveling osteotomy (TPLO) in dogs at the Ontario Veterinary College Health Sciences Centre..

Study Design: Retrospective case series

Animals: Dogs (n=184) undergoing TPLO (n=226)

Methods: Medical records were reviewed and data collected included timing and dosage of pre-, intra- and postoperative antimicrobial administration, method of stifle inspection, duration of surgery, duration of anesthesia, development of surgical site infection (SSI), microbiological investigation, implant removal, and possible co-morbidities. Univariable analysis was conducted, followed by stepwise forward logistic regression to determine factors associated with SSI.

Results: Of the 225 cases administered perioperative antimicrobials, only 96 (42.5%) received appropriate perioperative antimicrobial prophylaxis based on target times for preoperative and intraoperative dosing. Postoperative antimicrobials were administered to 54 (23.9%) of cases. Surgical site infection was documented in 30 (13.3%) cases. *Staphylococcus pseudintermedius* was isolated from 15/17 (88.2%) SSI from which a bacterium was isolated, with 6/15 (40%) being methicillin-resistant *Staphylococcus pseudintermedius* (MRSP). Postoperative administration of antimicrobials was protective for SSI (OR 0.1367; *P*= .0001; 95%CI= 0.021, 0.50). Duration of anesthesia time was associated with the likelihood of development of SSI. (OR = 1.0094; *P* =.001; 95%CI = 1.00, 1.02).

Conclusion: Current practices for administration of antimicrobial prophylaxis during TPLO can be improved. There was no association between timing of antibiotic administration that was inconsistent with the target and development of SSI. Further study into risk factors of TPLO SSI is required.

2.3: Introduction

Tibial plateau leveling osteotomy (TPLO) is one of the most commonly performed surgical techniques to stabilize a cranial cruciate insufficient stifle in dogs.[1] Despite being classified as a clean surgical procedure, TPLO has been associated with increased risk of surgical site infection (SSI) compared to other clean procedures, with incidences ranging from 0.8-14.3%.[2-9] Reasons for the apparently high rate of TPLO SSI are unclear and likely multifactorial. Potential factors include thermal damage by the saw blade used to perform the osteotomy, minimal soft-tissue coverage of the proximal aspect of the tibia, excessive soft tissue dissection at surgery, presence of an implant, aggressive periosteal dissection, prolonged surgery and anesthesia times, periosteal compression by the implant and increasing prevalence of opportunistic pathogens (particularly staphylococci) that are resistant to antimicrobials used for perioperative prophylaxis.[6-11] Whereas there are no accepted standards, perioperative prophylaxis is commonly used with TPLO and has been considered as a treatment to minimize SSI. Various factors affect the potential efficacy of antimicrobial prophylaxis. One is timing of administration, an area that receives major emphasis in human surgery.[12-14] The primary goal of antimicrobial prophylaxis is to have therapeutic levels present before incision and maintained throughout the surgical procedure. Standard recommendations from human medicine are to administer an appropriately selected antimicrobial at a maximum of 1 hour before first incision and then to discontinue the use of antimicrobials within 24 hours after procedure completion.[12] To maintain therapeutic levels during surgery, time-dependent antimicrobials such as beta-lactams are re-dosed intraoperatively every 2 half-lives.[12]

Similar guidelines are not available for veterinary surgery yet the concepts of antimicrobial prophylaxis should apply equally across species. However, there has also been limited scrutiny of current perioperative antimicrobial prophylaxis practices

performed in small animal surgery within the veterinary literature[16,17] and none specifically directed at TPLO. Thus our purpose was to evaluate perioperative antimicrobial use during TPLO at the Ontario Veterinary College Health Sciences Centre (OVCHSC).

2.4: Materials and Methods

Dogs

Medical records (January 1, 2008 - December 31, 2010) at the OVCHSC were reviewed to identify all dogs that had a unilateral TPLO. These dogs were eligible for study inclusion. Dogs that had 2 separate TPLO procedures on different dates were considered independent cases.

Data Collection

Data retrieved included timing and dosage of pre-, intra-, and postoperative antimicrobial administration, method of stifle inspection (open mini-arthrotomy or stifle arthroscopy or both), duration of surgery, duration of anesthesia, presence of postoperative SSI, microbial investigation (in cases of SSI), implant removal, and possible co-morbidities (e.g. atopic dermatitis, hypothyroidism). Criteria for diagnosis of SSI were based on standard definitions established by the US Centers for Disease Control and Prevention's (CDC) Hospital Infection Control Practices Advisory Committee[18] (Table 1).

A target time of antimicrobial administration was preoperative administration of antimicrobials 60 minutes before incision and every 90 minutes intraoperatively thereafter, based on established criteria in human surgical practice.[12] The 90 minute re-dosing interval was based on the ubiquitous use of cefazolin for perioperative prophylaxis at this facility and its half-life in dogs.[15]

Table 2.1: Criteria for diagnosis of surgical site infection (SSI).[18]

Category	Criteria
Superficial SSI	Within 30 days Skin and/or subcutaneous tissues 1 or more of: - pus - bacteria - diagnosis by a surgeon -heat, redness, pain OR localized swelling AND incision reopened by surgeon UNLESS culture negative
Deep SSI	Within 30d, 1 year if implant Deep soft tissues of the incision 1 or more of: - pus - spontaneous dehiscence of deeper incision OR incision is deliberately opened when patient has fever, localized pain or tenderness UNLESS culture negative - Abscess or other evidence of infection on imaging or histology
Organ/Space SSI	Within 30 days, 1 year if implant Any area other than the incision that was encountered during surgery 1 or more of: - pus - bacteria - Abscess or other evidence of infection upon exam, re-operation, histology or imaging

Data Analysis

Pearson's χ^2 or logistic regression analysis was used for univariable analysis of factors associated with SSI. Variables with a *P* value of <0.2 were selected for multivariate analysis. Stepwise forward logistic regression was performed. Insignificant variables were not retained in the model unless they were considered to be confounders. Confounders were identified by observing the changes in coefficients in other variables when the target variable was removed. If a change of >20% occurred for any variable, the confounder was forced into the final model. Two way interactions were tested and retained in the model if significant. Duration of anesthesia was forced into the model

because of its relationship with SSI.[19] A *P* value of < 0.05 was considered to be significant for the final multivariable model. Pearson's residuals were examined to identify any outliers that required confirmation that there was no data collection or entry error.

2.5: Results

Dogs (n = 184) undergoing TPLO (n = 226) ranged in age from 1 to 13.5 years (mean ± SD, 5.17 ± 2.45 years). Weight ranged from 16 - 108.1 kg (mean, 38.4 ± 12.85 kg). Perioperative cefazolin was administered to 225 (99.6%) cases; 1 case was not administered a perioperative antimicrobial. Of 225 cases administered cefazolin, only 96 (42.5%) received appropriate perioperative antimicrobial prophylaxis based on target times and dose for preoperative and intraoperative administration using guidelines established in human surgical practice.[12] Sixteen of 225 (7.1%) did not meet a minimum dose of cefazolin (20mg/kg) with doses ranging from 15.38 – 19.67 mg/kg (mean, 18.22 ± 1.39 mg/kg). Fifty-four of 225 (24%) cases received their initial dose within 30 minutes of the incision being made and 173 (76.9%) cases received their initial dose within 60 minutes of the start of the procedure. Preoperative dosing > 60 minutes before incision occurred in 37/225 (16.4%) cases, ranging from 60 - 100 minutes (mean, 78.24 ± 9.37 minutes). Fifteen (6.6%) cases received their first dose after incision (mean, 19 ± 25.7 minutes; range, 5-105 minutes).

Based on the time of initial administration and duration of surgery, intraoperative dosing of antimicrobials was indicated for 201 cases. One or more intraoperative doses were administered to 188/201 (93.5%) of these cases. A mean of 1.21 ± 0.42 intraoperative doses were administered (range, 0-3 doses). Intraoperative dosing was administered within 90 minutes of the previous dose for 134/188 (71.3%) cases. For

54/188 cases (28.7%), the intraoperative dose was administered >90 minutes after the previous dose. The range of late intraoperative dosing was between 105 – 165 minutes after the initial dose, with a mean of 118.4 minutes. When all cases are included, the mean interval for the first dose of intraoperative antimicrobials was 94.8 ±14.36 minutes (range, 45-165 minutes) from the previous dose.

Postoperative antimicrobials were administered to 54/226 (23.9%) of cases, all of which received cephalexin. Duration of prescribed treatment ranged from 5 - 30 days (mean, 11.32 ± 4.2 days).

SSI was documented in 30/226 (13.3%) dogs. Samples were submitted for bacterial culture in 26 cases, with bacteria recovered from 17 (65.4%) dogs (Table 2). *Staphylococcus pseudintermedius* was isolated from 15 (88.2%) SSI; 6 (40%) were methicillin-resistant *Staphylococcus pseudintermedius* (MRSP). Implant removal was performed in 24 (80%) SSI cases.

Table 2.2: Bacterial culture results for cases with surgical site infection after TPLO.

	# of cases	%
Staphylococcus pseudintermedius	9	30
Methicillin-resistant *Staphylococcus pseudintermedius*	6	20
Methicillin-resistant *Staphylococcus epidermis*	1	3.4
*Enterococcus spp.**	1	3.4
*Escherichia coli**	1	
No Growth	9	30
No Culture Submitted	4	13.3

* - Isolated from same SSI

Univariable data (Table 3) and the final multivariate model (Table 4) is presented. In the multivariate model, postoperative administration of antimicrobials was protective (OR 0.1367; *P* = .0001; 95% CI = 0.021, 0.50), while anesthesia time was associated with the likelihood of development of SSI (OR = 1.0094; *P* = .036; 95% CI = 1.00, 1.02). The combination of stifle arthroscopy + arthrotomy was forced into the model because it was acting as a confounder.

When logistic regression was performed, there was no impact of timing of the first antimicrobial dose on SSI occurrence, although the P value approached significance (P = .075). When plotted, an increase in SSI occurrence is observed when the first dose was administered over 100 minutes from the time of surgery (Figure 1). When dogs that received intraoperative antimicrobials are plotted separately from those that did not (Figure 2), there is an apparent earlier increase in SSI occurrence in dogs that did not receive intraoperative antimicrobials, with the occurrence appearing to increase when the first dose was administered ≥ 60 minutes before surgery.

