Surgical Site Infections in Veterinary Medicine; A Focus on the Tibial Plateau Levelling Osteotomy and an In Vitro Evaluation of the Elution of Amikacin and Dispersin B From a Local Drug Delivery System

by

Chris Hagen

A Thesis

presented to

The University of Guelph

In partial fulfilment of requirements

for the degree of

Doctor of Veterinary Science

in

Clinical Studies

Guelph, Ontario, Canada

©Chris Hagen, November, 2019
ABSTRACT

SURGICAL SITE INFECTIONS IN VETERINARY MEDICINE; A FOCUS ON THE TIBIAL PLATEAU LEVELING OSTEOTOMY AND IN VITRO EVALUATION OF THE ELUTION OF AMIKACIN AND DISPERSIN B FROM A LOCAL DRUG DELIVERY SYSTEM

Dr. Chris Hagen
University of Guelph, 2019

Advisor:
Dr. Tom Gibson

Surgical site infection (SSI) after tibial plateau leveling osteotomy (TPLO) is a common, frustrating, and expensive complication. The underlying reason for this increased risk of infection is poorly understood but is further complicated by biofilm formation. Emerging resistance, adverse effects of antimicrobials, and altered environments within an infection have revealed a need for local delivery of drugs including antimicrobials and anti-biofilm agents. The objectives of this study were to evaluate potential risk factors associated with SSI after TPLO and determine the in vitro elution profile of amikacin and Dispersin B from a novel polymer hydrogel. Administering pre-operative antibiotics greater than 60 minutes from the first incision and administration of post-operative antibiotics were protective against SSI. Patients younger than 6 and older than 12 years were at higher risk of developing an SSI. Amikacin and Dispersin B both rapidly eluted from the polymer hydrogel in the first 24 hours and there was no clinically significant difference in their total elution over ten days when compounded individually or together. These data suggest further research is required to determine optimal peri-operative antimicrobial regimens including timing and length of administration. Additionally, the
combination of amikacin and Dispersin B in a polymer hydrogel had promising results for local treatment of complex infections involving biofilms.
ACKNOWLEDGEMENTS

I would first like to thank my advisor Dr. Tom Gibson for guiding me through the last three years of this process. Your ability to keep the environment light through challenging situations, patience, and accessibility has made a potentially difficult thesis much more enjoyable.

I would also like to thank my DVSc committee members (Dr. Ameet Singh, Dr. Alex zur Linden, and Dr. Scott Weese) for their contributions and support in my comprehensive and thesis examinations, as well as the efficient and thorough assistance in the manuscript and thesis editing process. A sincere thank you to Dr. Kelley Thieman Mankin and Dr. Ron Johnson for taking time out of their busy schedules to participate in my thesis defense. Thank you to Gabrielle Monteith for her efforts in statistical analysis. Thank you to Joyce Rousseau, Michelle Beaudoin-Kimble, and Quinn Marshall for assistance in data collection. Thank you to Karen Lovetri and Kane Biotech for the generous donation of Dispersin B. Thank you to the Pet Trust Fund for financial support of this research.

A special thank you to my partner Briana Hagen, my children Benson and Quinn, and our dogs Lily and Gus for staying alongside me through this journey that has taken us across the country. Finally thank you to all my friends and family for your never ending support over the past 15 years. Although the time has flown by, we are ready to come home.
DECLARATION OF THE WORK PERFORMED

I declare that with the exception of the items below, all work reported in this thesis was performed by me.

Statistical analysis was performed by Gabrielle Monteith, of the Department of Clinical Studies, Ontario Veterinary College, University of Guelph.

Assistance with collecting amikacin and Dispersin B samples, performing the ELISA for Dispersin B concentrations, and collecting medical record data was given by Quinn Marshall, a summer research student of the Department of Clinical Studies, Ontario Veterinary College, University of Guelph,

Amikacin concentration analysis was performed by Helen Kocmarek and the Animal Health Laboratory at the University of Guelph.

I, Chris Hagen performed all the writing, graphing and table formatting in this thesis with editorial comments made by Dr. Tom Gibson, Dr. Ameet Singh, and Dr. Alex zur Linden of the Department of Clinical Studies, Ontario Veterinary College, University of Guelph and Dr. Scott Weese of the Department of Pathobiology, Ontario Veterinary College, University of Guelph.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iv</td>
</tr>
<tr>
<td>DECLARATION OF WORK PERFORMED</td>
<td>v</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>x</td>
</tr>
</tbody>
</table>

## CHAPTER I: Literature Review

1.1 Surgical site infections in veterinary medicine..........................1
  1.1.1 Definition of surgical site infection........................................1
  1.1.2 Infection rates and contributing factors....................................4
    1.1.2.1 Patient factors.......................................................................5
    1.1.2.2 Procedural factors....................................................................5
  1.1.3 Pathogens involved..........................................................................6
  1.1.4 *Staphylococcus pseudointermidius* - emerging resistance.................7

1.2 Post-operative infections from tibial plateau leveling osteotomy.........8
  1.2.1 Incidence and risk factors..........................................................8
  1.2.2 Impact.............................................................................................11

1.3 Peri-operative antibiotics...............................................................12
  1.3.1 Antibiotic selection........................................................................13
  1.3.2 Frequency, dose, and duration of antibiotic.....................................14
  1.3.3 Use in general practice....................................................................14

1.4 Biofilms...............................................................................................15
  1.4.1 Definition and bacteria implicated.................................................15
  1.4.2 Agents effective against biofilms..................................................17
  1.4.3 Dispersin B.......................................................................................18

1.5 Local drug delivery..............................................................................19
  1.5.1 Delivery systems..............................................................................21
    1.5.1.1 Coated implants.........................................................................21
    1.5.1.2 Polymethylmethacrylate...........................................................22
    1.5.1.3 Calcium sulfate..........................................................................24
    1.5.1.4 Gentamicin impregnated collagen sponges..................................25
    1.5.1.5 Poloxamer 407...........................................................................27
  1.5.2 Vetri-gel.........................................................................................28

1.6 Thesis objective and hypotheses......................................................28

1.7 Footnotes..............................................................................................30

1.8 References............................................................................................30

## CHAPTER II: Contributing factors to surgical site infection after tibial plateau leveling osteotomy; a follow up retrospective study

2.1 Abstract..............................................................................................45
2.2 Introduction........................................................................................................47
2.3 Materials and methods.....................................................................................48
   2.3.1 Dogs............................................................................................................48
   2.3.2 Data collection............................................................................................49
2.4 Data analysis.....................................................................................................50
2.5 Results...............................................................................................................50
2.6 Discussion.........................................................................................................59
2.7 Footnotes.........................................................................................................64
2.8 References.......................................................................................................64

CHAPTER III: In vitro elution of amikacin and Dispersin B from a polymer hydrogel...70
3.1 Abstract..........................................................................................................71
3.2 Introduction.....................................................................................................72
3.3 Materials and methods..................................................................................74
   3.3.1 Sample preparation....................................................................................74
   3.3.2 Sample analysis........................................................................................75
3.4 Data analysis....................................................................................................76
3.5 Results..............................................................................................................76
3.6 Discussion.......................................................................................................81
3.7 Footnotes.......................................................................................................86
3.8 References.....................................................................................................87

CHAPTER IV: Summary and conclusion..........................................................93
4.1 Overview of factors associated with surgical site infection after tibial plateau leveling osteotomy.................................................................93
4.2 Overview of local antibiotic therapy and the rationale for using a polymer hydrogel compounded with antibiotics and anti-biofilm agents........................94
4.3 Future investigations.......................................................................................96
4.4 References.....................................................................................................97
# LIST OF TABLES

Table 1.1: Definition of a surgical site infection modified from the CDC NHSN PSC Protocol Combined Manual 2018

Table 1.2: Summary of the veterinary literature documenting the rate of surgical site infection from TPLO

Table 1.3: Summary of the veterinary literature documenting a significant protective benefit of post-operative antibiotics after TPLO

Table 1.4: Summary of the veterinary literature documenting no significant benefit of post-operative antibiotics after TPLO. If the odds ratio and confidence interval were not provided, a p-value was recorded when available

Table 1.5: Advantages and disadvantages of local drug delivery

Table 2.1: Bacterial culture results for the 71/659 Cases with surgical site infection after TPLO

Table 2.2: Univariable analysis of variables predicted to be associated with surgical site infection (SSI) after TPLO. Exact conditional logistic regression was used to determine risk factors for SSI. P-values are presented with odds ratios when significant

Table 2.3: Summary of the association between age and developing a surgical site infection after TPLO. Exact conditional logistic regression was used for contrast estimation between select age categories to demonstrate the infection trend at the upper and lower age limits. P-values are presented with odds ratios

Table 2.4: Stepwise backward logistic regression analysis of variables predicted to be associated with surgical site infection after TPLO. Variables placed into the model were chosen based on a P-value <0.2 on univariable analysis (Table 2.2). P-values are presented with odds ratios when significant

Table 3.1: One-way analysis of variance (tukey adjusted) for repeated measures comparing the elution of Dispersin B from Vetri-Gel when added alone or in combination with amikacin over ten days. Concentration of Dispersin B (ng/mL) presented as the mean of ten samples (± standard deviation)

Table 3.2: One-way analysis of variance (tukey adjusted) for repeated measures comparing the elution of amikacin from Vetri-Gel when added alone or in combination with Dispersin B over ten days. Concentration of amikacin (µg/mL) presented as the mean of ten samples (± standard deviation)

Table 3.3: Student t-tests of the area under the elution curve (AUC) comparing the total elution over ten days of amikacin and Dispersin B from Vetri-Gel when added alone (A, D) or in combination (AD)
LIST OF FIGURES

Figure 1.1: Life cycle of a biofilm.................................................................16

Figure 2.1: Probability of developing a surgical site infection relative to timing of antibiotic re-administration after the first incision. Probability (blue) and 95% confidence limits (red) are included.................................................................56

Figure 2.2: Probability of developing a surgical site infection relative to age in patients that received post-operative antimicrobials. Four categories of pre-operative antimicrobial administration timing are presented relative to the first incision (Blue: 0-30 minutes, Red: 31-60 minutes, Green: >60 minutes, Purple: After incision).........................................................58

Figure 2.3: Probability of developing a surgical site infection relative to age in patients that did not receive post-operative antimicrobials. Four categories of pre-operative antimicrobial administration timing are presented relative to the first incision (Blue: 0-30 minutes, Red: 31-60 minutes, Green: >60 minutes, Purple: After incision).........................................................59

Figure 3.1: Dispersin B elution from Vetri-Gel over ten days. Group D contains Dispersin B alone while Group AD contains Dispersin B and amikacin..............................................77

Figure 3.2: Amikacin elution from Vetri-Gel over ten days. Group A contains amikacin alone while Group AD contains Dispersin B and amikacin. MIC$_{90}$ for methicillin resistant *Staphylococcus pseudintermedius* (MRSP) included for reference..............................................79
## LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLSI</td>
<td>Clinical &amp; Laboratory Standards Institute</td>
</tr>
<tr>
<td>TPLO</td>
<td>Tibial plateau leveling osteotomy</td>
</tr>
<tr>
<td>SSI</td>
<td>Surgical site infection</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
</tr>
<tr>
<td>MRSP</td>
<td>Methicillin resistant <em>Staphylococcus pseudintermedius</em></td>
</tr>
<tr>
<td>NHSN PSC</td>
<td>National Healthcare Safety Network Patient Safety Component</td>
</tr>
<tr>
<td>PMMA</td>
<td>Polymethylmethacrylate</td>
</tr>
<tr>
<td>GICS</td>
<td>Gentamicin impregnated collagen sponges</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimum inhibitory concentration to prevent growth of a bacterium</td>
</tr>
<tr>
<td>MIC(_{90})</td>
<td>Minimum inhibitory concentration to prevent growth of 90% of bacterial isolates</td>
</tr>
<tr>
<td>OVC-HSC</td>
<td>Ontario Veterinary College – Health Sciences Center</td>
</tr>
<tr>
<td>PNAG</td>
<td>(\beta)-1,6-(N)-acetyl-d-glucosamine polymer</td>
</tr>
</tbody>
</table>
CHAPTER I: Literature Review

Managing surgical site infections (SSIs) is an important part of any veterinary practice where surgical intervention is offered, as they represent a potentially devastating complication from any surgical procedure.\textsuperscript{1–3} With advances in medicine, the complexity and invasiveness of surgical procedures has followed along with an increased risk of exposure to microorganisms and subsequent increasing rates of antimicrobial resistance.\textsuperscript{4–6} Antimicrobials effective against these resistant strains have struggled to stay ahead of the rate of bacterial resistance development.\textsuperscript{7} Alternative strategies have therefore become important in managing development and spread of resistance from both a preventative and treatment standpoint. Furthering our understanding of perioperative antibiotic therapy and local antibiotic therapy to prevent or treat infections will contribute to these discussions moving forward.\textsuperscript{8–12}

1.1 – Surgical site infections in veterinary medicine

1.1.1 – Definition of surgical site infection

Unfortunately, the definition of an SSI lacks a universal description in veterinary medicine. There have been attempts to better describe and understand SSI based on human definitions developed by the US Centers for Disease Control and Prevention (CDC).\textsuperscript{13,14} Based on CDC guidelines, the recommended definition for veterinary SSI categorizes infections into superficial incisional, deep incisional, and organ/space infections (Table 1.1).\textsuperscript{15} Clarifying the definition of an SSI will help to better identify infections, facilitate active or passive surveillance in practice, and ultimately assist with developing a more thorough understanding of infection rates, successful interventions, and at risk populations.
Table 1.1: Definition of a surgical site infection modified from the CDC NHSN PSC Protocol Combined Manual 2018.\textsuperscript{15}

<table>
<thead>
<tr>
<th>Surgical Site Infection</th>
<th>Criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superficial Incisional</td>
<td>Date of event for infection occurs within 30 days after any NHSN operative procedure (where day 1 = the procedure date) AND involves only skin and subcutaneous tissue of the incision AND patient has at least one of the following: A. Purulent drainage from the superficial incision. B. Organisms identified from an aseptically-obtained specimen from the superficial incision or subcutaneous tissue by a culture or non-culture based microbiologic testing method which is performed for purposes of clinical diagnosis or treatment. C. Superficial incision that is deliberately opened by a surgeon, attending physician or other designee and culture or non-culture based testing is not performed. AND Patient has at least one of the following signs or symptoms: pain or tenderness; localized swelling; erythema; or heat. D. Diagnosis of a superficial incisional SSI by the surgeon or attending physician or other designee.</td>
</tr>
<tr>
<td>Deep Incisional</td>
<td>The date of event for infection occurs within 30 or 90 days after the NHSN</td>
</tr>
</tbody>
</table>
operative procedure (where day 1 = the procedure date)

AND

involves deep soft tissues of the incision (for example, fascial and muscle layers)

AND

patient has at least one of the following:

   A. Purulent drainage from the deep incision.

   B. A deep incision that spontaneously dehisces, or is deliberately opened or aspirated by a surgeon, attending physician or other designee.

