Prospective Evaluation of the Epidemiology and Microbiology of Surgical Site Infections

by

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in
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ABSTRACT

Prospective evaluation of the epidemiology and microbiology of surgical site infections

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University of Guelph, 2013

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Surgical site infections (SSIs) are an emerging cause of increased morbidity, mortality, and treatment cost, in veterinary medicine. Medical records were searched to evaluate for associations that could increase the risk of developing SSIs. Logistic regression was used to analyze the risk factors statistically, to determine their influence on SSI risk. An SSI incidence rate of 3.0% was found in this study for all small animal surgical procedures performed from September 2010 to July 2011, with implants, hypotension and surgical classification associated with increased likelihood of SSI. Active surveillance is crucial for the development of methods to prevent SSI’s.

Biofilms contribute to the antimicrobial resistance properties commonly found in bacteria such as methicillin-resistant Staphylococcus pseudintermedius, which is found in canines. An enzyme known as DispersinB was studied to assess its effect on biofilm formation and degradation. DispersinB prevented the formation and eradicated biofilm in vitro. *In vivo* testing is required to further assess the effects of DispersinB.
AKNOWLEDGEMENTS

I am very grateful to my thesis committee for their continuing support throughout my program. Drs. Scott Weese, Ameet Singh, and Paul Morley were constantly encouraging me to challenge myself and take my work to the next level.

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Finally I would like to thank my family, who always provided guidance and support throughout my entire academic career.
DECLARATION OF WORK PERFORMED

I, Ryen Turk, declare that all of the work completed during my MSc program included in this thesis was completed by me.
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CHAPTER 1

LITERATURE REVIEW
Introduction

Surgical site infection (SSI) is a potential complication to successful recovery in veterinary patients undergoing surgery; however, there has been inadequate study of their cause and prevention. All patients have an inherent risk of developing SSI, as they are colonized with bacteria, particularly on their skin [Eugster et al 2004, Vengust et al, 2006]. These opportunistic bacteria can potentially cause an infection in the perioperative period [Nicholson et al, 2002, Eugster et al, 2004, and Vengust et al, 2006]. Surgical site infections occur after a physical break in patient bodies’ physical barriers [Eugster et al, 2004, Weese et al, 2008]. In immune-compromised patients, the risk of infection is increased [Brown et al, 1997, Anderson et al, 2007]. Increased rates of morbidities related to infection are a common consequence of SSI [Eugster et al, 2004, Beal et al, 2000, Gallagher et al, 2010] and, there can also be significant economic burdens on animal owners or facilities. A more recent concern is the increase in multidrug resistant bacteria causing SSIs [Leonard et al, 2006, Kadlec et al, 2010, and Perreten et al, 2010]. Also, frustration of the owner and healthcare provider, the need for further surgery, legal action, as well as an overall negative perception of the hospital providing care, are possible. Human literature has estimated the economic impact of SSIs to be an average of $60,000 per patient depending on the type of surgical procedure [Hollenbeak et al, 2000]

SSI Definitions

Definitions of SSIs have not been consistent throughout the veterinary literature and can cause confusion, misdiagnosis and under- or over-estimation of their true rates. Standard SSI definitions are available from the US Centers for Disease Control and
Prevention (CDC) (Table 1) and are widely used in human medicine. These definitions both categorize whether an infection is present (differentiating inflammation from infection) and classify SSIs into superficial, deep and organ/space categories. Distinguishing between infection/inflammation is essential, however, many veterinary studies have taken inflammation and inferred infection from results. It is imperative to differentiate these two categories as according to the CDC they are fundamentally different. Inflammation is defined as clinical signs such as redness, swelling, tenderness and pain, whereas infection presents the bacteria in question through culture and diagnosis by a physician. There have been veterinary studies which group these two categories together, which will overestimate SSI rate.

There has been less use of standard definitions in veterinary medicine, complicating assessment of existing SSI data. The lack of standard definitions makes it difficult to universally recognize SSI, and makes it harder to implement general SSI prevention and treatment methods. Active surveillance is important for characterizing SSI rates and risk factors, which must be understood to undertake measures to reduce the incidence of SSIs.

Table 1: Standard definitions of SSIs as provided by the CDC.