Table 2.3: Univariable analysis of variables predicted to be associated with surgical site infection (SSI) after TPLO. Pearson's χ^2 Test and Logistic Regression analysis was used for their appropriate variables. Outcome variable is SSI.

Variable	**Number (Percentage)**	***P*-value**
Target timing of antimicrobials within 60 minutes of incision	96/226 (42.5%)	0.371
Target timing of antimicrobials within 30 minutes of incision	35/226 (15.6%)	.848
Prophylactic antibiotics administered	225/226 (99.6%)	0.695
Intraoperative dosing indicated	201/225 (89.3%)	.410
Intraoperative dosing administered	188/201 (93.5%)	.656
Arthroscopy and arthrotomy	35/226 (15.5%)	0.168
Arthrotomy	172/226 (76.1%)	.713
Arthroscopy only	19/226 (8.5%)	0.075
Postoperative antimicrobials	54/226 (23.9%)	0.018
Co-morbidities	15/226 (6.6%)	0.435
Duration of surgery		0.897
Duration of anesthesia		0.363

Table 2.4: Stepwise forward logistic regression analysis of variables predicted to be associated with surgical site infection (SSI). Arthroscopy and Arthrotomy were forced into the model because of being a confounding variable. Outcome variable is SSI.

Variable	**Odds Ratio**	***P*-value**	**95% Confidence Interval**
Postoperative Antimicrobials	0.1367	0.0001	0.02 – 0.50
Anesthesia Time (min)	1.0094	0.036	1.001 – 1.013
Arthroscopy + Arthrotomy	0.512	0.530	0.056 – 4.670
Arthroscopy	0.446	0.278	0.066 – 1.785

Figure 2.1: Logistic regression evaluating the impact of timing of the first antimicrobial dose on SSI occurrence (*P* = .075).

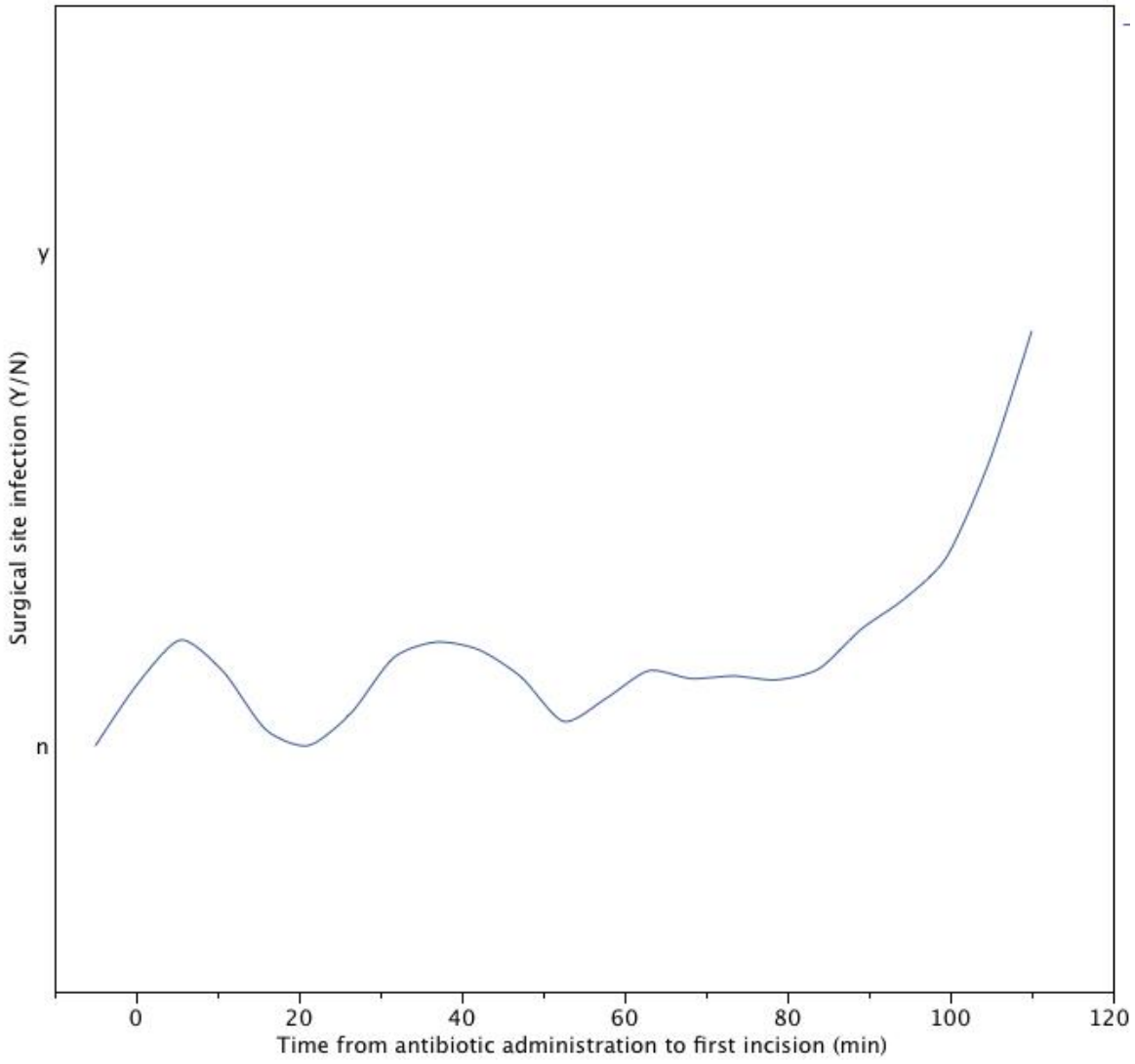
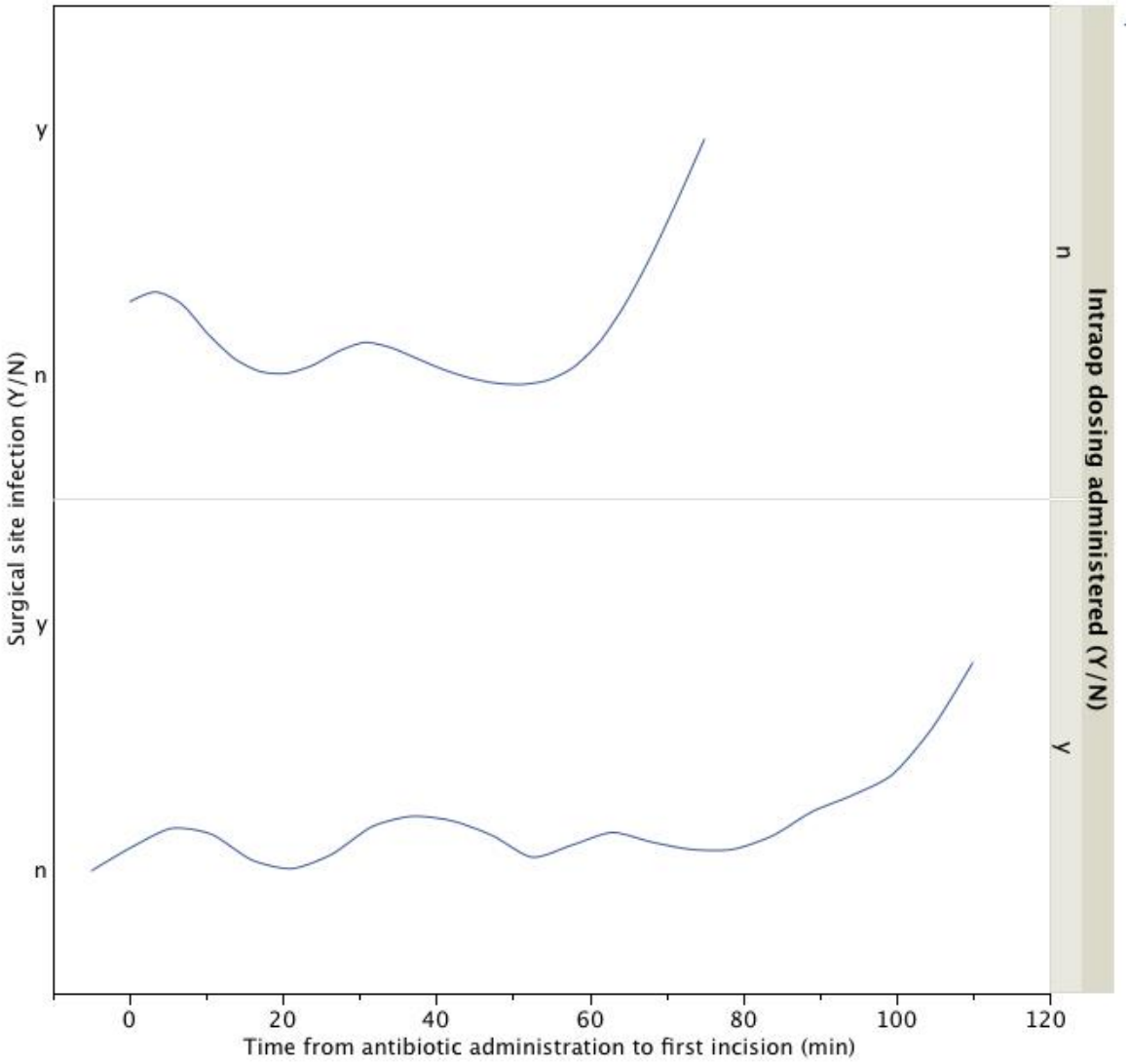


Figure 2.2: Logistic regression evaluating the impact of timing of the first antimicrobial dose on SSI occurrence with dogs receiving intraoperative dosing separated.



2.6: Discussion

It was unsurprising that perioperative antimicrobials were used in virtually every TPLO in this study. Whereas controlled studies have not been performed to indicate a need of perioperative antimicrobials in this clean procedure, antimicrobials are widely

used internationally for this procedure because of the high apparent SSI rate and the implications of implant-associated SSI.[2-9]

The 13.3% SSI rate reported here is consistent with other studies.[2,4,6,7] When the SSI incidence rates reported here and elsewhere are considered in the context of the commonness of the use of TPLO for stabilization of the cranial cruciate ligament insufficient stifle, the impact of TPLO SSI is clear. Thus, measures to reduce the incidence and impact of TPLO SSI are needed. Understanding how SSI develop and factors that are associated with SSI (either risk factors or protective factors) is important to develop and test effective interventions.