AND

Organism is identified by a culture or non-culture based microbiologic testing method which is performed for purposes of clinical diagnosis or treatment or culture or non-culture based microbiologic testing method is not performed

AND

Patient has at least one of the following signs or symptoms: fever (>38°C); localized pain or tenderness. A culture or non-culture based test that has a negative finding does not meet this criterion.

C. An abscess or other evidence of infection involving the deep incision that is detected on gross anatomical or histopathologic exam, or imaging test.
| Organ/Space | Date of event for infection occurs within 30 or 90 days after the NHSN operative procedure (where day 1 = the procedure date) AND infection involves any part of the body deeper than the fascial/muscle layers, that is opened or manipulated during the operative procedure AND patient has at least one of the following:

A. Purulent drainage from a drain that is placed into the organ/space (for example, closed suction drainage system, open drain, T-tube, drain, CT guided drainage)

B. Organisms are identified from fluid or tissue in the organ/space by a culture or non-culture based microbiologic testing method which is performed for purposes of clinical diagnosis or treatment.

C. An abscess or other evidence of infection involving the organ/space that is detected on gross anatomical or histopathologic exam, or imaging test evidence suggestive of infection.

AND

meets at least one criterion for a specific organ/space infection site listed in the Surveillance Definitions for Specific Types of Infections. |

### 1.1.2 – Infection rates and contributing factors

Communicating the impact of SSIs and the importance of reducing SSIs plays a vital role in implementing surveillance protocols in practice. Although the risk of SSI is relatively low, the consequences of infection are of considerable concern. Considering surgical site infections
across all clean veterinary surgical procedures, they occur across a wide range with documented rates of 1.3-12.9%. When considering the contributing factors of SSI, there are typically two broad categories discussed; patient and procedure factors. Within patient and procedure factors there are numerous variables and interplay between them making the cause of infections complex and multifactorial.

1.1.2.1 – Patient factors

Patient factors are more difficult for clinicians to control, but are important to understand so those patients at an increased risk can be identified and managed appropriately. Patient factors that have shown to increase the risk of infection include increasing body weight, dermatitis (at the surgical site or elsewhere), breed, hypotension, sex (intact male at higher risk), and concurrent endocrinopathies. Body weight as a risk factor has been shown to increase the risk of overall complications by up to 50% for every 10 pound increase in body weight where the majority of these complications encountered were SSI. With regards to breed, German Shepherd dogs have repeatedly been identified as a high risk of infection with up to 9 times higher risk relative to other breeds. Identifying at risk populations prior to surgery will help better prepare the surgery team and owner for pre-operative, intra-operative, and post-operative care including earlier intervention when necessary.

1.1.2.2 – Procedural factors

Procedural factors are in most cases under the control of the surgical team making their prevention more manageable. These factors include the wound classification, type of procedure performed, timing of surgical site clipping, perioperative and post-operative antimicrobial
administration, closure type, and duration of surgery and anesthesia.\textsuperscript{2,16,26–29} As expected, as you progress towards a more contaminated surgical site, the infection rate subsequently increases accordingly.\textsuperscript{2,14} Similarly, prolonged surgery and anesthesia times increase the risk of developing an SSI, likely related to increased exposure to the environment including increased risk of surgeon contamination.\textsuperscript{2,16,17,21,30} In an attempt to reduce the overall anesthetic time, pre-surgical clipping has been attempted. Unfortunately, clipping of the surgical site prior to the perioperative period is associated with a higher risk of SSI. This appears to be related to bacterial colonization in small cuts caused by the clipper blades which would not have sufficient time if clipped within four hours of the procedure.\textsuperscript{5,16}

Peri-operative and post-operative antimicrobial administration have had mixed results with regards to their effect on SSI. Peri-operative antibiotics are likely most effective when the surgery is not classified as clean and post-operative antimicrobials do not appear to have any effect on SSI. The one apparent exception to this is the decreased risk of SSI when post-operative antimicrobials are administered after tibial plateau leveling osteotomy (TPLO).\textsuperscript{2,17,27,30,31} The TPLO, specifically, has a higher documented infection rate compared to all other procedures when controlling for wound classification (Table 1.2). The literature on closure with suture versus surgical staples is also mixed with some finding a reduced infection rate when using staples and other literature finding the opposite.\textsuperscript{28,29} The mixed results from the veterinary literature on many of these contributing factors to SSI highlight the need for further research in the area including more rigorous study design.

1.1.3 – Pathogens involved
The most commonly identified bacterium in small animal patients include *Staphylococcus pseudintermedius*, *S. epidermidis*, *S. aureus*, *Pseudomonas aeruginosa*, *Enterococcus* species., *Streptococcus* species, and *Escherichia coli*.\(^1,30,32–35\) *Staphylococcus pseudintermedius* is one of the most commonly cited causes of SSI in dogs in North American veterinary hospitals accounting for 50-70% of the cultured bacteria.\(^1,18,32–34\) The source of bacteria is not usually identified but potential sources include skin commensals, fecal contamination, and environmental contamination. Unfortunately, nosocomial infections represent a major problem and veterinary medicine lacks a well-defined system for monitoring and prevention compared to human medicine. A review article encouraged development of systems in every hospital that included surveillance, communication with staff and clients, and appropriate staff training regarding handling of animals and equipment to help identify and prevent outbreaks.\(^18\) Unfortunately, eradication does not seem likely at this point so more appropriate alternatives are imperative.

1.1.4 – *Staphylococcus pseudintermedius* - emerging resistance

The gold standard for selecting an antibiotic to treat an SSI is bacterial culture and susceptibility testing. In most cases, clinicians rely on empirical antibiotic therapy until these results are available as it takes a minimum of 48 hours to obtain preliminary results. Empirical selection is therefore based on our knowledge of common bacteria, regional susceptibility trends, safety margin, location, and availability. Prophylactic or perioperative treatment follows the same guidelines. Commonly used antibiotics include potentiated amoxicillin and first generation cephalosporins.\(^20,30\) A concerning trend that affects both empirical and culture based selection of antimicrobials is the emergence of methicillin resistant *S. pseudintermedius* (MRSP).\(^4,36\)
Empirical antimicrobials will less frequently be effective against SSIs exposing animals to the potential adverse effects without benefit and clients to additional unnecessary costs. Culture based antimicrobial selection will be limited and less desirable antimicrobials (i.e. reduced availability, increased cost, adverse reactions) may be required more regularly. Prevalence of MRSP from cultured SSIs in these studies varied from 20-40%. Commonly used antimicrobials have limited to no efficacy against these resistant strains.

An antimicrobial family that is still typically effective in vitro against these resistant strains are the aminoglycosides; amikacin being a commonly used drug. Amikacin’s primary use in veterinary medicine is against aerobic gram-negative bacteria but it is rarely used as a first line antimicrobial as there are more appropriate, easier to administer, and safer broad spectrum antimicrobials available. Amikacin also has some effect against gram-positive bacteria including *S. pseudintermedius* but inherently lacks activity against anaerobes. With resistant infections such as MRSP, amikacin commonly becomes a last resort to treat complicated SSIs. The use of systemic aminoglycosides can be difficult due to the required route of administration and potential consequences. The two most concerning adverse effects from systemic administration are nephrotoxicity and ototoxicity and oral administration has limited absorption. Alternatively, there is the option to use amikacin locally which may become more common with the high rates of infection associated with the most frequently performed veterinary orthopedic procedure, the TPLO.

### 1.2 – Post-operative infections from tibial plateau leveling osteotomy

#### 1.2.1 – Incidence and risk factors
Multiple studies have found that one of the most commonly performed procedures in veterinary medicine, TPLO, has a significantly higher risk of SSI relative to other clean orthopedic procedures with documented infection rates varying from 2.9-25.9% (Table 1.2). The source of this increased risk is unknown (and likely multifactorial) but proposed mechanisms include periosteal dissection, prolonged surgery and anesthesia times, limited soft tissue coverage over a large orthopedic implant, and thermal bone necrosis associated with the tibial osteotomy. The factors involved in TPLO infection and possible intervention strategies, therefore, warrants further investigation.

Table 1.2: Summary of the veterinary literature documenting the rate of surgical site infection from TPLO.

<table>
<thead>
<tr>
<th>Number of Surgical Procedures</th>
<th>SSI (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>282</td>
<td>17.3</td>
<td>32</td>
</tr>
<tr>
<td>476</td>
<td>2.9</td>
<td>39</td>
</tr>
<tr>
<td>1000</td>
<td>6.6</td>
<td>3</td>
</tr>
<tr>
<td>549</td>
<td>6.7</td>
<td>34</td>
</tr>
<tr>
<td>247</td>
<td>8.5</td>
<td>19</td>
</tr>
<tr>
<td>2739</td>
<td>3.8</td>
<td>33</td>
</tr>
<tr>
<td>226</td>
<td>13.3</td>
<td>30</td>
</tr>
<tr>
<td>306</td>
<td>10.8</td>
<td>40</td>
</tr>
<tr>
<td>208</td>
<td>21.3</td>
<td>41</td>
</tr>
<tr>
<td>93</td>
<td>7.5</td>
<td>14</td>
</tr>
<tr>
<td>342</td>
<td>11.4</td>
<td>24</td>
</tr>
<tr>
<td>134</td>
<td>14.2</td>
<td>22</td>
</tr>
</tbody>
</table>
The literature evaluating the effect post-operative antibiotic administration has on SSI after TPLO has provided controversial results. Some literature has suggested postoperative antibiotics can significantly decrease the risk of infection for TPLO (Table 1.3).\textsuperscript{19,22,25,43} On the other hand, there are other studies finding no significant association between SSI and post-operative antibiotic administration (Table 1.4).\textsuperscript{30,34,39,41,42} To the author’s knowledge there is only one prospective study that has been performed looking at the effect post-operative antibiotics has on SSI after TPLO specifically in 150 dogs.\textsuperscript{22} Patients were randomized to a placebo or treatment (cefpodoxime) group for 7 days of post-operative therapy and the researchers were double blinded. This study did not find a significant difference in SSI between groups but noted that the number of patients needed to treat to prevent an SSI was 17.\textsuperscript{22} Based on the impact infections have on patients and their families, this may be sufficient cause to treat; however, there will continue to be surgeon preference.

Table 1.3: Summary of the veterinary literature documenting a significant protective benefit of post-operative antibiotics after TPLO.

<table>
<thead>
<tr>
<th># of cases</th>
<th>Odds Ratio</th>
<th>95% C.I.</th>
<th>Study Design</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>153</td>
<td>0.36</td>
<td>0.15-0.91</td>
<td>Retrospective</td>
<td>34</td>
</tr>
<tr>
<td>226</td>
<td>0.14</td>
<td>0.02-0.50</td>
<td>Retrospective</td>
<td>30</td>
</tr>
<tr>
<td>476</td>
<td>0.25</td>
<td>0.02-0.95</td>
<td>Retrospective</td>
<td>39</td>
</tr>
</tbody>
</table>
Table 1.4: Summary of the veterinary literature documenting no significant benefit of post-operatives antibiotics after TPLO. If the odds ratio and confidence interval were not provided, a p-value was recorded when available.

<table>
<thead>
<tr>
<th># of cases</th>
<th>Odds Ratio</th>
<th>95% C.I.</th>
<th>Study Design</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>240</td>
<td>Not Provided</td>
<td>Not Provided</td>
<td>Prospective</td>
<td>19</td>
</tr>
<tr>
<td>150</td>
<td>Not Provided; P=0.34</td>
<td>Not Provided</td>
<td>Prospective</td>
<td>22</td>
</tr>
<tr>
<td>405</td>
<td>0.5</td>
<td>0.22-1.14</td>
<td>Retrospective</td>
<td>25</td>
</tr>
<tr>
<td>683</td>
<td>Not Provided; P=0.66</td>
<td>Not Provided</td>
<td>Retrospective</td>
<td>43</td>
</tr>
</tbody>
</table>

1.2.2 – Impact

Consequences of an SSI in the veterinary field include patient, client, and clinician components. For patients, some possible considerations include additional hospitalization time, pain and suffering, wound management, additional surgeries, and in a worst case scenario euthanasia. For clients we typically consider the time commitment for return hospital visits, financial losses for time missed from work, additional costs associated with any follow up treatments required, and the emotional strain of having a pet away from home or making difficult decisions regarding additional treatments. A recent study by Nicoll et. al., found that the additional costs associated with SSI after TPLO were significantly higher than cases that did not develop an SSI. In the study, additional costs averaged $1559 in patients diagnosed with a SSI in
comparison to $212 in patients without.\textsuperscript{1} Additionally, patients with an SSI required an average of 4.6 follow-up visits compared to 1.4 for patients without an SSI and time to case closure was over 100 days longer for infected cases.\textsuperscript{1} Interestingly, studies of this nature likely underestimate the true costs associated with SSI from a financial perspective as many clients elect to follow up with their referring veterinarians and get lost to follow up. From a clinician’s perspective, managing an SSI can be stressful and time consuming. The strain they place on the client may also lead to redirected anger towards the clinician and hospital staff ultimately effecting job satisfaction and revenue production.\textsuperscript{44} Many of the above cannot be quantified in a meaningful way to get a full understanding of their effect on the client, patient, and clinician.

### 1.3 – Peri-operative antibiotics

Surgery exposes patients to the risk of bacterial infection primarily from commensal bacteria and environmental contaminants. In an attempt to reduce the risk of infection, there have been significant developments in perioperative preparation and treatment since the first surgical procedure was performed. Not all patients and procedures are treated equally and many of the current recommendations are extrapolated from human research which typically has larger databases to draw conclusions from, compared to animal research.\textsuperscript{45,10} It appears that most clean and clean contaminated surgical procedures have limited, if any, benefit from perioperative antibiotic prophylaxis.\textsuperscript{2,27,46} However, there is evidence that certain orthopedic surgeries and any contaminated or dirty surgery, may benefit from perioperative antibiotic administration.\textsuperscript{2,30,31}

Important considerations for prophylactic antibiotic use include appropriate antibiotic selection, dose timing, and duration of administration postoperatively.\textsuperscript{27} Prophylactic use of antimicrobials during surgery should be limited to procedures with a high risk of infection (e.g.
contaminated or dirty wounds) and those with catastrophic consequences if failure occurs (e.g. total hip replacement). Additionally, the antibiotic selected should be focused on expected bacterial contaminants based on location and given at appropriate times to sustain therapeutic drug levels at the surgical site throughout the entire period of risk (the entirety of surgery, +/- a short period of time after final closure).

1.3.1 – Antibiotic selection

Choosing an appropriate antibiotic for perioperative prophylaxis should consider the location of surgery and the expected bacterial population at that site. Endogenous sources of bacteria likely play a greater role than exogenous ones. Most surgery requires an incision through the skin, making skin commensals (S. pseudintermedius, Staphylococcus epidermidis, Staphylococcus schleiferi, Staphylococcus haemolyticus, Streptococcus canis, Pseudomonas aeruginosa) the most likely cause of an SSI. The oropharynx contains a mixed population of bacteria including gram-positive and negatives and anaerobes. The stomach, small intestine, and colon contain a rich and variable microbiota depending on the location within the gastrointestinal tract that ultimately turns any clean surgery into a clean contaminated surgery when their lumen is entered. Gram-negative bacteria predominate within the small intestine with anaerobes playing a smaller role; however, this ratio is reversed as you move aborally. Finally, the respiratory and genitourinary tract potentially contains both gram-positive and gram-negative organisms.

Based on the expected bacterial population discussed above, the majority of surgeries benefit from first generation cephalosporin administration (e.g. cefazolin) to provide broad spectrum coverage against gram-positive and gram-negative bacteria. Distal small intestinal,
large intestinal, and hepatobiliary surgery benefits from additional anaerobic coverage which can be provided by second generation cephalosporin’s (e.g. cefoxitin) that still provide coverage for typical skin commensals.\textsuperscript{10,27,35,45} These guidelines are based only on expected bacterial populations to guide empirical perioperative prophylaxis; however, it is always recommended that continued antibiotic administration be based on culture and susceptibility results collected from the infected surgical site.

\textbf{1.3.2 –Frequency, dose, and duration of antibiotic}

Logically, in order for prophylactic antibiotics to be effective, they must be present in effective concentrations at the start and through the duration of surgery. Recommendations for parenteral administration of the initial dose of any drug is within an hour of the first incision.\textsuperscript{10,27,45} Administration of the first dose after surgery has started may eliminate the opportunity to protect a patient from SSI.\textsuperscript{30,45} Timing for additional intra-operative doses is based on the chosen antimicrobial that is most appropriate. As an example, a commonly used perioperative antimicrobial is the beta lactam cefazolin. The recommended re-dosing interval is every 2 half-lives which is approximately 90 minutes.\textsuperscript{10,30,45} Human guidelines discourage administration of post-operative antibiotics beyond 24 hours and there is evidence that prolonged administration can contribute to resistance.\textsuperscript{10,19,45} Since these are guidelines and limited research has been performed in the veterinary field, there are obvious exceptions to consider. However, the ideal duration of post-operative administration (if any) has yet to be determined.

\textbf{1.3.3 –Use in general practice}
An article published in 2012 evaluated current veterinary perioperative antimicrobial use in Great Britain. Veterinarians in this study used antimicrobials in only 25.3-32.1% of clean surgical procedures but that number rose above 85% when the procedure was considered contaminated or dirty. The most commonly selected antimicrobials (in descending order) included potentiated amoxicillin, amoxicillin, clindamycin, enrofloxacin, cephalexin, and metronidazole. Important factors considered by these veterinarians when deciding to use antibiotics were patient immunosuppression, drain placement, degree of contamination, implant placement, and spillage of visceral contents. One of the major conclusions from the article was that perioperative antimicrobials are used inappropriately in many private practices with timing, duration of administration, and the chosen antimicrobial regularly not meeting recommended guidelines.

The use of perioperative prophylaxis has offered protection against SSI for many small animal patients. Unfortunately, there is still an inadequate amount of evidence-based research in the veterinary medical field to produce a guideline on prophylactic use of antimicrobials similar to the human medical field. Extrapolating from the human recommendations is a good first step but should be done cautiously as there are expected differences across species. Knowledge of the patient’s medical history and the procedure to be performed play an important role in not only selecting a perioperative antibiotic but whether or not it is even necessary. Once this decision has been made, considering each patient as an individual and developing a comfortable knowledge with the expected commensal bacterial population can help guide appropriate antibiotic selection.

1.4 Biofilms
1.4.1 – Definition and bacteria implicated

Biofilms have complicated prevention and treatment of SSIs further. Biofilms consist of a colony of bacterial cells tightly adhered to a surface and enclosed in a matrix of polysaccharide (Figure 1.1). Microorganisms typically only account for a small percent of the dry mass within the matrix, produced primarily by the cells within it, accounting for over 90% of the biofilm. The matrix consists of extracellular polymeric substances that play an important role in the structure of the biofilm and adhesion to surfaces.

Figure 1.1: Life cycle of a biofilm.

A concerning feature of biofilms is the altered behavior of organisms within them. Biofilm-associated organisms have different susceptibilities to antimicrobials and transcribe and exchange genetic material differently than their planktonic counterparts providing a mechanism
for spreading and promoting resistance.\textsuperscript{49,52,53} This can result in resistance to typical antimicrobial therapy. Additionally, it has been reported that bacteria within a biofilm require a 10-1000 fold increase in the required drug to be efficacious.\textsuperscript{54} In many cases this cannot be achieved by oral or injectable methods. The susceptibility of organisms within the biofilm to antimicrobials is also altered by variations in the metabolism of bacteria in the dormant or stationary phase.\textsuperscript{53,55} Antibiotics that rely on an active cell ultimately have little or no effect because their mechanism of action cannot be altered in a similar fashion.

Biofilm matrices provide a more efficient collection and storage of resources allowing them to thrive in environments with limited nutrient sources. The biofilm matrix is so efficient that it traps debris from lytic cells to recycle internally.\textsuperscript{50,53} Digestion of the available nutrients in a biofilm can be carried out individually or as a communal approach in that extracellular enzymes are retained within the matrix providing access to all members even those of a different genus or species.\textsuperscript{50}

As discussed above, \textit{S. pseudintermedius} is a commonly encountered bacterium within veterinary SSIs.\textsuperscript{1,18,32–34} Additionally, it has genetic coding for biofilm production and maintenance further complicating already difficult to treat SSIs.\textsuperscript{52,56} In a study by Singh et. al., 96\% of \textit{S. pseudintermedius} isolates tested were moderate or strong biofilm producers, with the ability to produce these biofilms being unrelated to methicillin-resistance.\textsuperscript{56} This problem highlights the need for addressing not only bacteria in surgical sites but also the formation of biofilm, specifically in procedures that require implants.

\textbf{1.4.2 – Agents effective against biofilms}
Therapy for biofilms has received attention and some products have shown efficacy in vitro. OligoG is an alginate oligomer that has shown promise in human literature at increasing the effect of antibiotics and the immune system against *Pseudomonas aeruginosa* in patients with chronic lung infections secondary to biofilms from cystic fibrosis.\(^{57-59}\) Nitric oxide has also demonstrated the capacity to encourage bacteria within a biofilm to undergo dispersal (release to a planktonic state) making them more accessible to the host immune system and antibiotics. The nitric oxide ultimately acts as a signalling molecule to increase the activity of phosphodiesterase which leads to biofilm dispersal.\(^{60-62}\) Although research has been primarily on *P. aeruginosa*, there have been similar effects demonstrated by other microorganisms through a similar pathway.\(^{60}\) In a study evaluating multiple anti-biofilm agents, they found that only Johnson Baby Soap\(^a\) in combination with an antibiotic effectively eradicated biofilms produced by both *Staphylococcus aureus* and *P. aeruginosa*.\(^{63}\) They also found that hydrogen peroxide alone and in combination with antibiotics is capable of eradicating *S. aureus* biofilm but only inhibited *P. aeruginosa* biofilms. Recombinant human deoxyribonuclease I alone reduced *S. aureus* biofilm but did not enhance bacterial activity of antibiotics. It also inhibited *P. aeruginosa* biofilm in combination with antibiotics.\(^{63}\) In the veterinary field, the enzyme Dispersin B has received the most attention as an anti-biofilm agent and is the focus of this research project.

### 1.4.3 – Dispersin B

Dispersin B\(^d\) (β-N-acetylglucosaminidase) has offered optimism in the field of biofilm research. It is an enzyme produced by the bacterium *Aggregatibacter actinomycetemcomitans* that causes the detachment of biofilm cells from surfaces in an attempt to spread the biofilm.\(^{64}\) This detachment occurs through degradation of a β-1,6-N-acetyl-d-glucosamine polymer
PNAG, an extracellular polysaccharide that mediates intercellular adhesion. PNAG is not produced by all biofilm producing organisms; therefore, Dispersin B is not effective against all biofilms. Based on the presence of PNAG during biofilm formation, Dispersin B may be effective against S. pseudintermedius, S. epidermidis, Staphylococcus lugdunensis, and Bordetella bronchiseptica.

A study by Turk et. al., demonstrated that Dispersin B was able to significantly reduce biofilm formation and degrade established biofilm in vitro but it had no effect on the growth of MRSP. Dispersin B was also found to efficiently remove biofilms produced by S. epidermidis on plastic surfaces and pre-coating surfaces with the Dispersin B prevented biofilm formation by the same bacteria. The next logical step would be the addition of Dispersin B with an appropriate antibiotic to combat implant-associated infections with superior efficacy.

1.5 – Local drug delivery

An ideal vehicle for administration of local antibiotics and other components has yet to be produced. Characteristics of an ideal local delivery system include: (1) prolonged elution of the chosen drug(s), (2) non-immunogenic to prevent a local inflammatory response, (3) biodegradable to reduce the risk of sub-inhibitory elution of antibiotics and consequently developed resistance, and (4) cost effective, readily available products to both general practitioners and referral hospitals. Since the introduction of local antibiotic administration in the 1970s, many new delivery systems have been proposed and tested with the addition of different drugs with each one having advantages and disadvantages. Many of the early products were not biodegradable and ultimately removal was recommended so more recent research has focussed on products available in liquid or gel formulations.
Biodegradable products available include poloxamer 407, Vetri-gel, calcium sulphate (plaster of paris), and collagen sponges. Polymethylmethacrylate (PMMA) is a non-biodegradable product broadly used for local antibiotic delivery. Additionally, direct coating of implants has also received attention. Among other benefits, the use of local delivery systems have the advantage of lower systemic concentrations of potentially harmful drugs while delivering higher concentrations to the site of concern which is ideal for a concentration dependent antibiotics such as amikacin (Table 1.5).

Table 1.5: Advantages and disadvantages of local drug delivery. Modified from Hayes et al.

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>High local concentrations of a drug at the site of concern not achievable systemically</td>
<td>Risk of cytotoxicity from high concentrations of drug or the carrier</td>
</tr>
<tr>
<td>Focussed delivery of drugs to the site of concern while minimizing systemic toxicity</td>
<td>Risk of carrier affecting wound healing, being a nidus for infection, or requiring surgical removal</td>
</tr>
<tr>
<td>Reduced systemic exposure and subsequent reduction in fecal output may reduce environmental exposure and selection for resistance traits</td>
<td>Risk of sub therapeutic drug concentrations (i.e. antibiotics) leading to development of resistance traits in bacteria</td>
</tr>
<tr>
<td>Delivery of drug is independent of the vascular nature of the tissue affected or the protective environment (i.e. biofilm, abscess)</td>
<td>Lack of research on appropriate dose ranges for different species or wounds and pharmacokinetic or pharmacodynamic profiles</td>
</tr>
<tr>
<td>Client compliance</td>
<td>Limited research on when systemic therapy is</td>
</tr>
</tbody>
</table>
1.5.1 – Delivery Systems

1.5.1.1 – Coated implants

Coating of medical implants serves as a preventive technique against infections in most cases; however, replacing medical devices in areas of active infection may also be necessary.\(^{84–86}\) Coating medical implants has garnered a lot of attention in primarily the human literature in an attempt to reduce the risk of infection associated with permanent implants as well as prolong the safety of short term implants. Examples of orthopedic applications include silver impregnated implants, hyaluronic acid-based antibiotic hydro gel coatings, and covalently bound antibiotic coatings.\(^{85–88}\) Studies of each of these techniques have shown efficacy against not only preventing or treating active infection at the site of implant but also preventing or reducing biofilm formation which plays an important role (as discussed above) in the efficacy of local and systemic antibiotics and potentially development or perpetuation of resistance traits.\(^{84–86,88}\) Drugs used with these coated implants include vancomycin, gentamicin, and caspofungin; however, many alternatives could be used based on the patient’s specific need.

Due to the high rate of nosocomial urinary tract infections and the frequency of urinary catheter usage, urinary catheters have received likely the most attention amongst interim use medical devices. An ideal device would be antimicrobial, biocompatible, and antifouling. Unfortunately, no such material has been identified despite decades of research.\(^{87}\) Clinically
tested materials include silver and a range of antibiotics. In a 2014 study, silver-alloy hydrogel catheters were shown to reduce the relative risk of urinary tract infection by 58%. The breadth of research into antibiotics available for urinary catheters in human medicine is beyond the scope of this project but a published review of the literature documents nitrofural, minocycline, rifampin, and sparfloxacyn as the most commonly used antibiotics. This review concluded that based on the available research, antibiotic coated urinary catheters are more efficient than silver-alloy coatings for commonly encountered urinary tract infections; however, with emerging antibiotic resistance this may change in the near future. An even larger set of materials have undergone research but lack clinical evidence at this time for their use.