In our study, variable administration of perioperative antimicrobials was noted. Considering only 96 (42.5%) dogs met targets of timing and dose of prophylactic antimicrobial administration, there is indication of much needed room for improvement in standard practices. Late initial doses ranged from 5 – 105 minutes after incision, with most being within 15 minutes after incision. Therefore, while later than desired, most dogs would have had adequate concentrations of antimicrobials at the surgical site at the time of implant placement. While disappointing, this is consistent with some reports from the human medical literature. For example, even when considering preoperative doses within 120 minutes before incision, only 60% of patients had been given adequately timed doses in a study of 2847 individuals.[12] Similarly, Braztler *et al* showed that only 55.7% of 34,133 surgical patients received antibiotics within 60 minutes before incision.[20] Whereas the impact of timing of perioperative antimicrobial therapy on SSI has not been determined for TPLO, it is reasonable to assume that deviation from standard human recommendations could be accompanied by some increase in SSI risk. Whereas timing was not identified as a risk factor in our study, it is possible that lack of statistical power rather than a true lack of influence was the reason. However, a recent study in over 32,000 people has shown that timing of antimicrobial administration was

not associated with increased SSI risk and adhering to timing protocols may not reduce their incidence.[21] Regardless, since improving timing of antimicrobials can potentially be achieved with little to no cost disruption, it should be considered. One potential method to improve antimicrobial timing is the use of a preoperative checklist, as is increasingly used in people,[22,23] which can help ensure prophylactic treatment be given before the start of the procedure.

An important aspect of perioperative prophylaxis is intraoperative re-dosing because the short half-life of commonly used drugs such as cefazolin, meaning that non-therapeutic levels would be present throughout much of the surgery if only a single preoperative dose was administered. Indeed, if an antimicrobial was administered 60 minutes before incision, there could be little to no effect left at the time of implant placement, a likely critical time. In our study, re-dosing compliance was excellent in terms of the incidence of re-dosing (93.5%); however, 28.4% of dogs received antimicrobials late, with the dose being administered ≥ 30 minutes later in 28% of those. It was interesting to note that when the time from administration of the first antibiotic dose increased from time to incision, the SSI rate appeared to increase (Figure 1). Whereas this was not statistically significant, the *P* value was suggestive and this result is consistent with a recent study in people that identified a similar trend when data were analyzed continuously compared with typical categorical analysis.[21] It was interesting that the graphs were different when intraoperative dosing was taken into consideration (Figure 3). Again, any conclusions must be tempered with the lack of statistical significance, but this requires further study. From a biological standpoint, this is plausible since the impact of early preoperative dosing would presumably be blunted or negated by proper intraoperative dosing to maintain therapeutic drug levels through the time of surgery. Conversely, early preoperative dosing in dogs that did not receive intraoperative

dosing would result in potentially extended periods of time during surgery, including the critical time of osteotomy and implant placement, of sub-therapeutic drug levels.

Postoperative administration of antimicrobials is a controversial subject, with increasing concern about excessive or inappropriate antimicrobial therapy. Routine postoperative treatment beyond 24 hr is not recommended in people undergoing clean surgical procedures[12,18,24,25] as this practice has not been shown to reduce SSI rates and may contribute to the development of antimicrobial resistance and additional morbidity.[25-27] However, whereas it is reasonable to look to well-designed human studies for guidance, there may be numerous differences in surgical procedures, patient factors, pathogen exposure and patient care between human and veterinary medicine. The protective effect of postoperative antimicrobials noted here is consistent with 2 recent canine TPLO studies[2,6] which indicated a protective effect of 3 – 14 days of postoperative antimicrobial administration. None of these studies were designed to specifically address the efficacy of postoperative antimicrobials, and the need for a proper controlled study is indicated. The importance of doing so is to understand both the potential impact on TPLO SSI and parallel concerns about antimicrobial use and antimicrobial resistance in animals.[25-27] We were unable to assess optimal postoperative practices (i.e. drug, duration), an area that also requires additional study, since minimizing duration of postoperative treatment is ideal to lessen concerns about antimicrobial resistance and adverse effects in patients.

Another consideration is whether postoperative antimicrobials are effective because of deficiencies in surgical practices and infection control. As a relatively well designed facility with highly trained surgical personnel and an established infection control program, no clear deficiencies in SSI prevention measures were apparent. It cannot be excluded that perioperative antimicrobials had an impact because of deficiencies in perioperative administration, but this seems unlikely given the lack of a

detectable effect of peri- or intraoperative dosing on SSI as well as recent data from people. It is possible, therefore, that there is a true protective effect of postoperative antimicrobial therapy, something that requires evaluation through a randomized controlled clinical trial.

Prolonged anesthesia time increased the likelihood of a dog developing a SSI. Studies have shown similar associations with increased surgical and anesthesia time. Vasseur *et al* showed that surgical procedures requiring > 90 minutes to complete have a greater risk of SSI possibly because of increased bacterial contamination, excessive tissue retraction, and tissue dehydration, which would decrease the host's own ability to fight infection.[28] Nicholson *et al* reported similar results where prolonged surgical time (not anesthesia time) was a risk factor for development of SSI.[30] Although rate of SSI could not be correlated to surgical time in 2 other studies, it was noted that prolonged anesthesia time was a significant risk factor.[19,30] Whereas rushing a surgical procedure should not be considered as a means to reduce anesthetic time, this is an area that could be improved by increased efficiency to reduce any post-induction delays associated with organizing the operating room or surgical personnel, or waiting for intraoperative diagnostic imaging.

Our study relied on retrospective review of the medical record to identify SSI. Reliance on medical record data is concerning because of the potential for underreporting of SSI, such as might occur if a patient is seen by their primary care veterinarian for treatment of SSI and this information is not passed on back to the surgical team. This would result in an underestimation of SSI rate and potentially reduce the ability to detect significant differences if large numbers of SSI cases were misclassified.

Microbial sampling was performed in 26/30 (86.7%) SSI in our study. The 4 cases in which microbial sampling was not performed were considered SSI based on the

criteria established by the CDC which states that a wound can be deemed infected if a surgeon decided to reoperate because of concerns of infection (Table 1).[18] A positive bacterial culture was obtained in 17/26 (65.4%) SSI that were sampled. Nine of 26 wounds were classified as SSI despite a negative culture as these cases were returned to surgery at time of re-evaluation because of clinical signs consistent with SSI.[18] Potential reasons for negative bacterial culture include difficulty obtaining a representative culture specimen from focal deep infections, the presence of biofilm-embedded bacteria, the presence of fastidious bacteria and loss of bacterial viability from sample collection to testing.

It was unsurprising that *S. pseudintermedius* was the main identified cause of SSI in our study because it is the leading canine opportunistic pathogen.[31-33] The high prevalence of methicillin-resistance was concerning because of the limited treatment options but unfortunately was unsurprising given the commonness of MRSP in SSI and other opportunistic infections in dogs internationally.[32-34] The combination of a high infection rate, presence of an implant which hampers medical therapy and highly drug resistant MRSP is of substantial concern.[8-11,31] The inherent resistance of MRSP to beta-lactams (and therefore the pre-, intra- and postoperative antimicrobials used in this study) raises another concern, since current perioperative prophylaxis practices will have no effect on this leading SSI pathogen.

There has been increasing attention paid to TPLO SSI and associated factors in recent years because of the commonness of this procedure, the high incidence of SSI and the potential patient health and economic implications of TPLO SSI. Studies such as this are required to evaluate current practices and identify potentially modifiable factors (e.g. perioperative antimicrobial timing, postoperative antimicrobial administration) that might be targeted for interventions to reduce SSI rates. It is unrealistic to think that TPLO

SSI will be eliminated; however, application of a good surgical and infection control plan may be able to reduce the incidence and impact of this common complication.

2.7: Disclosure

The authors report no financial or other conflicts related to this report.

2.8: References

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Chapter 3

The impact of methicillin-resistant *Staphylococcus pseudintermedius* carriage on surgical site infections in dogs undergoing tibial plateau leveling osteotomy

3.1: Acknowledgments

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3.2: Abstract

Objective: To evaluate preoperative methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) carriage and its effect on the development of surgical site infections (SSIs) following tibial plateau leveling osteotomy (TPLO).

Study Design: Prospective Multicentre Study

Animals: Dogs (n=549) undergoing TPLO

Procedures: Dogs admitted for TPLO were swabbed for MRSP in a prospective multicentre study involving seven hospitals from Canada and the United States. Data collected included preoperative antimicrobial administration, potential co-morbidities, dog contact and postoperative antimicrobial administration. Univariable analysis was conducted, followed by stepwise backward logistic regression to determine factors associated with preoperative MRSP carriage, MRSP SSI, overall SSI and postoperative MRSP carriage.

Results: Of the 549 dogs included in the study, 24 (4.4%) were preoperatively carrying MRSP at one or more body sites. Risk factors associated with MRSP carriage included bulldog breed (OR = 14.06, p = 0.001, 95% CI = 2.974 – 66.426) and increasing weight in kg (OR = 1.094, p = <0.0001, 95% CI = 1.030 – 1.096). Surgical site infection developed in 37 (6.7%) dogs, with MRSP responsible for 11 (29.7%) of SSIs. Preoperative MRSP carriage was the only identified risk factor associated with increased likelihood of MRSP SSI (OR = 14.8, p = <0.0001, 95% CI = 4.005 – 54.695). A protective effect of postoperative antimicrobials (OR = 0.285, p = 0.007, 95% CI = 0.088 – 0.711) against overall SSI was noted.

Conclusions and Clinical Relevance: It has been determined that MRSP carriage is a risk factor for MRSP SSI and therefore investigation into measure to rapidly identify MRSP carriers and develop interventions aimed at decreasing the risk of MRSP SSI in carriers are indicated. These data provide further support of the efficacy of postoperative antimicrobials for prevention of TPLO SSI.