1.5.1.2 – Polymethylmethacrylate

PMMA is a powdered bone cement polymer that when mixed with liquid methylmethacrylate forms a solid within 5-10 minutes. Human use of antibiotic impregnated bone cement became popular in the 1970’s as prophylactic antibiotic therapy in arthroplasties and for treatment of osteomyelitis. Antibiotics were incorporated into the powdered bone cement polymer prior to mixture with the liquid methylmethacrylate and then either used as an adhesive (fracture fixation, arthroplasty) or formed into beads to place in the surgical site. The chemical reaction that creates the PMMA is exothermic so the antibiotic used must be able to resist heat damage. Initially, it was thought that the antibiotic chosen must be available in powder form because liquid formulations had a negative effect on the polymerization process; however, more recent research has suggested similar efficacy in liquid or powder forms. Additionally, if the antibiotic impregnated PMMA is used as part of a fracture fixation, its dilution effect must be considered as it may reduce fatigue life.
Aminoglycosides and cephalosporins have received the most attention in the literature with several studies documenting their elution from PMMA.\textsuperscript{75,78} Mixed results regarding efficacy when compared to systemic therapy have been demonstrated in the literature. In a systematic review of the human literature looking at prophylactic use of local antibiotics on surgical site infections during total joint arthroplasties they found an eight percent absolute risk reduction and 81 percent relative risk reduction.\textsuperscript{93} Interestingly, when comparing antibiotic impregnated cement versus systemic antibiotics, there was no significant difference in clinical effect.\textsuperscript{93} On the other hand, a canine experimental model demonstrated a significantly higher rate of resolution for gentamicin impregnated PMMA compared to systemic therapy with gentamicin. The dogs in this study had artificially induced \textit{Staphylococcus aureus} osteomyelitis and were divided into two groups of treatment with the PMMA therapy group showing 89% resolution compared to 63% with systemic therapy when infection was evaluated post mortem 6-weeks after therapy was initiated.\textsuperscript{94} Another study evaluated in vitro biofilm formation from MRSP isolates when incubated with gentamicin, silver, or gentamicin and silver impregnated PMMA beads.\textsuperscript{72} They found that the gentamicin and gentamicin/silver combination could effectively reduce biofilm formation, compared to a control group, when isolates of MRSP were susceptible to gentamicin. The silver alone was not effective at reducing biofilm formation. This finding highlighted the importance of drug elution from PMMA which is reliant on the drug being present at high concentrations at the surface, where it is biologically active. Additionally, a larger proportion of drug can be eluted from smaller beads because they have a higher surface area-to-volume ratio effectively increasing exposure to the environment.\textsuperscript{95}

One of the major drawbacks to using PMMA is the need for an additional surgery to remove the beads to prevent bacterial colonization. Most concerning are the studies that have
found resistant strains of bacteria in surgical sites containing antibiotic impregnated beads at the
time of their removal.\textsuperscript{96,97} In one human study, 22\% of patients that had antibiotic impregnated
beads implanted to treat orthopedic infections had bacterial colonization at the time of explant.
Additionally, some of the bacterial strains that were originally susceptible to certain antibiotics
had become resistant.\textsuperscript{96} Beads removed up to 5 years after implantation, have continued to elute
sub-inhibitory concentrations of antibiotic which has been implemented as a factor in the
development of these resistant strains (if not all bacteria at the surgical site were eliminated prior
to reaching this concentration).\textsuperscript{97} With more recent attention on biodegradable alternatives
because of the potential consequences of using non-absorbable materials, there may a shift away
from using them in veterinary medicine in the future.

\textbf{1.5.1.3 – Calcium sulfate}

Calcium sulfate, also known as plaster of Paris, is biodegradable bone cement used in
both human and veterinary medicine in orthopedic and soft tissue surgery. One advantage to
using calcium sulfate as a local delivery system in orthopedic surgery is its osteoconductive
properties that provide an environment suitable for healing. Osteoconductive properties of bone
graft refer to the materials ability to act as a scaffold for bone healing to take place. It does not
provide cells for bone growth or factors involved in stimulating bone growth, but still play a
major role in healing.\textsuperscript{98} In human surgery, calcium sulfate is commonly used in maxillofacial
surgery and cases of chronic osteomyelitis because of its ability to act as a bone graft substitute
and deliver local antibiotics at high concentrations.\textsuperscript{98,81,99} In one human study, tobramycin-
impregnated calcium sulfate was radiographically resorbed at an average of 2.7 months and 23 of
25 cases of chronic osteomyelitis resolved.\textsuperscript{99} Five of the 7 of these fractures that were non-union
healed without the addition of any autogenous bone graft. However, it should be noted that some studies have shown that the osteoconductive properties can be negatively impacted with large volumes/doses of antibiotic so this must be considered when compounding these materials.

In veterinary medicine, in vitro and clinical studies have been performed evaluating the use of antibiotic-impregnated calcium sulfate. Elution varies significantly based on the antibiotic chosen with drugs like amikacin no longer inhibiting growth of Staphylococcus spp. within 24 hours and vancomycin inhibiting growth for up to 56 days. The combination of these drugs also dramatically altered their elution with both eluting faster and ultimately reducing their duration of bacterial growth inhibition. Gentamicin impregnated beads maintained their bactericidal activity against Escherichia coli for a minimum of 14 days in another study. In this study, the beads were sterilized using ethylene oxide and still effective up to 5 months after sterilization. A case series of 5 dogs with radiographic, clinical, or culture confirmed osteomyelitis showed resolution of osteomyelitis in all cases when tobramycin-impregnated calcium sulfate beads were implanted. The beads resorbed radiographically at a mean of 5 weeks in these cases and no adverse effects were documented.

1.5.1.4 – Gentamicin impregnated collagen sponges

Gentamicin-impregnated collagen sponges (GICS) are lyophilized collagen sponges impregnated with gentamicin to allow dosing based on the size of sponge used. Equine or bovine type I collagen is used in the manufacturing process and gentamicin is concentrated at 2mg/cm. Human use has been broadly applied to many areas including prophylaxis and treatment of infections in orthopedic, oncologic, and soft tissue surgery. A large systematic review of
randomized controlled trials including 15 studies and nearly 7000 patients found that there was a significant decrease in SSI when considering clean and clean contaminated procedures specifically. Veterinary use of the GICS has been primarily focussed on septic arthritis. Equine and canine research evaluating the use of GICS within the joint (tarsocrural and stifle, respectively) found that the drug concentration within the joint was up to 50 times above the MIC for *Staphylococcus* spp at the maximum concentration but fell below MIC within 24-48 hours, potentially conferring limited advantages over parenteral administration. Despite this rapid decrease in concentration within the joint, there are reports in the literature of successful use of these GICS to treat infection locally in both large and small animals which seems promising.

Unfortunately, the rapid rate of elution and persistence of foreign collagen has identified cause for concern in the canine population. In one canine study, local and systemic effects of a GICS implanted within a stifle with iatrogenically induced synovitis were evaluated. Canine patients had significantly higher joint inflammation compared to a control group for up to 35 days after implantation. Force plate analysis also showed significantly increased levels of lameness in the GICS group at 7 days post operatively but no difference at 14 days. The GFR in the GICS group was also significantly decreased but renal biopsies showed no difference in pathology. A second canine study implanted the GICS against the femur (outside the joint) and evaluated urea and creatinine in blood, urine specific gravity, urinalysis, and serum concentration of gentamicin daily for 7 days. Hematologic renal markers remained within normal limits, urine specific gravity was essentially unchanged, and no casts were identified on any urinalysis to suggest renal damage. Serum concentration of gentamicin was only detected at low levels on day 1. These studies demonstrate the importance of location of implantation
and the local environment. The first study suggests a more clinical picture for septic arthritis with inflammation present within the joint at the time of implantation which may increase the rate of antibiotic uptake but also increase the risk of renal damage with systemic inflammation also likely present. Additionally, the environment within a joint is considerably more mobile and may therefore increase the rate of breakdown. The second study represents a more prophylactic clinical use at the site of an implant and may be less deleterious to the patient’s health. However, inflammation was not evaluated at the site of implantation, so this cannot be assumed either way.

### 1.5.1.5 – Poloxamer 407

Poloxamer 407 is a triblock copolymer with a hydrophobic chain of polyoxypropylene and two identical lateral hydrophilic chains of polyoxyethylene that has recently gained substantial interest as a delivery system for local antibiotics. This product can be compounded in-house and is thermo-reversible so it readily transforms from a solution to a gel based on temperature without compromising its efficacy. Poloxamer 407 is compounded with other components at approximately 5°C while in a liquid state and typically concentrated at a 20-30% weight/weight (w/w) ratio. It is stored refrigerated in a liquid state until its use. Once introduced to warmer temperatures (i.e. the patient’s body), the Poloxamer 407 and compounded components transform to a semi-solid state at 22-31°C based on the w/w concentration. Previous studies have documented safe implantation of Poloxamer 407 and appropriate elution profiles of drugs over a period of time without clinically relevant effects on the rheological characteristics of the delivery system. The use of poloxamer 407 was initially attractive for this project because of its accessibility and low cost to produce; however, its dissolution was
too rapid for the proposed duration of effect dissolving within 3-5 days. This may still be attractive if high doses of drug are desired for only short periods of time.

1.5.2 – Vetri-Gel

Vetri-gel (previously R-gel when compounded with antibiotics) is a cross linked dextran polymer that is marketed for use as an absorbable wound dressing. The gel is purchased as a two syringe kit containing a dihydrazide cross linking reagent in one syringe and a solution of oxidized dextran in the other syringe. Mixture of the two syringes in a reciprocating fashion initiates the chemical reaction through addition of the hydrazide nitrogen to an aldehyde carbon which then dehydrates to form the gel over a two minute period. The gel degrades via hydrolysis over 4-5 weeks.73 Drugs can be added to the cross linking reagent, prior to mixing, providing local delivery of the chosen drug at the site of administration.

One study evaluated the safety of R-gel administration into the brain of rats and showed it had minimal effect on the surrounding tissues in comparison to a saline control.112 In another study, amikacin, vancomycin, and clindamycin eluted from the gel rapidly in the first 24 hours, similar to most local delivery systems.73 The antibiotic combinations maintained therapeutic drug levels against S. aureus for the 10 day duration of the study.73 To the author’s knowledge, this is the only article in the literature that evaluated the elution of antibiotics using R-gel, but this biodegradable product and drug combination is no longer available.73 Vetri-Gel contains the same polymer hydrogel as R-gel but does not contain any antibiotics. The inert nature of Vetri-
Gel, its ability to have drug combinations made in hospital, and its dissolvability make it a near perfect vehicle for local drug delivery but it requires additional research prior to clinical use.

1.6 – Thesis objectives and hypotheses

The purpose of this research is to determine the elution profile of amikacin and Dispersin B (individually and together) in a cross linked dextran polymer (Vetri-gel®). Prior to administering these products to a patient population it would be of value to evaluate the elution properties of these combined products in vitro. The elution profile of amikacin and Dispersin B dissolved in a cross linked dextran polymer (Vetri-gel) is the logical first step in determining whether these products have potential for preventing and/or treating SSIs in a clinical setting. Their combined effects against biofilm and bacteria make them an attractive pair with high potential for clinical efficacy.

Additionally, a follow-up retrospective study will help to further outline the effects of peri-operative patient care on surgical site infection in a single procedure; the TPLO. A previous retrospective study performed at the Ontario Veterinary College (OVC) by Nazarali, et al. found post-operative antibiotics significantly reduced the infection rate after TPLO. Since this study was published, more than 600 TPLO’s have been performed at the OVC offering a unique opportunity to evaluate the long term effects of a protocol change in antibiosis for this procedure at one institution. If administration of post-operative antibiotics is truly protective against SSI, there may be a role for local antibiotic administration at the time of TPLO.

A more thorough understanding of the effect post-operative antibiotics may have on reducing the SSI rate will play a vital role in making recommendations in the future. Additionally, treating infections with the emergence of resistant bacteria is becoming more
challenging with antibiotic therapy becoming more limited. Identifying an ideal vehicle for local antibiotic therapy as a prophylactic or preventive therapy is an important next step in managing these veterinary patients. Addressing complicating factors of these infections, (i.e. biofilms), will hopefully slow down emerging resistance patterns by more effectively killing the bacteria responsible.

1.7 – Footnotes

a – Johnson & Johnson, New Brunswick, NJ, US

b – Royer Biomedical, Frederick, MD, US

c – Royer Biomedical, Frederick, MD, US

d – Kane Biotech, Winnipeg, MB, Canada

1.8 – References


10. Bratzler, DW; Dellinger, EP; Olsen, KM; Perl, TM; Auwaerter, PG; Bolon, MK; Fish, DN; Napolitano, LM; Sawyer, RG; Slain, D; Steinberg, JP; Weinstein R. Clinical practice guidelines for antimicrobial prophylaxis in surgery. *American Journal of Health-System Pharmacy*. 2013;70:195-283. doi:10.2146/ajhp120568

doi:10.1097/SLA.0b013e31816c3fec

doi:10.1111/vsu.12738


28. Etter SW, Ragetly GR, D P, Bennett RA, Schaeffer DJ, D P. Effect of using triclosan-impregnated suture for incisional closure on surgical site infection and inflammation


35. Boothe DM, Jr HWB. Antimicrobial Considerations in the Perioperative Patient Antimicrobial prophylaxis Surgical site infection Antiseptics Disinfectants. *Veterinary
doi:10.1016/j.cvsm.2015.01.006

doi:10.1016/j.vetmic.2014.02.008


42. Hans EC, Barnhart MD, Kennedy SC, Naber SJ. Comparison of complications following tibial tuberosity advancement and tibial plateau levelling osteotomy in very large and

43. Brown G, Maddox T, Siles MMB. Client-assessed long-term outcome in dogs with surgical site infection following tibial plateau levelling osteotomy. *Veterinary Record*. 2016;October. doi:10.1136/vr.103688

44. Bartram OJ, Baldwin DS. Veterinary surgeons and suicide: A structured review of possible influences on increased risk. *Veterinary Record*. 2010;166(13):388-397. doi:10.1136/vr.b4794


47. Knights CB, Mateus A, Baines SJ. Papers Current British veterinary attitudes to the use of perioperative antimicrobials in small animal surgery. *Veterinary Record*. 2012. doi:10.1136/vr.100292


2010;8(9):623-633. doi:10.1038/nrmicro2415


73. Thomas LA, Bizikova T, Minihan AC. In vitro elution and antibacterial activity of


doi:10.1007/s11999-014-3558-1

doi:10.1097/WON.0000000000000056


doi:10.1302/0301-620X.90B7.20498


Walenkamp G. Small PMMA beads improve gentamicin release. *Acta Orthopaedica*. 
1989;60(6):668-669. doi:10.3109/17453678909149599


106. Summerhays G. Treatment of traumatically induced synovial sepsis in horses with gentamicin-. *The Veterinary Record*. 2000;147:184-188.


doi:10.1002/jbm.a.33207
CHAPTER II: Contributing factors to surgical site infection after tibial plateau leveling osteotomy; a follow up retrospective study

Chris R.M. Hagen BSc, DVM¹, Ameet Singh DVM, DVSc¹, DAVCS, J. Scott Weese DVM, DVSc, DACVIM², Quinn Marshall BSc¹, Alex zur Linden DVM, DACVR¹, Thomas W.G. Gibson DVM, DVSc, DACVS, DACVSMR¹

¹Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, Canada

²Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Canada

This manuscript has been submitted for publication in *Veterinary Surgery*, August, 2019
2.1 – Abstract

Objective: Identify factors associated with surgical site infection (SSI) after tibial plateau leveling osteotomy (TPLO).

Study Design: Retrospective case series.

Animals: Dogs (n=541) that underwent TPLO (n=659)

Methods: Medical records of dogs that underwent TPLO from 2011-2018 at the OVCHSC were reviewed. Data collected included peri- and post-operative antimicrobial administration, method of stifle inspection, duration of surgery and anesthesia, co-morbidities, and development of SSI including timing, microbiological investigation, and implant removal. Referring veterinarians were contacted and asked to fill out a survey for all patients without a return visit. Risk factors for SSI were assessed using a multivariable logistic regression model built using a stepwise approach.

Results: SSI was documented in 71/659 (10.77%) cases, with methicillin-resistant Staphylococcus pseudintermedius accounting for 20/71 (28.17%). Protective factors against SSI included administration of post-operative antibiotics (OR 0.263; 95% CI=0.157, 0.442) and timing of pre-operative antibiotic administration. Pre-operative antibiotic timing was protective against SSI when administered greater than 60 minutes before the first incision when compared to within 30 minutes (OR 0.275; 95% CI= 0.112, 0.676) or within 60 minutes (OR 0.419; 95% CI = 0.189, 0.929). Dogs under the age of six and over the age of twelve were at increased risk of developing an SSI (OR 0.969; 95% CI = 0.941, 0.997)

Conclusion: Post-operative antibiotic administration was protective against SSI after TPLO. Optimal pre-, intra- and post-operative antimicrobial regimens including timing and length of administration requires further research.
Clinical Significance: Administration of antibiotics after TPLO should be considered to reduce SSI.

2.2 – Introduction

Surgical site infection (SSI) after tibial plateau leveling osteotomy (TPLO) is a common, frustrating, and expensive complication, and for reasons that have not been discerned, SSI rates after TPLO (2.9-25.9%) tend to be higher than those for other orthopedic surgeries. The source of this increased risk is unknown but likely multifactorial. Factors hypothesized to contribute, related to the procedure itself, include periosteal dissection, thermal bone necrosis associated with the tibial osteotomy, prolonged surgery and anesthesia times, and limited soft tissue coverage on a large orthopedic implant. The literature currently lacks sufficient agreement on these contributing factors or preventative measures that could help to reduce SSI.

One controversial finding specific to the TPLO is the use of post-operative antibiotics to reduce SSI. In general, use of post-operative antibiotics is not considered necessary for clean orthopedic surgeries. Several studies, however, have documented post-operative antibiotics after TPLO as protective against SSI. In contrast, several studies have found no protective benefit of post-operative antibiotics following TPLO.

Emergence of resistant bacteria has reinforced the need for antibiotic stewardship. *Staphylococcus pseudintermedius* has been cultured in 50-70% of North American veterinary hospitals making it one of the most commonly cultured bacteria. Unsurprisingly, with the frequency of this bacteria in hospitals, resistant strains have become common with methicillin resistant *S. pseudintermedius* (MRSP) at the forefront. Several studies have documented a 20-40% prevalence of SSI caused by MRSP. With the TPLO being one of the most
commonly performed procedures in veterinary orthopedic surgery, reducing SSI could have a
dramatic effect on the perpetuation of MRSP infections. Treating these SSI’s is often challenging
with limited effective antibiotic treatment options, increased cost to the client, and increased
patient morbidity. In one study, post-operative costs for patients with a SSI following TPLO
were, on average, $1559 compared to $212 in a control population. The authors also noted that
these costs underestimate the actual cost to a client with additional time off work and travel
expenses not considered. A study previously published from this institution found shorter anesthesia and use of
post-operative antibiotics significantly reduced the risk of infection. Additionally, there was a
trend toward appropriate peri-operative antibiotic timing reducing SSI but this was not
significant. Since this study was published, more than 600 TPLO’s have been performed
implementing changes in patient protocols primarily during post-operative management. This
provided a unique opportunity to further evaluate the findings and possibly add power to future
recommendations. The objective of this study was to identify factors associated with SSI after
TPLO including the association between SSI and post-operative antibiotic administration. We
hypothesized that appropriate peri-operative antibiotic timing and post-operative antibiotic use
would be protective against SSI and prolonged surgical and anesthetic time would be a risk
factor for developing a SSI.

2.3 – Materials and Methods

2.3.1 - Dogs

Dogs with complete medical records that had a unilateral TPLO from 2011-2018 at the
Ontario Veterinary College (OVC) Companion Animal Hospital and at least one follow up visit
were considered eligible for inclusion. Referring veterinarians were contacted and asked to fill out a survey for all patients without a return visit to the OVC after their TPLO to identify SSIs that were unrecorded in the medical record. If no follow up was available or medical records were incomplete, these dogs were excluded. If dogs had staged bilateral TPLO’s performed on separate dates, they were considered independent cases for inclusion.

2.3.2 – Data Collection

Data collected included timing and dosage of pre, intra, and postoperative antimicrobial administration, method of stifle inspection (arthrotomy, arthroscopy, both), duration of surgery and anesthesia, development of surgical site infection including timing, microbiological investigation, implant removal, and possible comorbidities. SSI was identified from the medical record using Center for Disease Control definitions as a guideline. Briefly, while reviewing the medical record, a dog was considered to have developed an SSI if a veterinarian evaluated the incision site and documented one or more of the following: (1) pus (2) bacteria, (3) heat, redness, pain, fever, or localized swelling AND spontaneous dehiscence or surgical re-exploration with evidence of infection, or (4) evidence of abscess or infection on cytology or diagnostic imaging. Ideally, a bacterial culture was performed and available for review but patients with clinical signs but no culture performed or a negative culture were diagnosed with a SSI. Peri-operative antibiotic re-administration was considered necessary every 90 minutes based on this institutions recommended protocol.
2.4 – Data Analysis

Information from each patient’s medical record was entered into a spreadsheet on Microsoft Excel\textsuperscript{a}. Descriptive statistics (mean, median, standard deviation, range) were calculated. Data was transferred to SAS\textsuperscript{b} for further analysis.

Exact conditional logistic regression was used to assess risk factors for surgical site infection. Continuous variables were assessed for linearity and any that did not meet these assumptions were transformed appropriately. Univariable analysis was used to identify variables with a P value of <0.2 to be further assessed in a multivariable model. Forward and backward stepwise logistic regression analysis was performed to find the best fit model. Significance was set at p<0.05. The pre-operative antibiotic timing, peri-operative antibiotic timing (0-30 or 0-60 minutes, every 90 minutes thereafter), and antibiotic re-dose timing after the first incision variables were co-linear, therefore only one could be used in the final model.

2.5 – Results

Five hundred and forty one dogs who underwent a total of six hundred and fifty nine TPLO’s met the inclusion criteria. Of the 659 dogs, there were 273 (41.4%) castrated males, 14 (2.1%) intact males, 360 (54.6%) spayed females, and 12 (1.8%) intact females. The mean age of the dogs was 5.4 ± 2.5 (range: 0.9-14.1) years and their average weight was 39.0 ± 12.0 (range: 10-94) kg. Mean anesthesia time was 216.9 ± 41.7 (range: 110-535) minutes. Mean surgery time was 94 ± 27.4 (range: 40-285) minutes. During surgery, the stifle joint was evaluated via arthroscopy alone in 263 (39.9%) patients, arthrotomy alone in 82 (12.4%) patients, and arthroscopy and arthrotomy in 314 (47.7%) patients. Co-morbidities of any kind were documented in 45 (6.8%) patients.
All patients received peri-operative antibiotics with 657 (99.7%) receiving cefazolin and two (0.3%) receiving clindamycin. The timing of pre-operative antibiotics (prior to the first incision) was within 30 minutes in 112 (17.0%) dogs, within 31-60 minutes in 379 (57.5%) dogs, greater than 60 minutes in 153 (23.2%) dogs, and after the incision in 15 (2.3%) dogs. Re-dosing of antibiotics (every 90 minutes) intra-operatively was required in 622 patients with 480 (77.2%) dogs receiving it within 90 minutes of the first dose. An additional dose was required in 97 dogs with 81 (83.5%) receiving it within 90 minutes of the previous dose. Target peri-operative antibiotic dosing (within 60 minutes of the first incision, every 90 minutes thereafter as required) was obtained in 374 (56.75%) dogs.

Seventy-one (10.8%) dogs developed an SSI after TPLO with infection identified a median of 17 days (range: 3-1147 days) post-operatively. 29/71 (40.8%) infections were identified within 14 days, 51/71 (71.8%) within 30 days, 57/71 (80.3%) within 90 days, and 65/71 (91.5%) within one year. Sixty-nine (97.2%) SSIs were detected at the referral hospital and two (2.8%) during referring veterinarian follow up. Specimens were submitted for bacterial culture in 64/71 (90.1%) cases with bacteria recovered in 59/64 (92.2%) (Table 2.1). MRSP was the most commonly cultured bacteria accounting for 20/71 (28.2%) cases. Of the 71 documented cases of SSI, 42 (59.2%) ultimately had their implants removed.
Table 2.1: Bacterial culture results for the 71/659 Cases with surgical site infection after TPLO.

<table>
<thead>
<tr>
<th>Bacteria Cultured</th>
<th># of Cases</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methicillin resistant <em>Staphylococcus pseudintermedius</em></td>
<td>20</td>
<td>28.2</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>12</td>
<td>16.9</td>
</tr>
<tr>
<td><em>Staphylococcus pseudintermedius</em></td>
<td>11</td>
<td>15.5</td>
</tr>
<tr>
<td>Multiple organisms</td>
<td>4</td>
<td>5.6</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>2</td>
<td>2.8</td>
</tr>
<tr>
<td><em>Pasteurella spp</em></td>
<td>2</td>
<td>2.8</td>
</tr>
<tr>
<td><em>Actinomyces</em></td>
<td>2</td>
<td>2.8</td>
</tr>
<tr>
<td><em>Streptococcus canis</em></td>
<td>1</td>
<td>1.4</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>1</td>
<td>1.4</td>
</tr>
<tr>
<td><em>Enterococcus faecium</em></td>
<td>1</td>
<td>1.4</td>
</tr>
<tr>
<td><em>Brevibacterium casei</em></td>
<td>1</td>
<td>1.4</td>
</tr>
<tr>
<td><em>Arcanobacterium</em></td>
<td>1</td>
<td>1.4</td>
</tr>
<tr>
<td><em>Pseudomonas Aeruginosa</em></td>
<td>1</td>
<td>1.4</td>
</tr>
<tr>
<td>No growth</td>
<td>5</td>
<td>7.0</td>
</tr>
<tr>
<td>Culture not performed</td>
<td>7</td>
<td>9.9</td>
</tr>
</tbody>
</table>

Post-operative antibiotics were administered to 455 dogs (69.0%) with cephalexin accounting for 426 of those cases (93.6%). Other antibiotics patients were discharged with included amoxicillin/clavulanic acid (17; 3.7%), doxycycline (5; 1.1%), clindamycin and cefovecin (2; 0.4%), and chloramphenicol, and amoxicillin (n=1 each; 0.2%). Antibiotics were
administered for a mean of 6.2 ± 2.2 (Range: 2-15) days. Of the 455 dogs that received post-operative antibiotics, 30/455 (6.6%) developed an SSI compared to 41/204 (20.1%) that did not receive antibiotics.

Univariable data are presented in Table 2.2. The relationship between age and SSI is presented in Table 2.3. The relationship between intraoperative antibiotic administration relative to the first incision and SSI is represented in Figure 2.1. When timing of the first intra-operative re-dose was evaluated, administering this dose within 30 minutes after the first incision was protective against SSI when compared to within 31-60 minutes (OR 0.4; P = 0.038; 95% CI 0.173, 0.963). This was not entered into the final multivariable model due to co-linearity with pre-operative antibiotic timing.

Table 2.2: Univariable analysis of variables predicted to be associated with surgical site infection (SSI) after TPLO. Exact conditional logistic regression was used to determine risk factors for SSI. P-values are presented with odds ratios when significant.

<table>
<thead>
<tr>
<th>Variable</th>
<th>P-value</th>
<th>Odds ratio (95% confidence limits)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>0.328</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>0.362</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.004*</td>
<td>0.589 (0.413, 0.841)</td>
</tr>
<tr>
<td>Age (quadratic)</td>
<td>0.013*</td>
<td>1.037 (1.008, 1.068)</td>
</tr>
<tr>
<td>Timing of pre-operative antimicrobial administration</td>
<td>0.0356*</td>
<td></td>
</tr>
<tr>
<td>0-30 min vs 31-60 min</td>
<td>0.278</td>
<td></td>
</tr>
<tr>
<td>Timing of repeat antimicrobial dosing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>intraoperative</td>
<td>0.979</td>
<td></td>
</tr>
<tr>
<td>Target peri-operative dosing (within 30 minutes, every 90 minutes)</td>
<td>0.040*</td>
<td>2.0 (1.032, 3.704)</td>
</tr>
<tr>
<td>Target peri-operative dosing (within 60 minutes, every 90 minutes)</td>
<td>0.417</td>
<td></td>
</tr>
<tr>
<td>Appropriate peri-operative dose of antibiotics</td>
<td>0.987</td>
<td></td>
</tr>
<tr>
<td>Duration of surgery</td>
<td>0.868</td>
<td></td>
</tr>
<tr>
<td>Duration of anesthesia</td>
<td>0.272</td>
<td></td>
</tr>
<tr>
<td>Stifle evaluation technique</td>
<td>0.142</td>
<td></td>
</tr>
<tr>
<td>Co-morbidities</td>
<td>0.907</td>
<td></td>
</tr>
<tr>
<td>Post-operative antibiotics</td>
<td>&lt;0.0001*</td>
<td>0.281 (0.164, 0.479)</td>
</tr>
<tr>
<td>Post-operative antibiotic duration</td>
<td>0.244</td>
<td></td>
</tr>
<tr>
<td>Appropriate post-operative dose of antibiotics</td>
<td>0.469</td>
<td></td>
</tr>
<tr>
<td>Antibiotic re-dose timing after incision</td>
<td>0.083</td>
<td></td>
</tr>
<tr>
<td>0-30 min vs 31-60 min</td>
<td>0.038*</td>
<td>0.435 (0.173, 0.963)</td>
</tr>
<tr>
<td>0-30 min vs &gt;60 min</td>
<td>0.084</td>
<td></td>
</tr>
<tr>
<td>31-60 min vs &gt;60 min</td>
<td>0.830</td>
<td></td>
</tr>
<tr>
<td>Implant Removal</td>
<td>&lt;0.0001*</td>
<td>34.483 (17.857, 66.667)</td>
</tr>
</tbody>
</table>
Table 2.3: Summary of the association between age and developing a surgical site infection after TPLO. Exact conditional logistic regression was used for contrast estimation between select age categories to demonstrate the infection trend at the upper and lower age limits. P-values are presented with odds ratios.