3.3: Introduction

One of the most commonly performed surgical techniques to stabilize a cranial cruciate insufficient stifle in dogs is the tibial plateau leveling osteotomy (TPLO).[1] Tibial plateau leveling osteotomy is considered a clean surgical procedure, but has been associated with high surgical site infection (SSI) rates compared to other clean procedures, with published rates ranging from 2.5-15.8%.[2-11] The impact of TPLO SSI can be devastating, with consequences affecting patient recovery, limb function, treatment costs and causing frustration for the client and clinician alike. [4,5,7,8,10-12] A recent study by Nicoll *et al* reported an average postoperative cost of $1559 for dogs that suffered from a SSI following TPLO, compared to an average cost of $212 for dogs that recovered without complication.[12] It is currently unclear as to why TPLO is plagued by such a high SSI rate, but it is likely multifactorial and may include factors such as periosteal dissection, presence of an implant, prolonged surgery and anaesthesia times and increasing prevalence of antimicrobial-resistant opportunistic pathogens that are not affected by perioperative prophylaxis. [6-8,11,13]

The most common bacteria isolated from TPLO SSI are coagulase positive *Staphylococcus spp.*, predominantly *Staphylococcus pseudintermedius*.[2,7-10] Recently, methicillin-resistant *S. pseudintermedius* (MRSP) has emerged as a predominant cause of TPLO SSI in some regions[7,14], which can complicate treatment because of the extensively resistant nature of many MRSP isolates and resistance to drugs typically used for initial or empirical treatment. All MRSP isolates are resistant to cefazolin, the main perioperative antimicrobial used in canine orthopaedic procedures and is of particular concern given the apparent establishment of MRSP carriage in dogs in the general population, with reported prevalences ranging from 2-7.4%.[15-17]

In humans, the epidemiology of MRSA SSI has been extensively studied and issues pertaining to MRSA SSIs are comparable to those with MRSP SSIS in dogs. A small percentage of humans are MRSA carriers and the role of perioperative colonization on MRSA SSI has received much attention.[18,19] MRSA carriage rates of 0% to 6.8% have been reported for human surgical patients[18-23], and preoperative MRSA carriage is a well identified risk factor for the development of MRSA SSI.[18,19,23] In some regions, this association has led to the practice of preoperatively testing of elective surgical patients, with preoperative decolonization therapy prescribed for colonized

individuals.[24,25] This approach can be effective and one study has shown a 1.8 times reduction in MRSA SSI risk following preoperative treatment with mupirocin nasal ointment[24] Another study, assessing a 5 day preoperative treatment of mupirocin nasal ointment and clorhexidine impregnated wash cloths for MRSA carriers, showed a 72% decrease in the development of MRSA SSI over a three year period.[25]

While MRSP carriage is present in dogs in the population and MRSP is a leading cause of TPLO SSI, the influence of preoperative MRSP colonization on MRSP SSI is unknown. The objectives of this research were to determine the prevalence and site specific patterns of MRSP carriage in dogs undergoing TPLO and to evaluate the influence of preoperative MRSP carriage on SSI following TPLO.

3.4: Materials and Methods

Study Population

A prospective multicentre study involving seven veterinary teaching (n=2) or private referral hospitals (n=5) from Canada (n=6) and the United States (n=1) was performed. All dogs that had a TPLO performed from September 2012 to March 2014 were eligible for inclusion in this study. Dogs that underwent two separate TPLO procedures on different dates were considered independent cases. This study was approved by the University of Guelph Animal Care Committee

Sample collection and processing

Using an aerobic sterile culture swab (Starplex, Etobicoke, ON, Canada), preoperative samples from one naris, pharynx, rectum and skin at the surgical site were individually obtained at the time of admission. A preoperative questionnaire was administered to owners regarding patient information such as preoperative antimicrobial exposure, possible co-morbidities (e.g. atopic dermatitis, hypothyroidism), and amount of interaction with other dogs (e.g. dog contact, visits to dog parks). A second set of swabs was collected, as described above, from patients from three facilities at the time of postoperative recheck (6-8 weeks) to determine postoperative MRSP carriage status.

Microbiological Analysis

Sterile aerobic culture swabs were placed in a test tube containing an enrichment broth consisting of 10g tryptone/L, 75g sodium chloride/L, 10g D-mannitol/L and 2.5g yeast extract/L and incubated at 35^{o}C for 24 hours. One loopful (~10 µl) of broth was then inoculated onto mannitol salt agar with 2µg/mL oxacillin and incubated at 35^{o}C for 48 hours. Colonies that were suspected to be *Staphylococcus pseudintermedius* were then sub-cultured onto Columbia blood agar with 5% sheep blood and incubated at 35^{o}C for 24 hours.

Isolates were presumptively identified as *S. pseudintermedius* by colony morphology, gram stain appearance, catalase and coagulase reactions and negative *S. aureus* latex agglutination test (Pastorex Staph-plus, Bio-Rad, Mississauga, Canada). DNA was isolated through extraction (InstaGene™ Matrix, Bio-Rad ,Hercules, CA) and identification was confirmed by *S. pseudintermedius*-specific polymerase chain reaction (PCR).[26] Positive and negative controls were included with every PCR run.

Methicillin-resistance was confirmed by penicillin binding protein 2a latex agglutination test (MRSA latex agglutination test, Denka Seiken, USE, Inc., Campbell, CA).

MRSP Characterization

MRSP isolates were characterized by sequence analysis of the mec-associated direct repeat unit (dru) typing[27], with dru repeats and types assigned by the Dru-typing.org database (http://www.dru-typing.org/search.php).

Data Collection

Data recorded included timing and dosage of pre, intra and postoperative antimicrobial administration, duration of surgery, duration of anaesthesia, presence of postoperative SSI, culture results (when applicable), and the need for implant removal. Criteria for diagnosis of SSIs were based on standard definitions established by the United States Centers for Disease Control and Prevention (CDC).[28] This consists of incisions with pus, incisions with heat, redness and swelling that have been re-opened by a surgeon and incisions with positive bacterial culture results with 30 days post-operation (1 year if implant was placed). Active surveillance was performed by contacting owners of all animals that underwent TPLO by telephone 30 days following their pet's procedure. This information, combined with recheck appointments, was used

to identify cases that fulfilled the SSI definition criteria. One year followup was performed on a subset of patients, consisting of 286 dogs from 4 of the participating hospitals that had surgery between September 2012 and July 2013 (or: that had recovered for a year by June 2014).

Data Analysis

Pearson's chi squared, Fischer's exact test and/or logistic regression analysis were used for univariable analysis of factors associated with preoperative MRSP colonization, postoperative MRSP colonization, MRSP SSI development and overall SSI development. Variables with a *P* value of <0.20 were selected for multivariable analysis. Stepwise backward logistic regression was performed. Insignificant variables were not retained in the model unless they were deemed to be confounders. Confounders were identified by observing the changes in coefficients in other variables after removing the target variable. The confounder was forced into the final model if a change of >20% occurred for any variable. Two way interactions were tested and were retained in the model if they were deemed significant. A P value of < 0.05 was considered to be significant for the final multivariable model. Due to the small number of events per outcome variable, a multiple subset logistic regression was also conducted and compared to the backwards stepwise logistic regression.[29] Pearson's residuals were examined to identify any outliers that required confirmation that there were no errors made during data collection or entry.

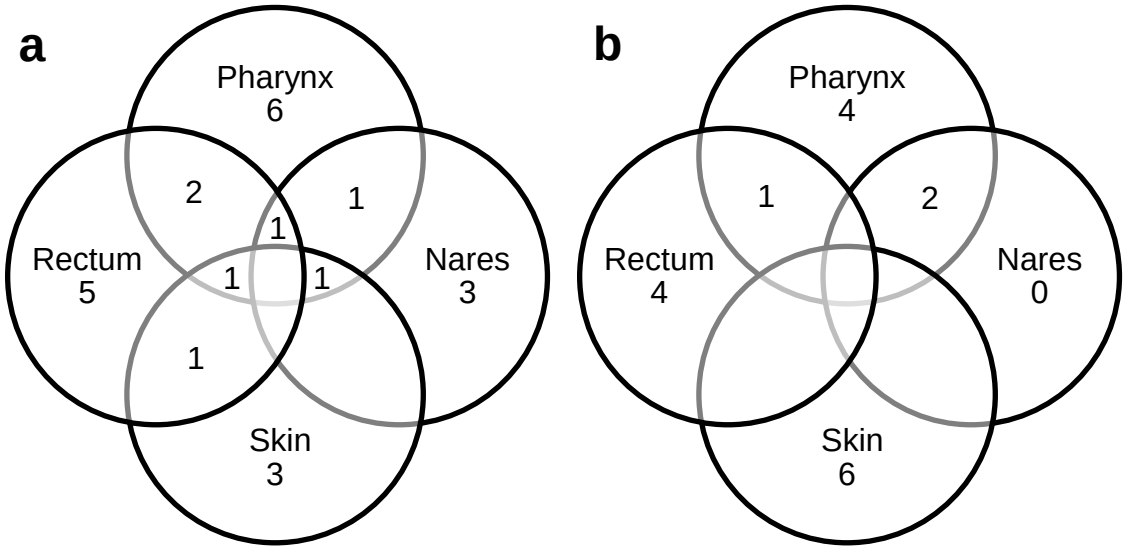
3.5: Results

Five hundred and forty nine dogs were enrolled. The age of dogs ranged from 11 months to 13.1 years (Mean +/- SD, 5.53 +/- 2.65). Weights ranged from 5.6kg – 81kg (37.4 +/- 11.8). Seventy-four breeds were represented, with the most common being mixed breeds (113, 20.6%), Labrador retrievers (101, 18.4%) and golden retrievers (38, 6.9%). The right leg was operated on in 256 (46.7%) cases, the left leg in 258 (46.9%) cases, both legs in 27 (4.9%) cases, and information on the operated side could not be obtained for 9 cases (1.6%). Patients in the study included 277 (50.5%) spayed females, 250 (45.5%) castrated males, 11 (2%) intact females, 9 (1.6%) intact males and for 2 cases (0.4%) the sex was not obtained. Perioperative antimicrobials were used for all

procedures and this parameter was not evaluated, despite its potential relevance to TPLO SSI. Postoperative antimicrobials were administered to 398/549 (72.5%) dogs, with a median of 10 days postoperatively (range: 12 hours to 21 days).

Twenty-four dogs (4.4%) were preoperatively carrying MRSP, 12 (2.2%) in the pharynx, 6 (1.1%) in the nares, 10 (1.8%) in the rectum and 6 (1.1%) on the skin. In 17/24 (70.1%) animals, MRSP was isolated from only one body site, the pharynx (n=6), nares (n=3), rectum (n=5) and skin (n=3) while the other 7 (29.9%) dogs were positive for MRSP at multiple sites (Figure 3.1).