<table>
<thead>
<tr>
<th>Age Comparison (years)</th>
<th>P-value</th>
<th>Odds Ratio (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 vs 3</td>
<td>0.005*</td>
<td>0.643 (0.473, 0.874)</td>
</tr>
<tr>
<td>5 vs 6</td>
<td>0.004*</td>
<td>0.863 (0.780, 0.954)</td>
</tr>
<tr>
<td>8 vs 9</td>
<td>0.595</td>
<td>1.049 (0.880, 1.251)</td>
</tr>
<tr>
<td>10 vs 11</td>
<td>0.214</td>
<td>1.195 (0.902, 1.583)</td>
</tr>
<tr>
<td>12 vs 13</td>
<td>0.124</td>
<td>1.361 (0.919, 2.016)</td>
</tr>
<tr>
<td>14 vs 15</td>
<td>0.090</td>
<td>1.551 (0.935, 2.574)</td>
</tr>
</tbody>
</table>
Figure 2.1: Probability of developing a surgical site infection relative to timing of antibiotic re-administration after the first incision. Probability (blue) and 95% confidence limits (red) are included.

The final multivariable model is presented in table 2.4. Post-operative antibiotic administration (OR 0.3; P < 0.0001; 95% CI = 0.157, 0.442) and administration of the peri-operative antibiotics greater than 60 minutes before the first incision compared to within 30 minutes (OR 0.3; P = 0.005; 95% CI = 0.112, 0.676) or within 31-60 minutes (OR 0.4; P = 0.032; 95% CI = 0.189, 0.929) were protective against SSI. Age had a quadratic relationship with SSI with dogs younger than six and older than twelve being at higher risk of developing an SSI (OR 0.97; P = 0.033; 95% CI = 0.941, 0.997). The relationship between SSI, age, pre-operative antibiotic timing, and post-operative antibiotic administration are presented in Figure 2.2-2.3.
Table 2.4: Stepwise backward logistic regression analysis of variables predicted to be associated with surgical site infection after TPLO. Variables placed into the model were chosen based on a P-value <0.2 on univariable analysis (Table 2.2). P-values are presented with odds ratios when significant.

<table>
<thead>
<tr>
<th>Variable</th>
<th>P-value</th>
<th>Odds Ratio (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.008*</td>
<td>1.645 (1.142, 2.370)</td>
</tr>
<tr>
<td>Age (quadratic)</td>
<td>0.033*</td>
<td>0.969 (0.941, 0.997)</td>
</tr>
<tr>
<td>Timing of pre-operative antimicrobial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>administration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;60 mins vs 0-30 mins</td>
<td>0.005*</td>
<td>0.275 (0.112, 0.676)</td>
</tr>
<tr>
<td>&gt;60 mins vs 31-60 mins</td>
<td>0.032*</td>
<td>0.419 (0.189, 0.929)</td>
</tr>
<tr>
<td>Post-operative antibiotics</td>
<td>&lt;0.0001*</td>
<td>0.263 (0.157, 0.442)</td>
</tr>
</tbody>
</table>
Figure 2.2: Probability of developing a surgical site infection relative to age in patients that received post-operative antimicrobials. Four categories of pre-operative antimicrobial administration timing are presented relative to the first incision (Blue: 0-30 minutes, Red: 31-60 minutes, Green: >60 minutes, Purple: After incision).
Figure 2.3: Probability of developing a surgical site infection relative to age in patients that did not receive post-operative antimicrobials. Four categories of pre-operative antimicrobial administration timing are presented relative to the first incision (Blue: 0-30 minutes, Red: 31-60 minutes, Green: >60 minutes, Purple: After incision).

2.6 – Discussion

Our study identified peri-operative antibiotic timing and post-operative antibiotic administration were protective against SSI. Age was also identified as a risk factor for developing an SSI with the model predicting an increased risk of infection under six or over twelve years of age.

An impact of pre-operative antimicrobial timing on SSI was identified. However, the association of administration beyond 60 minutes of the first incision being protective is, at first
glance, counter to standard recommendations and data from other species. There is no consensus on the ideal peri-operative antibiotic protocol in the veterinary literature so many of the current recommendations are extrapolated from human literature. The current peri-operative human recommendations include a pre-operative antimicrobial dose within 60 minutes of the first incision and re-dosing within every two half-lives of the chosen antibiotic.\textsuperscript{21–25} One veterinary study had similar recommendations based on their findings.\textsuperscript{17} However, a small number of human studies, however, have found that administration of the pre-operative antibiotic dose too close to the time of the first incision is not protective when compared to dosing further from the first incision.\textsuperscript{26–28} Here, it is possible that this result does not truly reflect the importance of administering antimicrobials greater than 60 minutes prior to surgery, but is a proxy for re-dosing of antimicrobials early in the surgical procedure. Because intra-operative dosing is routinely performed, dogs that received a first dose 60 minutes prior to incision would receive an intra-operative dose within the first 30 minutes after incision, and the SSI rate in dogs receiving antimicrobials within the first 30 minute period after incision was the lowest of any time period.

Dogs that received a pre-operative antibiotic dose closer to the time of the incision would be due for their first intraoperative dose near the time of proposed high risk periods of the surgery (stifle evaluation, tibial osteotomy, or plate placement).\textsuperscript{3,10,18} This theory is reinforced by the lower SSI rate in dogs that received a second dose of antibiotics within 30 minutes of the first incision when compared to those who received it 31-60 minutes after the first incision. In a recent pharmacokinetic study looking at interstitial cefazolin concentrations after intravenous administration, the peak concentration was at one hour with a steady decline thereafter.\textsuperscript{29} However, the measured concentrations remained above the minimum inhibitory concentrations of \textit{S. pseudintermedius, Streptococcus} spp and \textit{Escherichia coli} for greater than two hours.\textsuperscript{29} It is
important to note that these patients were not having a TPLO performed and no pharmacokinetic studies look at this surgery specifically and the effect the osteotomy or elevation of different muscles has on antibiotic concentrations in the surgical site. This highlights the need for more detailed study of peri-operative antimicrobial prophylaxis timing.

Post-operative antibiotic administration after TPLO has garnered attention recently with conflicting research on whether they can offer protection against SSI. Peri-operative antimicrobials, when indicated, should be at therapeutic levels during the period of risk. That is assumed to start with the first incision but the endpoint is not clear, as it likely extends for an undefined period after wound closure. In general, post-operative antimicrobials are not indicated because once the period of risk has ended, there is no benefit and unnecessary use can lead to concerns about adverse events, cost and antimicrobial resistance. In human surgery, post-operative administration rarely extends beyond 24 hours. These data, along with other studies of TPLOs demonstrated a protective effect of post-operative antimicrobials.

However, studies have not clarified what type of post-operative approach is indicated. The average duration of post-operative antibiotic administration was approximately six days but duration was not significantly associated with developing an SSI. Therefore, it is unclear whether the relatively long durations used here are needed for protection, or whether shorter durations, even the 24h period that is widely used in human medicine, might be adequate. Further research is therefore needed to determine the ideal duration of post-operative antibiotic administration to gain the protective benefit of reducing SSI without encouraging resistance or exposing patients to their adverse effects. Accordingly, these data should be taken as indication of support for some form of post-operative antimicrobial treatment for TPLO (not necessarily other procedures), but not necessarily an indication for the durations that were used in these patients.
Age of dogs developing an SSI in this study was not linear with younger and older dogs at highest risk of developing an SSI (Figure 1). The gradual decrease in infection risk from age two to age six was significant; however, the gradual increase in infection risk from age twelve and older was not significant. One limitation to this finding is the small number of patients in the age categories beyond twelve years. A larger patient population may have exposed this as a significant risk factor. Age has not been a well-documented risk factor for SSI in veterinary medicine; however, in human medicine extremes of age have documented higher rates of SSI. These may be explained by alterations in the immune system of younger and older patients as well as their general differences in personality and activity. Younger patients may be more prone to over activity after surgery and wound dehiscence which can then become infected. Co-morbidities are also more likely in older patient populations.

Co-morbidities were not associated with SSI in this study despite previous research. This may represent a type II error due to the small number of co-morbidities among the dogs in our study. With a larger sample size of co-morbidities such as endocrine diseases or dermatitis, we may have appreciated an association with SSI. The TPLO is not a representative sample population to assess co-morbidities; however, because the population of patients typically affected by ruptured cranial cruciate ligaments are young to middle aged and otherwise healthy. This study documented a similar finding with the median age of affected animals being 5.4 years.

Limitations of the study include the potential for errors in data collection with medical record use not primarily intended to capture SSI. This leads to a potential for missed SSI rather than identifying those that are not truly SSI. Patients did not require long term follow up to be included in the study but just required a single visit back to the OVC. Although patients who did
not return to the OVC following their TPLO had a follow up survey performed by their referring veterinarian, it is possible that infections developed in patients who only returned for suture removal and therefore no follow up was performed with the referring veterinarian. However, infections were identified at a median of 17 days in this study which is the period of closest observation considering most patients return to their veterinarian or surgeon at approximately two weeks for suture removal or incision evaluation. In one study, only 65% of the SSI were documented in the medical records when patient follow up was performed via telephone conversation at 30 days and one year. In our study, two (2.82%) additional SSI were identified on referring veterinarian follow up but only cases with no follow up at the primary surgical facility had these surveys filled out.

Conversely, identifying an SSI incorrectly may also be a limitation with the retrospective nature of this study. Relying on medical records to describe the physical exam findings and the subjective nature of these descriptions may lead to categorizing surgical site inflammation as infection. In veterinary medicine, the definition of SSI is not as clearly defined and surveillance systems are lacking. Fortunately, in this study, 90% of the cases categorized as a SSI based on clinical signs and physical exam had a culture performed with less than 10% having a negative culture. These additional diagnostics in addition to patient evaluation and follow up by a board certified surgeon or a surgical resident add confidence to the data collected.

In conclusion, this study found that post-operative antibiotics were protective against the well documented higher risk of SSI following TPLO. The duration of post-operative antibiotic administration requires further research but can likely be limited to several days based on these findings. Additionally, administering a pre-operative antibiotic dose further from the first incision was identified as a protective factor against SSI for the first time in veterinary literature.
The timing of intra-operative antibiotic re-administration (within 30 minutes) may better explain this pre-operative finding with peak concentrations at potentially high risk periods of the procedure. The optimal timing of pre, intra-, and post-operative antimicrobial regimens requires further research before making any further recommendations in a clinical setting.

2.7 – Footnotes

a – Microsoft, Redmond, WA, US


2.8 – References


11. Pratesi A, Moores AP, Downes C, Grierson J, Maddox TW. Efficacy of Postoperative Antimicrobial Use for Clean Orthopedic Implant Surgery in Dogs: A Prospective...


19. Weese JS, van Duijkeren E. Methicillin-resistant Staphylococcus aureus and
68


CHAPTER III: In vitro elution of amikacin and Dispersin B from a polymer hydrogel

Chris R.M. Hagen BSc, DVM\textsuperscript{1}, Ameet Singh DVM, DVSc\textsuperscript{1}, DAVCS, J. Scott Weese DVM, DVSc, DACVIM\textsuperscript{2}, Quinn Marshall BSc\textsuperscript{1}, Alex zur Linden DVM, DACVR\textsuperscript{1}, Thomas W.G. Gibson DVM, DVSc, DACVS\textsuperscript{1}

\textsuperscript{1}Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, Canada

\textsuperscript{2}Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Canada

This manuscript has been submitted for publication in Veterinary Surgery, August, 2019
3.1 – Abstract

Objective: To characterize the in-vitro elution of amikacin and Dispersin B in a dissolvable polymer. Group A – amikacin; Group D – Dispersin B; Group AD – amikacin and Dispersin B.

Study Design: In-vitro, experimental study.

Methods: Amikacin (40mg/mL), Dispersin B (70ug/mL), or amikacin (40mg/mL) and Dispersin B (70ug/mL) were added to a polymer. Ten aliquots per group were incubated in phosphate buffered saline that was exchanged at 1, 4, 8, 12, and 24 hours then once daily for 10 days. The eluted amikacin and Dispersin B was quantified using a commercially available amikacin reagent kit and Dispersin B ELISA kit. Drug concentrations were compared between groups using repeated measures ANOVA and area under the curve calculation.

Results: Amikacin and Dispersin B alone (A and D) and compounded together (AD) underwent rapid elution in the first twenty-four hours followed by a gradual decrease over ten days. The total elution of amikacin was significantly greater from group AD compared to A (P = 0.02, 95% CI = -30239.0, -2618.92). The total elution of Dispersin B was not significantly different between group D and AD (P= 0.50, 95% CI = -2310.75, 4505.52).

Conclusions: Total elution of Dispersin B was not effected by combination with amikacin. Total elution of amikacin was significantly greater in group AD compared to group A, but is likely of no clinical significance.

Clinical Significance: The combination of amikacin and Dispersin B in a dissolvable polymer could allow local treatment of complex infections.
3.2 – Introduction

With antimicrobial resistance on the rise globally, techniques designed to reduce development of resistance while treating infections should be prioritized. Systemic administration of a chosen drug may fail to achieve effective concentrations at the site of concern. Additionally, the environment at the site of infection is unique, even to the type of bacteria responsible, and can impact the efficacy of the chosen drug. Possible solutions for treatment of complex, resistant, or polymicrobial infections include local delivery of antimicrobials at high concentrations independent of the wound microenvironment and vascularity and alterations to the wound environment enhancing antimicrobial penetration.