Figure 3.1: Site-specific (a) preoperative and (b) postoperative carriage of methicillin-resistant *Staphylococcus pseudintermedius* in dogs undergoing tibial plateau leveling osteotomy.



Thirty-day followup information was available for all cases, while one year followup data were available for 223/286 (78%). Surgical site infection was identified in 35 (6.4%) dogs within 30 days of surgery, with facility-specific rates ranging from 0% to 15.7% (Table 3.1). A further 2 SSIs were identified at the time of 1 year surveillance, one

at 3 months post-operation and the other at 10 months post-operation. Implants were removed from 25/37 (67.6%) dogs with SSI.

Table 3.1: Incidence of SSI and duration of postoperative antimicrobial use, separated by clinic.

Clinic	Incidence of 30d SSI (%)	Post-op Antimicrobial Use: Range (Mean)
A	24/153 (15.7%)	None: 74 cases 12h – 21d (7d): 79 cases
B	2/129 (1.6%)	10d: all cases
C	0/97 (0%)	4 – 14d (12d): all cases
D	0/57 (0%)	14d: all cases
E	5/41 (12.2%)	7 – 14d (10d): 5 cases
F	1/40 (2.5%)	None: all cases
G	3/32 (9.4%)	</= 24h: all cases

Culture specimens were submitted from 32 (86.5%) SSI cases, and bacteria were isolated from 27 (84.4%) of those. *Staphylococcus pseudintermedius* was the most commonly identified cause of SSI, being isolated from 19/37 (51.4%) cases overall (59% of cases from which a culture was submitted) and MRSP accounted for 57.9% of *S. pseudintermedius* isolates and 34.4% of all culture-confirmed SSIs (Table 3.2).

Postoperative culture swabs were collected from 193/549 (35.2%) dogs at the time of recheck, and MRSP was isolated from 17 (8.8%); 7 (3.6%) from the pharynx, 2 (1%) from the nares, 5 (2.6%) from the rectum and 6 (3.1%) from the skin (Table 3.3). In 14/17 (82.4%) animals, MRSP was isolated from only one body site, the pharynx (n=6), nares (n=3), rectum (n=5) and skin (n=3) while the other 3 dogs were positive for MRSP at multiple sites (Figure 3.1). Twelve of the twenty-four (50%) dogs that were carrying MRSP preoperatively were swabbed at time of recheck, with MRSP isolated from 10/12 (83%) of those dogs versus 7/181 (3.9%) dogs from which MRSP was not initially isolated (P= <0.0001). The prevalence and test-sensitivity of overall site-specific MRSP positive carriage sites (pre and postoperative) was calculated (Table 3.4). No statistically significant difference was identified between the preoperative and postoperative MRSP prevalence values for any of the three clinics that participated in postoperative screening.

Table 3.2: Microbiological evaluation of isolates recovered from surgical site infections in dogs following tibial plateau leveling osteotomy. *Multiple bacteria were isolated from some SSI.

	N /37	**Percentage**
Staphylococcus pseudintermedius (methicillin-resistant)	19 (11)	51.4 (29.7)%
Staphylococcus aureus (methicillin-resistant)	4 (2)	10.8 (5.4)%
Streptococcus spp.	4	9.5%
Enterococcus faecalis	1	2.7%
Enterococcus faecium	1	2.7%
Escherichia coli	1	2.7%
Actinomyces spp.	1	2.7%
Pasturella canis	1	2.7%
No Growth	5	13.5%
No Culture Submitted	5	13.5%

Table 3.3: Preoperative prevalence and postoperative prevalence and incidence of MRSP in dogs undergoing TPLO, separated by clinic.

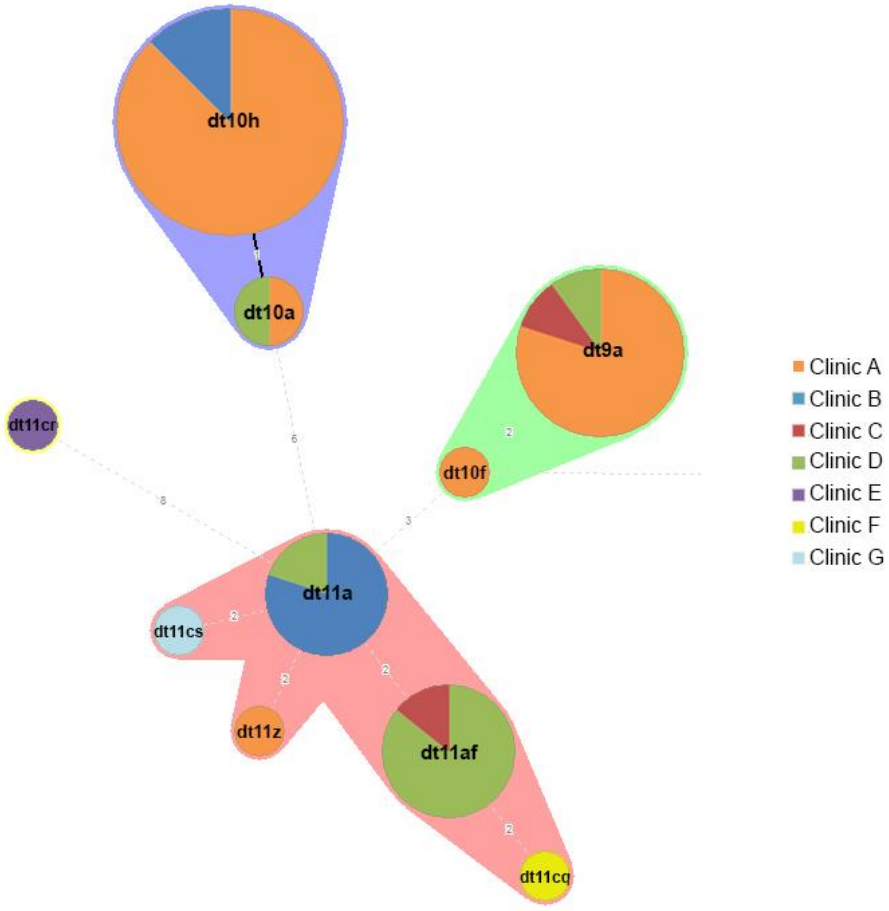
Clinic	**Pre-op MRSP Prevalence (%)**	**Post-op MRSP Incidence (%)**	**Post-op MRSP Prevalence (%)**
A	10/153 (6.5%)	5/138 (3.6%)	12/138 (8.7%),
B	5/129 (3.9%)		
C	2/97 (2.1%)		
D	4/57 (7%)	2/31 (6.5%)	5/31 (16.1%),
E	1/41 (2.4%)	0/24 (0%)	0/24 (0%)
F	1/40 (2.5%)		
G	1/32 (3.1%)		

Table 3.4: Overall site-specific MRSP colonization (pre and post-op) and site-specific sensitivity for isolating MRSP from a positive patient.

Body Site	Site-Specific MRSP carriage / Overall MRSP Carriage = Test Sensitivity (%)
Pharynx	18/41 (44%)
Nares	8/41 (19.5%)
Rectum	15/41 (36.6%)
Skin	12/41 (29.3%)

The most common MRSP dru types were dt9a, dt10h and dt11af (Figure 3.2). Nine of ten dogs that were colonized both pre- and postoperatively harboured the same dru type. Nine of ten dogs that were positive on multiple sites at one sampling time harboured the same dru type at all sites.

Figure 3.2: Minimum spanning tree of dru types for recovered MRSP isolates.



Univariable data are presented in Table 3.5. In the multivariable model for preoperative MRSP carriage, bulldog breed (OR = 14.06, p = 0.001, 95% CI = 2.97 – 66.4), hypothyroidism (OR = 5.02, p = 0.05, 95% CI = 1 – 25.1) and increasing weight in kg (OR = 1.09, p = <0.0001, 95% CI = 1.03 – 1.1) were associated with increased odds of MRSP carriage. Dogs that visited dog parks (OR = 0.33, p = 0.024, 95% CI = 0.12 – 1.01) were associated with a lower odds of MRSP carriage, although unfortunately the confidence interval bridges 1.

When factors associated with postoperative MRSP carriage were assessed, increasing weight in kg (OR = 1.07, p = 0.023, 95% CI = 1.01 – 1.13) preoperative MSRP carriage (OR = 97.2, p = <0.0001, 95% CI = 16.3-578) were associated with increased odds of MRSP carriage. When site-specific preoperative MRSP carriage was used instead of overall preoperative MRSP carriage, preoperative MRSP carriage of the pharynx (OR = 58.9, p = 0.001, 95% CI = 5.42 – 641) was identified as a risk factor for the development of MRSP SSI. The former model had a smaller AIC and better model fit and was considered as the final model.

The multivariable model for the development of MRSP SSI identified preoperative MRSP carriage (OR = 14.8, p = <0.0001, 95% CI = 4 – 54.7) as a risk factor. When site-specific preoperative MRSP carriage was used instead of overall preoperative MRSP carriage, preoperative MRSP carriage of the nares (OR = 14.4, p = 0.015, 95% CI = 1.68 – 124) and rectum (OR = 13.5, p = 0.03, 95% CI = 2.07 – 88.1) were identified as risk factors for the development of MRSP SSI. In this second model, the bulldog breed (OR = 12.2, p = 0.008, 95% CI = 1.91 – 77.5) remained as a statistically significant risk factor for the development of MRSP SSI. The latter model had a smaller AIC and better model fit and was considered as the final model.

The multivariable model for overall SSI is represented in Table 3.6. The three clinics identified as risk factors for SSI either did not administer antimicrobials or administered postoperative antimicrobials for no longer than 24 hours for the majority of cases (Table 3.1). A protective effect of administration of postoperative antimicrobials was identified (OR = 0.29, p = 0.007, 95% CI = 0.09 – 0.71), where patients that were administered postoperative antimicrobials were 3.5 times less likely to develop infection. When site-specific preoperative MRSP carriage was used instead of overall preoperative MRSP carriage, preoperative MRSP carriage of the skin (OR = 26.5, p = 0.002, 95% CI

= 3.29 - 214) was identified as a risk factor for the development of SSI following TPLO. The initial model had a smaller AIC and better model fit and was considered as the final model.

Table 3.5a: Univariable analysis of potential factors associated with outcome variables pre and postoperative MRSP carriage.