Biofilms alter the environment at the site of an infection by providing a protective barrier between bacteria and antimicrobials. Biofilms consist of aggregates of bacteria attached to a surface, in a matrix of polysaccharide. When compared to their planktonic counterparts, bacteria within a biofilm have different susceptibilities to antimicrobials due to variations in their metabolism and enhanced transcription and exchange of genetic material. In some cases, this altered bacterial metabolism of drugs increases the concentration of antimicrobials required for bacterial cell death by 10-1000 fold. One of the most commonly cultured bacteria in veterinary hospitals, Staphylococcus pseudintermedius, is capable of producing a biofilm. Furthermore, there has been an emergence of methicillin resistant S. pseudintermedius (MRSP) with documented rates in surgical site infections of 20-40%. Methicillin resistant S. pseudintermedius is not inherently resistant to amikacin, however, amikacin has significant adverse effects when administered systemically, including nephrotoxicity and ototoxicity, which may limit its dose or duration in certain patients.
Dispersin B (β-N-acetylglucosaminidase) has received attention for its capability to breakdown implant associated biofilms.\textsuperscript{22,23} Dispersin B is an enzyme produced by the bacterium Aggregatibacter actinomycetemcomitans that degrades N-acetylglucosamine, an extracellular polysaccharide that mediates intracellular adhesion within a biofilm.\textsuperscript{23} Dispersin B has efficacy in vitro against biofilm formation but lacks antibacterial properties.\textsuperscript{16,24} In one study, 20 μg/mL of Dispersin B prevented biofilm formation and degraded established biofilm from MRSP.\textsuperscript{16} Another study found pre-coating surfaces with 40 μg/mL of Dispersin B could effectively prevent biofilm formation, and treating biofilm-coated surfaces lead to degradation of the biofilm produced by S. epidermidis.\textsuperscript{24}

An ideal vehicle for administration of local antimicrobials and other components has yet to be produced, however, Vetri-gel\textsuperscript{b}, has shown encouraging results in the literature.\textsuperscript{25,26} Vetri-gel is a polymer hydrogel that is commercially available as a 2-syringe kit. One syringe contains an oxidized dextran solution and the other a cross-linking reagent in powder form that after mixing, gradually turns to a gel within two minutes. The dehydrated gel does not migrate once injected and is degraded via hydrolysis within 4-5 weeks in vivo.\textsuperscript{25} This gel provides a unique opportunity to administer drugs that not only manage bacterial load but also provide a means for environmental modifications of biofilms. To address the need for alternative antimicrobial therapy, including treatment and prevention of biofilm production, this studies objective was to evaluate the in vitro elution of amikacin and Dispersin B from Vetri-gel. Our hypothesis was that there would not be a difference in the elution of amikacin or Dispersin B when combined or added individually.
3.3 – Materials and Methods

3.3.1 – Sample Preparation

Amikacin sulfate and Dispersin B (595 ug/mL) were added to Vetri-gel to produce three groups (A, D, AD) described below. Amikacin concentrations in group A and AD were 40 mg/mL and Dispersin B concentrations in group D and AD were 70 ug/mL. These concentrations were chosen based on previous research on elution of Amikacin from a similar gel and Dispersin B preparations.\textsuperscript{7,16,25} All calculations were determined using the commercially available 2 mL Vetri-Gel formulations. Prior to connecting the syringes, group A had 80 mg of Amikacin sulfate in powder form added to the cross-linking reagent (80 mg/2 mL) and group D had 158.87 ug of Dispersin B in liquid form added to the dextran solution (158.87 mg/2.267 mL). Group AD had 158.87 ug of Dispersin B in liquid form added to the dextran solution and 90.68 mg of Amikacin sulfate in powder form added to the cross-linking reagent (90.68 mg/2.267 mL) prior to connecting the syringes.

For each group, aliquots of 0.5 mL were pipetted into individual sterile 2 mL cryogenic vials. Ten replicates for each group (30 samples total) were prepared. Vials were left to sit for a minimum of five minutes and then evaluated by overturning the vial to ensure gelation was complete. After confirming gelation, 1 mL of sterile phosphate-buffered saline (PBS) with a pH of 7.4 was pipetted on top of the gel, being careful not to disrupt it. Two distinctly separate layers (gel and PBS) were visible from the outside of the vial. The vials were then sealed with a sterile plastic cap and stored in an incubator at 37°C without agitation between sampling. The PBS was removed in its entirety at each sampling period with a pipette and stored in individual 2 mL sterile cryogenic vials at 4°C until analyzed (within 72 hours). The PBS was replaced with
an additional 1 mL of sterile PBS and returned to the incubator until the next sampling. Samples were collected at 1, 4, 8, 12, and 24 hours then once daily for a total of 10 days.

### 3.3.2 Sample Analysis

The concentration of amikacin in groups A and AD were determined using a Roche Cobas 6000 c501 analyzer\(^6\) and the AMIK2 reagent kit\(^7\). The measuring range of the AMIK2 reagent kit was 0.8-40 \(\mu\)g/mL. All calibrations, controls, and samples were performed according to the manufacturer’s specifications. When results were above the upper limits of quantification, dilutions were performed using sterile PBS.

The concentration of Dispersin B in groups D and AD were determined using a Dispersin B ELISA kit\(^8\). All samples were analyzed in triplicate according to the product information sheet. Briefly, 50 \(\mu\)L of sample or standard (100 \(\mu\)g/mL serially diluted to within the range of 20-500 ng/mL) was added to each well in a 96 well flat bottom plate\(^8\) and incubated at 4°C overnight to coat the well. The residual sample was then removed and each well washed three times with 200 \(\mu\)L washing buffer (PBS, 0.05% (v/v) Tween-20\(^9\)). After the final wash, 200 \(\mu\)L of well blocking solution (2% bovine serum albumin in wash buffer) was added to each well and incubated at room temperature for 2 hours with agitation. After incubation, the wells were washed with 300 \(\mu\)L of washing buffer again then 100 \(\mu\)L of Detection Antibody (anti-Dispersin B monoclonal antibody with alkaline phosphatase conjugate\(^a\) diluted 500-fold in washing buffer) was added to each well and incubated for an additional two hours at room temperature with agitation. After incubation, the wells were washed with 600 \(\mu\)L of washing buffer before adding 50 \(\mu\)L of substrate solution (4-nitrophenyl phosphate bis(cyclohexylammonium) salt (Sigma) in substrate buffer (5% diethanolamine\(^c\), 0.5mM MgCl\(_2\)_\(^{b}\)). Optical density of the wells
was read at 405 nm after 30 minutes on an Epoch microplate spectrophotometer. Serial dilutions of the standard provided in each kit were prepared for each set of samples that were analyzed. A linear curve was prepared in Microsoft Excel using the standards to extrapolate the concentration in each sample analyzed using the equation of the line.

3.4 – Data Analysis

Data were analyzed using SAS. To compare the change in concentration of amikacin and Dispersin B over time for AD vs A and AD vs D, respectively, a repeated measures ANOVA was modelled. Fixed effects of treatment and time as well as their interaction were included in the model. Data were checked for normality with a Shapiro Wilk test and examination of the residuals. Data were log transformed to meet the assumption of normality. Correlation structures were tested using the lowest Akaike information criterion (AIC) as best. Post hoc tests back to baseline within a treatment were based on a Dunnetts adjustment.

To compare the total amikacin or Dispersin B eluted, an area under the curve calculation using an integration approach was carried out for each sample. The data were run through a general linear model and met the assumptions of normality. Post hoc tests comparing the two treatment groups per drug were based on student’s T-tests. Significance was set at p<0.05.

3.5 – Results

Dispersin B rapidly eluted from the Vetri-gel in the first 24 hours with 1826.82 ng/mL (73.8% of total drug eluted) and 1624.94 ng/mL (67.2% of total drug eluted) eluted in group D and group AD, respectively (Figure 3.1). Time point comparisons for Dispersin B elution are presented in Table 3.1. The concentration of Dispersin B eluted in D was significantly higher at 1 day as compared to the AD and significantly lower from day 5 to 10. The total drug eluted from
D and AD was 2473.97 ng/mL (7.1%) and 2416.81 ng/mL (6.9%), respectively. Using the area under the elution curve, the total Dispersin B eluted over 10 days (Table 3.3) was not significantly different between D (28,428 (ng/mL)*hours) and AD (27,330 (ng/mL)*hours) (P = 0.50, 95% CI = -2310.75, 4505.52).

Figure 3.1: Dispersin B elution from Vetri-Gel over ten days. Group D contains Dispersin B alone while Group AD contains Dispersin B and amikacin.
Table 3.1: One-way analysis of variance (tukey adjusted) for repeated measures comparing the elution of Dispersin B from Vetri-Gel when added alone or in combination with amikacin over ten days. Concentration of Dispersin B (ng/mL) presented as the mean of ten samples (± standard deviation).

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Dispersin B</th>
<th>Dispersin B in Amikacin</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hour</td>
<td>349.11 (41.86)</td>
<td>455.06 (37.21)</td>
<td>0.089</td>
</tr>
<tr>
<td>4 hours</td>
<td>510.17 (66.12)</td>
<td>485.64 (69.42)</td>
<td>0.99</td>
</tr>
<tr>
<td>8 hours</td>
<td>312.67 (71.97)</td>
<td>276.44 (68.46)</td>
<td>0.957</td>
</tr>
<tr>
<td>12 hours</td>
<td>184.47 (26.83)</td>
<td>169.40 (17.40)</td>
<td>0.342</td>
</tr>
<tr>
<td>1 day</td>
<td>470.40 (65.86)</td>
<td>238.40 (35.78)</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td>2 days</td>
<td>271.39 (53.36)</td>
<td>236.50 (24.95)</td>
<td>0.140</td>
</tr>
<tr>
<td>3 days</td>
<td>84.59 (31.46)</td>
<td>68.57 (7.36)</td>
<td>0.804</td>
</tr>
<tr>
<td>4 days</td>
<td>59.45 (4.86)</td>
<td>82.57 (39.23)</td>
<td>0.137</td>
</tr>
<tr>
<td>5 days</td>
<td>35.83 (12.93)</td>
<td>69.22 (26.18)</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td>6 days</td>
<td>32.32 (1.26)</td>
<td>61.10 (20.04)</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td>7 days</td>
<td>39.95 (6.26)</td>
<td>82.81 (37.92)</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td>8 days</td>
<td>33.54 (2.20)</td>
<td>57.76 (12.60)</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td>9 days</td>
<td>39.53 (5.79)</td>
<td>65.92 (15.90)</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td>10 days</td>
<td>50.55 (1.54)</td>
<td>67.42 (6.26)</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

*Significant difference in elution between formulations (p<0.05)
Amikacin rapidly eluted from the Vetri-gel in the first 24 hours with 4750.38 ug/mL (51.4% of total drug eluted) when alone (A) whereas when in combination with Dispersin B (AD) was delayed with only 3350.89 ug/mL (39.5% of total drug eluted) (Figure 3.2). Time point comparisons for amikacin elution are presented in Table 3.2. The concentration of amikacin eluted in A, compared to AD, was significantly higher at 1, 4, and 8 hours and on day 10, and it was significantly lower on day 1. The total drug eluted from A and AD was 9249.09 ug/mL (46.2%) and 8491.18 ug/mL (42.5%), respectively. Using the area under the elution curve, the total amikacin eluted over 10 days (Table 3.3) was significantly less from A (144 950 (ug/mL)*hours) when compared to AD (161 378 (ug/mL)*hours) (P = 0.022, 95% CI = -30 239.0, -2618.92).

Figure 3.2: Amikacin elution from Vetri-Gel over ten days. Group A contains amikacin alone while Group AD contains Dispersin B and amikacin. Amikacin MIC$_{90}$ for methicillin resistant Staphylococcus pseudintermedius (MRSP) included for reference.
Table 3.2: One-way analysis of variance (tukey adjusted) for repeated measures comparing the elution of amikacin from Vetri-Gel when added alone or in combination with Dispersin B over ten days. Concentration of amikacin (ug/mL) presented as the mean of ten samples (± standard deviation).

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Amikacin</th>
<th>Amikacin in Dispersin</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hour</td>
<td>857.57 (98.58)</td>
<td>148.01 (91.68)</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td>4 hours</td>
<td>1013.64 (49.67)</td>
<td>488.78 (186.24)</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td>8 hours</td>
<td>843.33 (70.73)</td>
<td>480.62 (122.31)</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td>12 hours</td>
<td>681.93 (49.59)</td>
<td>523.26 (238.87)</td>
<td>.5106</td>
</tr>
<tr>
<td>1 day</td>
<td>1353.91 (80.65)</td>
<td>1710.22 (197.18)</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td>2 days</td>
<td>1709.60 (86.83)</td>
<td>2017.31 (254.22)</td>
<td>0.696</td>
</tr>
<tr>
<td>3 days</td>
<td>1000.06 (112.25)</td>
<td>1214.43 (135.13)</td>
<td>0.0566</td>
</tr>
<tr>
<td>4 days</td>
<td>606.99 (72.68)</td>
<td>605.81 (53.61)</td>
<td>1.0</td>
</tr>
<tr>
<td>5 days</td>
<td>372.07 (71.57)</td>
<td>416.12 (103.71)</td>
<td>0.997</td>
</tr>
<tr>
<td>6 days</td>
<td>248.04 (59.65)</td>
<td>296.59 (91.41)</td>
<td>0.982</td>
</tr>
<tr>
<td>7 days</td>
<td>184.41 (39.56)</td>
<td>234.06 (80.63)</td>
<td>0.957</td>
</tr>
<tr>
<td>8 days</td>
<td>164.50 (37.26)</td>
<td>185.29 (61.84)</td>
<td>0.946</td>
</tr>
<tr>
<td>9 days</td>
<td>126.90 (53.07)</td>
<td>109.02 (25.64)</td>
<td>0.954</td>
</tr>
<tr>
<td>10 days</td>
<td>86.14 (15.46)</td>
<td>61.66 (9.57)</td>
<td>0.033*</td>
</tr>
</tbody>
</table>

*Significant difference in elution between formulations (p<0.05)
Table 3.3: Student t-tests of the area under the elution curve (AUC) comparing the total elution over ten days of amikacin and Dispersin B from Vetri-Gel when added alone (A, D) or in combination (AD).

<table>
<thead>
<tr>
<th></th>
<th>Amikacin</th>
<th>Dispersin B</th>
<th>Combination</th>
<th>P value (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin (ug/mL)*hours</td>
<td>144 950</td>
<td>N/A</td>
<td>161 378</td>
<td>0.02* (-30 239.0, -2618.92)</td>
</tr>
<tr>
<td>Dispersin B (ng/mL)*hours</td>
<td>N/A</td>
<td>28 428</td>
<td>27 330</td>
<td>0.50 (-2310.75, 4505.52)</td>
</tr>
</tbody>
</table>

*Significant difference in elution between formulations (p<0.05)

CI – Confidence interval

**3.6 – Discussion**

Amikacin rapidly eluted from Vetri-gel in group A and AD in the first 24 hours with AD initially having a slower release than A in the first 12 hours but ultimately a significantly larger concentration of amikacin was eluted over ten days in group AD. Dispersin B rapidly eluted from Vetri-gel in group D and AD in the first 24 hours with no significant difference in the total elution of Dispersin B between groups over ten days. Due to differences in the total elution of amikacin between group A and AD and time point differences between groups (A vs AD and D vs AD) we reject our hypothesis that there would no difference in the elution of amikacin or Dispersin B.
The significant difference at certain time points and of the total elution of amikacin between group A and AD likely has limited clinical significance due to the high concentrations present in both groups at all time points. In vivo concentrations of 8-10 times the MIC have been recommended for amikacin, similar to other concentration dependent drugs. In one study, the amikacin minimum inhibitory concentration (MIC) to inhibit growth in 50% (MIC_{50}) of the *S. pseudintermedius* isolates was 3 ug/mL regardless if it was methicillin sensitive or resistant. However, the MIC to inhibit growth in 90% (MIC_{90}) of the *S. pseudintermedius* isolates was substantially higher for the MRSP isolates at 24 ug/mL versus 3 ug/mL for methicillin susceptible strains. This suggests that some MRSP isolates require substantially higher concentrations of amikacin to inhibit their growth. To further complicate matters, *S.pseudintermedius* strains within a biofilm had an MIC (lowest amikacin concentration able to remove biofilm from a well plate) in excess of 2000 ug/mL. The concentration of amikacin in our study remained above the MIC_{90} of *S. pseudintermedius* (3-24 ug/mL) for the duration of sample collection. Additionally, the concentration in both groups remained ten times above the low and high end of the MIC_{90} of *S. pseudintermedius* for the duration of sample collection and six days, respectively. If similar concentrations are obtained in vivo, this would provide concentrations sufficient to inhibit growth in 90% of planktonic MRSP isolates at all time points but this reinforces the need for biofilm degradation in addition to antibiosis.