		Preoperative MRSP colonization			Postoperative MRSP colonization		
Variable	**N(%)**	**P-value (P)**	**Odds Ratio (OR)**	**Confidence Interval (CI)**	**P**	**OR**	**CI**
Breed: Bulldog (referent: Airdale Terrier)	11/549 (2%)	0.002	9.18	2.27-37.1	0.09		
Breed: Labrador Retriever	101/549 (18.5%)	0.28			0.08		
Sex: Intact male (referent: intact female)	11/547 (2%)	0.08			0.31		
Sex: Female spayed	277/547 (50.6%)	0.19			0.98		
Dog contact	425/455 (93.4%)	1			0.23		
Visit dog parks	285/538 (53%)	0.027	0.36	0.15-0.89	0.3		
Kennel boarded	64/539 (11.9%)	0.34			0.37		
Groomer visits	183/538 (34%)	0.19			0.79		
Diarrhea	22/539 (4.1%)	0.62			0.49		
Hospitalization	29/538 (5.4%)	1			1		
Preoperative infection	14 (2.6%)	0.09			0.09		
Corticosteroids	21/539 (3.9%)	1			0.54		
Immunosuppressive drugs	4/539 (0.7%)	1			1		
Cushing's	7/539 (1.3%)	1			1		
Hypothyroidism	18/537 (3.4%)	0.184			0.55		
Pyoderma	27/539 (5%)	0.34			0.021	2.2	0.78-6.36
Atopy	33/539 (6.1%)	0.39			1		
Age (years)	5.54 +/- 2.67 (0.9-13.1)	0.031	0.829	0.04-0.57	0.04	0.79	0.63 - 0.99
Weight (kg)	37.34 +/- 11.81 (5.6-82.9)	0.002	1.05	1.02-1.09	<0.0001	1.08	1.04-1.13
Preoperative antimicrobials (y/n)	103/549 (19.1%)	0.2			0.21		
Postoperative antimicrobials (y/n)	399/549 (72.7%)				0.8		
Postoperative antimicrobial duration (days)	3.56 +/- 5.35 (0-21)						
Clinic A	153/549 (27.8%)	0.006	5.4	1.6-18.2	0.93		
Clinic B	129/549 (23.5%)	0.74					
Clinic C	97/549 (17.7%)	0.14					
Clinic D	57/549 (10.4%)	0.62			0.16		
Clinic E	41/549 (7.5%)	1			0.14		
Clinic F	40/549 (7.3%)	1					
Clinic G	32/549 (5.8%)	0.15					
Preoperative MRSP colonization	24/549 (4.4%)				<0.0001	124	22.8-678
Pre-op MRSP pharynx	12/549 (2.2%)				<0.0001	72.9	7.88 - 675
Pre-op MRSP nares	6/549 (1.1%)				0.012	23.3	2-272
Pre-op MRSP rectum	10/549 (1.8%)				omitted		
Pre-op MRSP skin	6/549 (1.1%)				omitted		

Table 3.5b: Univariable analysis of potential factors associated with outcome variables surgical site infection and MRSP surgical site infection.

		Surgical Site Infection (SSI)			MRSP SSI		
Variable	**N(%)**	**P-value (P)**	**Odds Ratio (OR)**	**Confidence Interval (CI)**	**P**	**OR**	**CI**
Breed: Bulldog (referent: Airdale Terrier)	11/549 (2%)	0.001	8.69	2.42-31.2	0.004	11.7	2.23-61.1
Breed: Labrador Retriever	101/549 (18.5%)	0.28			0.136		
Sex: Intact male (referent: intact female)	11/547 (2%)	0.17			1		
Sex: Female spayed	277/547 (50.6%)	0.61			0.57		
Dog contact	425/455 (93.4%)	0.033	0.33	0.12-0.92	0.14		
Visit dog parks	285/538 (53%)	0.145			0.56		
Kennel boarded	64/539 (11.9%)	0.6			0.63		
Groomer visits	183/538 (34%)	0.93			0.08		
Diarrhea	22/539 (4.1%)	0.39			1		
Hospitalization	29/538 (5.4%)	1			1		
Preoperative infection	14 (2.6%)	0.24			1		
Corticosteroids	21/539 (3.9%)	1			1		
Immunosuppressive drugs	4/539 (0.7%)	1			1		
Cushing's	7/539 (1.3%)	1			1		
Hypothyroidism	18/537 (3.4%)	0.62			1		
Pyoderma	27/539 (5%)	1			1		
Atopy	33/539 (6.1%)	1			0.51		
Age (years)	5.54 +/- 2.67 (0.9-13.1)	0.07	0.89	0.78-1.01	0.043	0.77	0.6-0.99
Weight (kg)	37.34 +/- 11.81 (5.6-82.9)	0.14	1.02	0.99-1.05	0.016	1.05	1.01-1.1
Preoperative antimicrobials (y/n)	103/549 (19.1%)	0.66			0.45		
Postoperative antimicrobials (y/n)	399/549 (72.7%)	<0.0001	0.18	0.09-0.36	0.1		
Postoperative antimicrobial duration (days)	3.56 +/- 5.35 (0-21)	0.1	0.91	0.81-1.02	0.1	0.85	0.71-1.03
Clinic A	153/549 (27.8%)	<0.0001	6.25	3.05-12.8	0.006	5.4	1.61-18.2
Clinic B	129/549 (23.5%)	0.017	0.17	0.04-0.73	0.74		
Clinic C	97/549 (17.7%)	0.004			0.14		
Clinic D	57/549 (10.4%)	0.024			0.62		
Clinic E	41/549 (7.5%)	0.17			1		
Clinic F	40/549 (7.3%)	0.65			1		
Clinic G	32/549 (5.8%)	0.54			0.15		
Surgery time	90.21 +/- 38.76 (25-285)	0.78	1.001	0.99-1.01	0.68	1	0.98-1.01
Anaesthesia time	178.50 +/- 64.72 (50-405	<0.0001	1.01	1.01-1.02	0.07	1.01	1-1.02
Preoperative MRSP colonization	24/549 (4.4%)	<0.0001	8.55	3.38-21.6	<0.0001	12.9	3.4-46.5
Pre-op MRSP pharynx	12/549 (2.2%)	0.021	4.93	1.276-19.1	0.24		
Pre-op MRSP nares	6/549 (1.1%)	0.025	7.26	1.29-41	<0.0001**	26.7	4.366-162.679
Pre-op MRSP rectum	10/549 (1.8%)	0.009	6.366	1.575-25.720	0.002**	13.225	2.487-70.325
Pre-op MRSP skin	6/549 (1.1%)	0.001	15	2.911-76.986	0.125*		

Table 3.6: Multivariable analysis of potential factors associated with overall SSI by backwards stepwise logistic regression. *indicates site-specific MRSP carriage used as parameters.

30 Day Postoperative Surgical Site Infection (SSI)				
Variable	**N (%)**	**P – value (p)**	**Odds Ratio (OR)**	**Confidence Interval (CI)**
Bulldog	2/11 (18.2%)	0.005	11.1	2.07-59.3
Postoperative antimicrobials (y/n)	13/400 (3.3%)	<0.0001	0.36	0.15-0.91
Preoperative MRSP carriage	8/24 (33.3%)	0.001	6.72	2.12-21.4
Clinic A	24/153 (15.7%)	<0.0001	15	3.91-57.5
Clinic E	5/41 (12.2%)	0.007	10	1.87-54
Clinic G	3/32 (9.4%)	0.001	18.9	3.31-109

Assessment of antimicrobials was hampered by relatively homogenous practices at most facilities. However, at one location, patients were evenly distributed between whether or not postoperative antimicrobials were administered (79 – yes, 74 – no). At that facility, SSI developed in, 7/79 (8.9%) dogs that received postoperative antimicrobials versus 18/74 (24.3%) dogs that did not (OR = 0.3, p = 0.015, 95% CI = 0.12 – 0.76).

3.6: Discussion

The preoperative prevalence of MRSP carriage of 4.4% identified in this study is consistent with previous reports of MRSP carriage.[15,16,30] A risk factor for preoperative MRSP carriage in the final multivariable model of this study was Bulldog breed. A reason for this was not directly investigated. Bulldogs are considered to be at a high risk of skin-fold dermatitis[31,32], and MRSP carriage rates can be high in dogs with active or recent pyoderma.[16] Antimicrobial administration and pyoderma were not identified as risk factors in these models, but it is possible that Bulldogs in this study may have had

undiagnosed pyoderma, MRSP carriage from previously diagnosed pyoderma and subsequent antimicrobial exposure not identified during the study questionnaire. Weight was also identified as a risk factor for pre and postoperative MRSP carriage in the final multivariable model where the risk of carrying MRSP increases by 5% – 9% for every kg heavier the dog is. Reasons for this, and whether this is a function of increased lean mass or obesity, are unknown. The pharynx was the most common preoperative carriage site in this study, as has been reported in a German study.[17] Isolation of MRSP from the four carriage sites was variable, with site-specific test sensitivity being highest for the pharynx, although it was only 44%. Since no single site showed high test sensitivity for isolating MSRP, it may be important to include all four sites in order maximize likelihood of isolating MRSP from carriers.

The prevalence of postoperative MRSP carriage did not significantly change from the prevalence of preoperative MRSP carriage in any of the hospitals that conducted postoperative screenings. Although it was not identified in this study, reports of higher colonization rates in hospitalized animals are available.[16,30] Antimicrobial administration and hospitalization have been identified as risk factors for MRSP carriage,[17] both of which occurred in this population. Sources of MRSP acquisition were not investigated but would potentially include the contamination of general waiting rooms, examination rooms and treatment rooms. Most dogs that were identified as MRSP carriers upon admission were still carriers at the postoperative recheck, and the majority of those were shedding the same MRSP strain. This finding is consistent with recent evidence of long term MRSP shedding by dogs. Laarhoven *et al* followed 12 MRSP positive dogs for 6 months and found that two dogs were continuously MRSP positive, five dogs were intermittently positive and five dogs became negative.[33] They also found that four households had MRSP in the environment without a carrier in the house.

The overall incidence of TPLO SSI identified in the veterinary literature varies between clinics and ranges from 2.5% to 15.8%,[3,11] and the SSI rate mean (6.7%) and range (0-15.7%) in this study are consistent with those data. The variability in SSI rates between facilities was striking. No clear reasons for this were evident. The clinic with the lowest SSI rate administered postoperative antimicrobials to all patients while the facility with the highest SSI rate administered limited postoperative antimicrobials, had low SSI rates for other procedures and an active infection control program.

As is consistent with MRSA in humans,[18,19,23] dogs that carried MRSP preoperatively had an increased risk of MRSP SSI (and correspondingly an overall increased risk of SSI). More specifically, carriage in the nares and rectum increased likelihood of developing MRSP SSI by 14 and 13 times respectively. It was surprising that carriage in the pharynx was not identified as a risk factor, as it was the most commonly isolated carriage site for MRSP. There is a possibility that colonization of the pharynx poses less of a risk for development of SSIs when compared to the nares and rectum. This could be possible because the pharynx is a deeper site than nares or the rectum, or due to the rectum Preoperative screening and decolonization strategies for MRSA have proven to be both clinically and financially effective in most human studies.[24,25,34,35] One study demonstrated a 0% SSI rate after following a MRSA screening and decolonization protocol with a MRSA positive population of 3%, compared to patients that did not participate in the intervention that had an SSI rate of 1.2%.[34] This raises questions about whether preoperative screening and decolonization might be considered in dogs. However, there are a few issues that must be considered. An eradication program must be effective and efficient in order to be beneficial and clinically relevant. The identification of carriers should be done quickly and the screening test should also have a high sensitivity and specificity in order to rule out error.[36] Assessment of screening methods for MRSA in humans was conducted by Paule *et al* in 2009 and it was observed that real-time PCR provided the fastest results and demonstrated the highest sensitivity when being compared to broth-enriched culture (2 vs. 48 hours).[36] It was also mentioned that labour time was neutral for all screening methods and the monetary cost for real-time PCR was 2.27 times more than broth-enriched culture.[36] However, real time PCR assays for MRSP are not currently available. The turnaround time for culture is such that it might be logistically challenging, or at least inconvenient, to culture patients prior to surgery, especially if owners have to travel to the surgical facility. Further, optimal screening methods for MRSP carriage have not been adequately investigated. Development of real time PCR or other rapid assays such as loop-mediated isothermal amplification (LAMP)[37] could facilitate screening in the near future. However, another complicating factor is what to do with the results. Decolonization regimens have been described and studied in humans, but no comparable data are available for animals. The limited antimicrobial options for most MRSP isolates, inability to topically treat the nasal passages or pharynx of most dogs, multisite colonization and concerns about furthering antimicrobial resistance complicate potential decolonization

approaches. Another preventive measure that could be considered would be modified perioperative prophylaxis in MRSP carriers (e.g. addition of amikacin to the preoperative regimen), something that might be more practical and reduce overall antimicrobial resistance pressure, but objective assessment of any such approach is required.

Postoperative administration of antimicrobials was associated with a protective effect against the occurrence of overall SSI. This finding is in agreement with four other studies that identified postoperative antimicrobial administration as a protective factor against the development of TPLO SSI.[2,5,6,14] These data contradict guidelines in human medicine where no or short term (≤24 h) postoperative antimicrobials are recommended for similar procedures.[38] Although studies in human surgery have documented that this practice may lead to antimicrobial resistance, additional morbidity and treatment costs[39-41], there are likely many differences in patient and treatment factors, especially when considering pathogen exposure and patient care. A controlled clinical trial thoroughly assessing the effect of postoperative antimicrobial administration in dogs undergoing TPLO is warranted.

It was documented that the swabbing of a single body site was not effective enough to isolate MRSP reliably on its own. The findings in this study provide evidence to suggest that multiple body sites should be swabbed if screening for MRSP, with the nares and rectum being the most influential and the pharynx being the most reliable. The increased risk of developing SSI for Bulldog breeds and decreased risk for patients administered postoperative antimicrobials should be further investigated. The identification of preoperative MRSP carriage as a risk factor for the occurrence of MRSP SSI and overall SSI is an important finding that provides justification for assessing preoperative MRSP screening and decolonization protocols in dogs undergoing TPLO as well as other surgical interventions.

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Chapter 4

General Discussion

In chapter 2, it was identified that administration of perioperative antimicrobials was variable and most cases did not received adequate dosing according to guidelines previously established in human medicine and extrapolated to veterinary medicine. Considering that only 42.5% of dogs were administered antimicrobials appropriately in our study, it is suggested that improvements in standard perioperative antimicrobial administration protocols me made. There have been similar reports of perioperative antimicrobial administration non-compliance in the human medical literature. Braztler *et al* determined that only 55.7% of 34133 surgical patients received antibiotics within 60 minutes prior to incision.[1] Even when considering preoperative doses within 120 minutes before incision, one study showed that only 60% of patients had been given adequately timed doses in a study of 2847 individuals.[2] Although timing of perioperative antimicrobial administration was not identified as a risk factor in our study, there is a chance that lack of statistical power may have been the reason for it to not have any influence in our model. It is more likely that the hypothesized strength of association of perioperative antimicrobial prophylactic timing with SSI may have been overestimated. There are reports in the human literature of single-dose preoperative antimicrobial administration between 20 to 30 minutes prior to incision being equally as effective as multi-dose perioperative antimicrobial prophylaxis.[3,4] This helps to suggest that the preoperative dose is the most important one to consider and if administered within 30 minutes of the incision being made, may be adequate enough to defend against SSI. In chapter 2, 93% of patients received their preoperative antimicrobial dose on time and most of the untimely doses were given during the perioperative period. If the above theory is correct, then the majority of patients in our study may have received adequate antimicrobial prophylaxis even though over half of them did not receive adequate perioperative doses. There are other suggestions as well, that speculate whether the window for timely dosing is too narrow for us to notice a level of non-compliance or that untimely antimicrobial dosing is usually confounded with factors such as being an inpatient, having a higher ASA score or long duration of surgery or anesthesia.[5,6]

There are methods to improve perioperative antimicrobial administration timing compliance without any increase in costs or labour. The use of a preoperative checklist is one potential method of ensuring that patients are administered antimicrobials at the appropriate time and dose. The preoperative checklist can help ensure prophylactic antimicrobial treatment is given prior to the start of the procedure and that appropriate

intraoperative doses are administered. This technique is being increasingly used in human medicine and has showed promising results in many aspects of protocol compliance while performing surgery.[7] One study decreased complication rates in surgery from 11% to 7% by introducing a Surgical Safety Checklist.[7] Another study identified that perioperative antimicrobial administration compliance increased from 65% to 99.1% after introducing a “time out” period where it was ensured that antimicrobials had been administered appropriately.[8] There are potential pitfalls to having a checklist and would most likely be evident when a checklist program is not designed for the objective. In the case of timely antimicrobial prophylaxis, it may be wise to only have a pre-induction checklist to ensure that the preoperative antimicrobial dose is ready for administration after induction. It would increase anaesthesia time by having multiple checklists at the pre-induction, pre-surgical, pre-closure and post-surgical stages which would be detrimental to a patient`s risk of developing SSI. A different, but fairly simple and uncostly way to ensure intraoperative antimicrobial administration is given according to protocol is to introduce a timer on the anaesthesia machine. A simple egg timer would be able to alert staff that 90 minutes has passed and an intraoperative dose is required. Although the association of timing of perioperative antimicrobial administration with SSI has not been identified for TPLO and may be less impactful than hypothesized, it is rational to assume that deviation from standard human recommendations could be accompanied by the increase in likelihood of developing SSI and therefor intervention to improve protocol is warranted.

Postoperative administration of antimicrobials is a controversial subject and there is concern for excessive or inappropriate antimicrobial use. It was identified that postoperative antimicrobial administration was associated with decreasing the likelihood of SSI occurrence in both chapter 2 and 3. Neither study was able to assess optimal postoperative practices such as which drug is optimal or what duration should be sufficient. Since it is important to minimize duration of postoperative antimicrobial use in order not to select for antimicrobial resistance and lessen adverse effects in patients, these are important questions that require additional study to answer. In clean surgical procedures in humans, postoperative treatment beyond 24h is not recommended.[1,2] Administering postoperative antimicrobials following clean procedures has not been shown to reduce the occurrence of SSI and may contribute to the development of antimicrobial resistance and additional morbidity.[9-11]

Looking to well-designed human studies for guidance is important, although there may be a number of differences between human and veterinary medicine in certain areas such as types of surgical procedures, patient factors, varied pathogen exposure and patient care. The protective effect of postoperative antimicrobials noted in these studies is consistent with the findings of three canine TPLO studies,[12-14] where they indicated a protective effect of 3 – 14 days of postoperative antimicrobial administration. None of the studies mentioned, or the studies in this thesis, were designed to assess the most ideal methods of postoperative antimicrobial use. Therefore, the need for a proper controlled study is indicated so that it can be determined if there truly is a protective effect against SSI when administering postoperative antimicrobials and the ideal duration should be determined to address concerns about excessive use of antimicrobials and antimicrobial resistance in animals. A clinical trial assessing duration of postoperative cephalexin administration in TPLO patients would be ideal and a number of different durations can be implemented to randomized groups from 24 hours to 14 days. This would be beneficial in minimizing excessive postoperative antimicrobial use. The use of postoperative antimicrobials may have a protective effect against the occurrence of SSI, but it is unable to protect patients from MDR SSI. In chapters 2 and 3, the incidence of MRSP SSI development was 35.3% and 29.7% respectively and was the leading cause of infection next to MSSP. The use of broad-spectrum postoperative antimicrobials such as cephalexin have no effect on MRSP which means a third of SSIs that developed in dogs underdoing TPLO were unpreventable with postoperative antimicrobials. These hypotheses are enforced with data from chapter 3, where postoperative antimicrobial duration was identified as a protective effect against the development of overall SSI, but not MRSP SSI specifically. This means that a surveillance system to identify MRSP carrier could also help identify patients that should potentially not receive perioperative antimicrobials to which the pathogen is resistant to.