The eluted concentrations of Dispersin B and the relevance of significant differences in Dispersin B concentrations at certain time points is more difficult to interpret because of the limited research on the required concentrations to prevent or treat biofilm formation. From previous research on *S. pseudintermedius*, biofilm growth was prevented and treated in a tryptic soy broth containing 20 ug/mL of Dispersin B. The eluted concentrations in our study are
substantially lower than 20 ug/mL. In another study, urinary catheters were coated with 100 ug/mL of Dispersin B in combination with triclosan. This coating significantly inhibited biofilm formation on the urinary catheters from *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Escherichia coli*. Similarly, a third study found saturating polyurethane discs with Dispersin B, at a range of 20-600 ug/mL, significantly reduced the *S. epidermidis* colony forming units and biofilm formation at all concentrations. The concentration of Dispersin B within the Vetri-gel was chosen based on these previous studies that adsorbed the drug to a surface. Unfortunately, these studies did not determine the eluted concentrations of Dispersin B that were effective against biofilm and there is no literature evaluating the required concentration to prevent or treat biofilms, so it is not known if the concentrations eluted in this study would be sufficient.

Dispersin B elution from the Vetri-gel was considerably lower than expected with a total of approximately 7% eluted over ten days from both groups D and AD. Possible explanations include Dispersin B is truly released slowly from polymer hydrogels, the ELISA used to measure Dispersin B is insensitive and there is more released than detected, the combination of Dispersin B in Vetri-gel somehow inactivates the drug making it undetectable to the ELISA, or amikacin inhibits the ELISA. An inaccurate ELISA is possible; however, controls were used for every optical reading with a known concentration which was then plotted to extrapolate the concentration of the samples. The most likely explanation may relate to the nature of Dispersin B. It is a large molecule (40 kDa) compared to amikacin sulfate (782 Da), something that could play a role in its release from the gel and the small amount of total drug released.

The total concentration of amikacin eluted (42.5 -46.2%) was lower than previously documented from an R-gel study (73.0-74.0%). In this study, amikacin was combined with clindamycin or clindamycin and vancomycin and sample collection was similar, except they
used the commercially available R-gel which came with amikacin and clindamycin already in solution. The concentration of amikacin was also determined using fluorescence polarization immunoassay. The addition of Dispersin B may also play a role in the elution time of amikacin since there was a notable initial delay in our study. Regardless of the total drug eluted, a rapid initial release was documented in both studies which could play an important role in early bacterial kill since amikacin is a concentration dependent antimicrobial.\(^{21}\) Additionally, the drug concentration in solution was consistently above the MIC\(_{50}\) and MIC\(_{90}\) (3-24 ug/mL) for \(S.\) pseudintermedius in both studies as discussed above.\(^{15}\)

Vetri-gel was chosen for this study because it meets the majority of the criteria that make a local delivery system ideal; it is biodegradable preventing prolonged sub-inhibitory elution of drugs and the need for a subsequent procedure for removal, cost effective, and easy to prepare.\(^{8,29}\) Additionally, Vetri-gel does not undergo an exothermic reaction, like PMMA, making it a good vehicle for thermally unstable drugs.\(^{30}\) Vetri-gel was well tolerated in a group of 20 dogs that had it placed at the time of implant removal for treatment of surgical site infection with no dogs having documented signs of infection or inflammation at 12 weeks post-operative.\(^{26}\) Unfortunately, Vetri-gel has limited research on the effects different concentrations and environments have on its elution profiles. Other gels, such as thermo-reversible poloxamer 407, have identified a maximum added volume of 20-30% before the physical properties of the gel are affected.\(^{31,32}\) Intuitively, a maximum volume of drug would also affect the physical properties of Vetri-gel ultimately leading to more rapid degradation and therefore drug elution but this has not been evaluated.

Alternative delivery systems have not been evaluated for Dispersin B. Amikacin, however, has been compounded with several other local delivery systems including
polymethylmethacrylate (PMMA). In one study, the elution of amikacin from PMMA over 30 days was substantially less than our study at only 5.62% of the total incorporated drug. Concentrations of the amikacin did exceed MIC\(_{90}\) for the duration of the study when compounded individually, however, co-elution of amikacin (with cefazolin) led to concentrations below antimicrobial efficacy by day six exposing residual bacteria to sub-inhibitory concentrations that may lead to production of resistant bacterial strains. In another study, elution of amikacin from plaster of paris beads was rapid, whether compounded alone or with vancomycin, with sub-inhibitory concentrations reached within 24 hours. In-vitro elution of amikacin from ferric-hyaluronate was not as rapid with inhibitory concentrations present until day eight. In our study, co-elution of amikacin from Vetri-gel did not identify a decrease in concentration below MIC\(_{90}\) by day ten.

This study has several limitations. An in vitro elution model does not necessarily recreate a normal biological environment where there is serum, tissue, movement and other physiological variables that must be considered. These variables could alter the elution profile of both amikacin and Dispersin B, the interaction between the two drugs in solution or when released into the environment, and the efficacy of the drugs against biofilm formation and bacteria, as seen in other studies. To measure the concentration of drug released over time in this study, the PBS was exchanged which effectively removed it from the in vitro environment. In vivo, this drug would accumulate until systemically absorbed and metabolized. The balance between absorption and accumulation in vivo could significantly impact its efficacy and potential toxicity. Another limitation is compounding of Vetri-gel with other drugs has not been evaluated previously so its effect on the gel’s stability requires additional testing to determine if
the dilution effect reduce the gel’s residence time and ultimately the duration of drug administration locally.

In conclusion, the results of this study suggest that Dispersin B can be combined with amikacin without having an impact on the amikacin concentrations eluted to prevent microbial growth. By combining an antimicrobial with an anti-biofilm agent, this local delivery system has the potential for both prevention and treatment of implant-associated infections. Future research should focus on determining the Dispersin B concentrations required to prevent and treat biofilm formation and evaluating the additive effects of drugs on the Vetri-gel prior to in vivo implementation.

3.7 – Footnotes

a – Kane Biotech, Winnipeg, MB, Canada

b – Royer Biomedical, Frederick, MD, US

c – Sigma, St. Louis, MO, US

d – Corning Incorporated, Corning, NY, US

e – Roche, Basel, Switzerland

f – Roche Diagnostics, Indianapolis, IN, US

g – Corning Incorporated, Kennebunk, ME, US


i – Biotek, Winooski, VT, US

3.8 – References


8. Hayes G, Moens N, Gibson T. A review of local antibiotic implants and applications to veterinary orthopaedic surgery. *Veterinary and Comparative Orthopaedics and*


24. Kaplan JB, Raganath C, Velliyagounder K, Fine DH. Enzymatic Detachment of
**Staphylococcus epidermidis** biofilms. *Antimicrobial agents and chemotherapy.*


doi:10.1111/j.1532-950X.2009.00632.x


CHAPTER IV: Summary and conclusion

4.1 – Overview of factors associated with surgical site infection after tibial plateau leveling osteotomy

Infection is a potential consequence of any surgery, with the tibial plateau leveling osteotomy (TPLO) having a documented higher risk than other veterinary procedures.\textsuperscript{1–7} Theories for this increased risk include periosteal dissection, prolonged surgery and anesthesia times, limited soft tissue coverage over a large orthopedic implant, and thermal bone necrosis associated with the tibial osteotomy.\textsuperscript{4,8,9} With cranial cruciate ligament injuries being one of the most common canine injuries, the TPLO is subsequently one of the most commonly performed procedures making SSI an unfortunately regular occurrence in practices providing advanced surgical intervention.\textsuperscript{10,11} One of the management strategies that has received considerable attention in an attempt to address this, is administration of post-operative antibiotics, despite human and veterinary literature on other clean surgical procedures having no proven benefit.\textsuperscript{3,12–16} Support for the use of post-operative antibiotics after TPLO has grown in the last decade with several sources in the literature showing a reduction in SSI, although this remains a controversial subject.\textsuperscript{3,5,6,17,18}

One objective of our study was to evaluate peri-operative TPLO risk factors for SSIs because of this higher rate of infection than other clean orthopedic procedures despite being one of the most commonly performed procedures in veterinary medicine.\textsuperscript{19,20} A previous study published at the Ontario Veterinary College (OVC) in 2014 showed a significant reduction in SSIs when post-operative antibiotics were administered.\textsuperscript{3} The OVC has since implemented a protocol prescribing post-operative antibiotics following TPLO. This provided a similar study
population and environment to evaluate the outcome of this protocol change. Our study was somewhat unique when compared to the current literature because it followed a previous study with limited changes to the peri-operative protocol except for the regular administration of post-operative antibiotics.

We found that post-operative antibiotics and administration of peri-operative antibiotics greater than 60 minutes from the first incision were protective against SSI. Additionally, younger and older patient populations were at higher risk of developing an SSI. The findings related to pre-operative antibiotic timing were unexpected and therefore evaluated further. It is suspected that this earlier pre-operative dose led to an earlier intra-operative re-dosing of antibiotics. This intra-operative dose timing may correspond to generating peak antibiotic concentrations during times of the TPLO that have been theorized as the cause of higher SSI rates. These include stifle evaluation, tibial osteotomy, or plate placement. One limitation to this study is that the medical records do not document timing for each part of the TPLO and therefore we cannot directly evaluate the relationship between intra-operative antibiotic dosing and SSI. All of these steps are crucial parts of performing a TPLO so evaluating when they are performed relative to peri-operative antibiotic timing in a prospective manner may help us better understand their impact on TPLO SSI rates. Ultimately, these findings should form the foundation of future research into optimal peri-operative antibiotic timing to assist in reducing SSI rates, specifically after TPLO.

4.2 – Overview of local antibiotic therapy and the rationale for using a polymer hydrogel compounded with antibiotics and anti-biofilm agents

With antimicrobial resistance emerging and limited production of new antimicrobials, new strategies to manage or prevent infections are mandatory.21–23 Local delivery systems will
likely be a part of these future strategies and the pursuit for an ideal material is already underway. Polymer hydrogels have received attention more recently with Vetri-gel still being commercially available in veterinary medicine. Although limited research is available with this product, it is attractive as a local delivery system because it is cost effective, drugs can be compounded into the formulation prior to mixing and forming a solid, it is biodegradable, and non-immunogenic.\textsuperscript{24,25} Discovery of biofilms has highlighted the need for local adjunctive therapy when managing certain SSIs. In vitro research with Dispersin B has provided evidence to support its use to prevent or treat these biofilm associated infections.\textsuperscript{26,27} Our study addressed the need to evaluate the use of Dispersin B in a local delivery system by compounding it into a polymer hydrogel.

Our study aimed to determine the elution profile for amikacin and Dispersin B when combined or individually compounded within a polymer hydrogel because there was no literature evaluating their effect on one another. We hypothesized that their combination may provide a superior therapy for commonly implicated bacteria in implant associated SSI’s but in vitro investigation was necessary prior to clinical studies. Our study showed that the combination of these two drugs did not have a significant effect on their total in vitro elution. Additionally, the concentrations of drug eluted were maintained at high levels throughout the ten day collection period. The concentration of amikacin on day ten was at least ten times above the minimum inhibitory concentration for susceptible isolates of \textit{S. pseudintermedius}, \textit{Escherichia coli}, and \textit{Pseudomonas aeruginosa}, as recommended for concentration dependent antibiotics.\textsuperscript{28,29} Clinically, this combination may provide high concentrations of antimicrobial to the site of concern and our in vitro research has shown that the concentrations should be sufficient to kill common pathogens cultured from an SSI. Dispersin B has not been evaluated previously in
combination with an antimicrobial but we found it eluted in high concentrations in vitro. Without previous research looking at minimum required concentrations of Dispersin B to prevent or treat biofilm formation, we cannot conclude these concentrations would be sufficient in a clinical application. Our findings should guide future studies evaluating varying concentrations of Dispersin B on biofilm treatment to determine its efficacy prior to clinical studies.

4.3 – Future investigations

In vivo elution studies evaluating polymer hydrogels such as Vetri-gel are indicated prior to recommending for clinical use. This is the first study to evaluate the in vitro elution of antibiotics with an anti-biofilm agent. Dispersin B has documented efficacy against bacteria that produce biofilms with PNAG.\textsuperscript{26,27,30-32} The most commonly cultured bacteria in veterinary SSIs, \textit{Staphylococcus pseudintermedius}, are also biofilm producers containing PNAG.\textsuperscript{17,27,33} TPLO infections are also commonly caused by \textit{S. pseudintermedius}, which make the implants susceptible to biofilm formation. With our research showing a benefit of post-operative antibiotics following TPLO, an alternative option to parenteral administration would be to use coated implants or incorporate local antibiotics in the surgical site prior to closure. Further, the addition of Dispersin B in the polymer hydrogel would potentially prevent biofilm formation possibly increasing the effectiveness of antimicrobial therapy. In vivo studies evaluating efficacy and safety of local administration from these polymer hydrogels are required first; however, this study provides a foundation for future research.

Since local therapy may not be available to all clinicians, further research is indicated to determine the ideal duration of post-operative antibiotics to provide the protective benefit from SSIs without exposing the patient to prolonged and unnecessary adverse effects. It is possible
patients only require 24 hours of post-operative antibiotics to yield similar results, similar to humans. Additionally, peri-operative antibiotic protocols require further evaluation to determine the necessity, frequency, timing, dose, and duration of antibiotics during TPLO (among other procedures). Findings from this study are unique to the veterinary literature as administration of pre-operative antibiotics further from the incision resulted in a protective effect. At this time, it may be an indirect measure of a different finding, such as re-dosing antibiotics intraoperatively to provide peak concentrations during certain parts of the procedure. Well-designed prospective veterinary studies are necessary to broaden the literature base and add power to future recommendations for both local drug delivery and peri- and post-operative antimicrobial protocols during TPLO.

4.4 – References