In chapter 3, carriage of MRSP was identified as a risk factor for MRSP SSI as well as overall SSI. It was also seen that the site-specific carriage of MRSP on the skin, nares or rectum increased the likelihood of developing SSI, although the pharynx was the most common source of contamination. This association is similar to the association that MRSA has with the development of MRSA SSI in humans.[15,16] It is important to act on important findings such as this and begin to construct a preoperative screening and decolonization strategy. These preoperative intervention protocols have been created for

MRSA carriage in human surgical patients and are both clinically and financially effective in most human studies.[17-19] Methods of decolonization can include mupirocin nasal ointment, clorhexidine soap or wash cloths, or both treatments given simultaneously.[17,20] Optimal timing for these decolonization treatments have not been determined, but have been reported to be administered anywhere from 24 hours to 7 days prior to surgery.[17,20] It was demonstrated that a 0% SSI rate was achieved after following a MRSA screening and decolonization protocol with a MRSA positive population of 3% in one study.[19] This was compared to patients that did not participate in the intervention that had an SSI rate of 1.2%.[19] Despite these findings, the prevalence of MRSP carriage in dogs in this study was low (4.4%) and therefore intervention programs should be thoroughly assessed for efficacy and efficiency before being put in place. When considering intervention programs for dogs, the methods for MRSP isolation in this study are not fast enough to identify MRSP in a timely manner. It would be difficult to have clients comply with screening when they are hassled to make extra trips to the hospital in order for the patient to be swabbed and then be placed on a decolonization treatment protocol. This process would take a minimum of eight days and may unnecessarily increase client frustration. The ideal screening method should be rapid enough to allow a patient to be cleared for TPLO or to be sent home with a decolonization treatment package with a rescheduled surgery date. An intervention strategy must be effective and efficient (both financially and timely) in order to be beneficial and clinically relevant.

The identification of carrier patients should be done rapidly and at the lowest cost possible in order to start patients on a decolonization program and the screening test should have a test high sensitivity and specificity so any test error can be ruled out.[21] When comparing real-time PCR to broth-enriched culture, a study in the human literature observed that MRSA was identified much faster and with a higher test sensitivity when using PCR (2 vs. 48 hours).[21] The financial cost for real-time PCR was 2.27 times more than broth-enriched culture, but was 24 times faster at identifying MRSA. [21] It is important to note that labour time was neutral between the screening methods.[21] This data helps support that the novel method of screening for *Staphylococcus pseudintermedius* using LAMP that Diribe *et al* have recently developed may aid in producing a viable MRSP screening program when combined with a PBP_{2a} test for antimicrobial resistance.[22] Their method demonstrated when testing DNA that was extracted directly from clinical surgical site swabs, *S. pseudintermedius* was determined

within 15 minutes and had a diagnostic sensitivity of 94.6%.[22] This means that compared to waiting for 7 days for MRSP identification in this study's current methodology, identification of MRSP using LAMP would be over 600 times faster. Even if the monetary cost of LAMP was 5 times more than the cost of traditional plating methods, its clinical benefit would far outweigh the financial cost. It is of importance to further investigate this technique and attempt to integrate into an MRSP screening or intervention program for dogs undergoing TPLO in order to reduce development of SSI.

Decolonization protocols have been reported to be effective in human medicine, but there have not been any similar studies conducted dogs. There is no evidence to support that dogs can be adequately decolonized of MRSP from the skin and nasopharynx by using conventional methods identified in human strategies. The best method to eliminate bacteria is to use disinfectants and soaps as opposed to antimicrobials in order to minimize the increase in prevalence of antimicrobial resistance. If decolonizing dogs prior to surgery is not possible, then other intervention strategies could be considered. Patients that are carriers of MRSP could be administered an effective antimicrobial perioperatively, rather than cefazolin, order to reduce the occurrence of MRSP SSI. This option would have to be carefully assessed as the risk of increasing antimicrobial resistance within the population may outweigh the potential for minimizing SSI.

Tibial plateau leveling osteotomy is a fairly new surgical procedure and there is still much to learn regarding patient risks and outcomes for development of SSI. The importance of understanding what factors are involved in TPLO SSI is enhanced by the fact that it is one of the most commonly performed techniques to treat CCLI. Although there are certain risk factors such as MRSP and protective effects such as the use of postoperative antimicrobials emerging from studies, consideration must be taken to further investigate these findings in trails specifically designed for them. The effectiveness of other strategies such as screening and decolonization of MRSP would also have to be more thoroughly assessed since there has yet to be any reports of this taking place in animals. There is still much to understand regarding factors associated with the development of SSI following TPLO in dogs.

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Appendix A: Client Consent Form

Impact of preoperative colonization with methicillin-resistant *Staphylococcus pseudintermedius* on surgical site infection (SSI) in dogs undergoing tibial plateau leveling osteotomy (TPLO)

Principal Investigator: Ameet Singh BSc, DVM, DVSc, DACVS, University of Guelph, Ontario, Canada.

You are asked to participate in a study investigating whether dogs harboring a bacteria termed *Staphylococcus pseudintermedius* have a higher rate of surgical site infections. Participation in this study is voluntary and confidential.

Your dog is going to have TPLO surgery to eliminate the instability in their knee as a result of cranial cruciate ligament injury. Surgical site infection is an inherent risk of any type of surgical procedure and the risk of infection following TPLO has been discussed with you. It is known that dogs undergoing TPLO have a higher infection rate (~ 6%) compared with other types of orthopedic surgeries (repair of broken bones, joint surgery). Although 6% is not that high, TPLO is commonly performed on dogs around the world and investigating possible methods to reduce the infection rate will benefit a large number of dogs worldwide.

As part of the pre-surgical evaluation in humans undergoing surgery, a nasal swab is obtained to determine if they are harboring a bacteria termed *Staphylococcus aureus*. It has been shown that nasal carriers of *S. aureus* are almost 10x more likely to get an infection. Based on this information, humans carrying *S. aureus* in their noses will be treated with an antibiotic to get rid of this bacteria prior to surgery. Similar type of information does not exist in veterinary medicine.

S. pseudintermedius is now emerging as an opportunistic bacteria in dogs and is normally found in the ears, skin and gastrointestinal tract of healthy dogs. Although this bacteria is normally found on dogs, if there is a break in the skin (i.e. surgical incision) or if a dog is sick, it can turn into an invasive bacteria and lead to an infection.

If you consent to allowing your dog to participate in this study, a swab will be taken from its nose, throat, rectum and skin overlying its surgical site during pre-surgical evaluation. These swabs are very small in size and will not cause your dog any discomfort during sampling. These swabs will then be tested in our lab for the presence of *S. pseudintermedius.* Our hospital staff will contact you by phone at 30 days and 1 year after surgery to ask you questions about your dog's surgical site and determine if an infection occurred.

*Following surgery, if you have any concerns regarding healing of the surgical incision,

or whether an infection is present, please contact us immediately.

Commonly asked questions

Why should I enter my dog into this study?
By entering your dog into this study, it will provide valuable information on whether dogs that already harbor *S. pseudintermedius* are at higher risk for infection. Unfortunately, cranial cruciate ligament injury is a very common occurrence and, therefore, the information gathered from this study will have a tremendous impact on dogs worldwide.

Are there additional costs for entering the study?
No. The cost for surgery, whether you enter your dog in the study or not, will be the same.

Are there any additional risks to my dog if it enters the study?
There are no additional risks to taking swabs for samples of your dog's nose, throat, rectum and skin.

Will I find out if my dog has methicillin-resistant S. pseudintermedius before surgery?
No, all samples will be collected before surgery and will take a minimum of 30 days to process. If your dog is found to harbor this bacteria, we will contact you by mail and provide you some additional information on this bacteria. Do not be alarmed as this bacteria is found in a small proportion of healthy dogs.

Thank you very much for considering entering your dog in this study. By signing this form, you acknowledge reading the information for the above-described study and agree to participate in this study. You will be given a copy of this form.

I, _____ consent to enroll my dog in this study.
(please print name)

_____ _____
(sign) (date)

Appendix B: Preoperative Questionnaire

Preoperative questionnaire

1) Has your dog received antimicrobials in the last 6 months (antibiotics for any type of infection)? YES NO UNSURE

If yes, which drug? _____

When? _____

2) Has your dog received corticosteroids in the last 4 weeks (reduces inflammation for allergic reactions or arthritis)? YES NO

3) Has your dog received any other immunosuppressive drugs in the last 4 weeks?

e.g. atopica (cyclosporine), chemotherapy, azathioprine YES NO

4) Has your dog stayed in a veterinary hospital overnight in the last 4 weeks? YES NO

5) Has your dog had diarrhea in the last 4 weeks? YES NO

6) Is your dog being treated for or suspected to have hypothyroidism? YES NO

7) Is your dog being treated for or suspected to have diabetes? YES NO

8) Is your dog being treated for or suspected to have cushings? YES NO

9) Was your dog diagnosed with pyoderma in the last 6 months (bacterial skin infection)? YES NO

If so, when? _____

10) Was your dog diagnosed with atopy (allergic dermatitis – skin allergies) in the last 6 months? YES NO

11) Is your dog currently being treated for any other infection? YES NO

12) Has your dog been in boarding kennel in the last 6 months? YES NO

If yes, when was the last time? _____

13) Has your dog been to a groomer? YES NO

If yes, when was the last visit? _____

14) Does your dog go to dog parks?

NEVER UNCOMMONLY

AT LEAST WEEKLY DAILY

15) Does your dog come into contact with other dogs (family, friends, etc) ?

NEVER UNCOMMONLY

AT LEAST WEEKLY DAILY

16) Is your dog currently receiving any medications? YES NO

If yes, which drug(s)?

Appendix C: Client Calling Script

Calling Script for TPLO SSI Clients

Hello is *client name* there please?

Hi my name is _____ and I am a graduate student at the University of Guelph. I'm calling to follow up on *patient name*'s TPLO surgery. It will take less than 3 minutes and *patient name* will be helping future TPLO patients.

My research is looking at how TPLO surgical sites heal after surgery. I have a few quick questions to ask you about *patient name*'s recovery.

30 day:

1. Was your pet wearing an Elizabethan collar after surgery?

2. Did you see your pet licking or rubbing the surgical site

3. Did you notice any problems with your pet's surgical site, such as oozing, redness, tenderness, or pain?

   If YES, ask these questions:

   When did you notice the problems?
   What specifically did you see?
   Did you see a veterinarian?
   Were any treatments prescribed? (If so, what was given?)
   Was there any need for further surgery?
   Has the problem resolved?

1 Year:

1. How is *patient's name* doing on the operated leg(s)?

2. Currently, are there any problems with the surgical site?

   If YES, ask these questions:
   Describe the problem
   Did you have to take your pet to a vet because of this?

Thank you for enrolling *patient's name* in this study. This study would not be possible without you and *patient's name*, so please thank him/her with a treat for me! You and *patient's name* take care! Thank you!