<table>
<thead>
<tr>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Superficial SSI</strong></td>
</tr>
<tr>
<td>- Within 30days.</td>
</tr>
<tr>
<td>- Skin and/or subcutaneous tissues</td>
</tr>
<tr>
<td>- 1 or more of:</td>
</tr>
<tr>
<td>Pus</td>
</tr>
</tbody>
</table>
Bacteria
Heat, redness, pain OR localized swelling AND incision reopened by surgeon UNLESS culture negative.

| Deep SSI | -Within 30days, 1 year if implant is present.  
|          | -Deep soft tissues of the incision  
|          | -1 or more of:  
|          | Pus  
|          | Spontaneous dehiscence of deeper incision OR incision is deliberately opened when patient has fever, localized pain OR tenderness UNLESS culture negative  
|          | Abscess of other evidence of infection on imaging or histology. |

| Organ/Space SSI | -Within 30days, 1 year if implant is present.  
|                 | - Involves any part of the body, excluding the skin incision, fascia, or muscle layers, that is opened or manipulated during the operative procedure  
|                 | -1 or more of  
|                 | Pus  
|                 | Bacteria  
|                 | Abscess or other evidence of infection on exam, re-operation, histology or imaging. |

In veterinary medicine, clean and clean-contaminated wounds have shown to produce fewer infections than do contaminated and dirty wounds [Brown et al, 1997, Nicholson et al, 2002 and Eugster et al, 2004]. The reason may be due to the complexities of the surgeries associated with the wound classifications as surgeries involving GI tract or
genitourinary tract, for example carry an inherent risk of bacteria laden contents spilling into wounds causing severe contamination and often sepsis and death. [Vassuer et al, 1998, Nicholson et al, 2002].

**Human SSI Incidence**

There have been extensive studies of SSI rates in various procedures in human medicine (Table 2). In contrast to veterinary studies, human studies have large sample sizes and use standard SSI definitions.

Table 2: Procedure specific SSI rates in humans according to previous studies.

<table>
<thead>
<tr>
<th>Author</th>
<th>Procedure</th>
<th>SSI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hafez et al, 2012.</td>
<td>Cardiothoracic</td>
<td>27.1</td>
</tr>
<tr>
<td></td>
<td>Urologic</td>
<td>8.8</td>
</tr>
<tr>
<td>Reilly et al., 2006.</td>
<td>Breast surgery</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>Coronary bypass graft</td>
<td>5.79</td>
</tr>
<tr>
<td></td>
<td>Cesarean section</td>
<td>1.17</td>
</tr>
<tr>
<td></td>
<td>Hip replacement</td>
<td>1.75</td>
</tr>
<tr>
<td></td>
<td>Knee replacement</td>
<td>1.46</td>
</tr>
<tr>
<td></td>
<td>General vascular</td>
<td>11.65</td>
</tr>
<tr>
<td>Abdul-Jabbar et al, 2012.</td>
<td>Arthrodesis</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>Osteotomy</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td>Decompression(thoracic/lumbar)</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>Corpectomy (cervical)</td>
<td>1.5</td>
</tr>
<tr>
<td>Teija-Kaisa et al, 2013.</td>
<td>Mastectomy</td>
<td>8.9</td>
</tr>
<tr>
<td></td>
<td>Lumpectomy</td>
<td>4.7</td>
</tr>
<tr>
<td>Mirbagheri et al, 2013.</td>
<td>Ileostomy</td>
<td>8.9</td>
</tr>
<tr>
<td></td>
<td>Colostomy</td>
<td>13.5</td>
</tr>
<tr>
<td>Brandt et al, 2006.</td>
<td>Appendectomy</td>
<td>2.12</td>
</tr>
<tr>
<td></td>
<td>Nephrectomy</td>
<td>6.33</td>
</tr>
<tr>
<td></td>
<td>Prostatectomy</td>
<td>3.84</td>
</tr>
<tr>
<td></td>
<td>Coronary artery bypass</td>
<td>2.50</td>
</tr>
<tr>
<td></td>
<td>Hysterectomy</td>
<td>2.25</td>
</tr>
<tr>
<td></td>
<td>Proximal femur fracture repair</td>
<td>2.63</td>
</tr>
<tr>
<td>Bowman et al, 2013.</td>
<td>Aortic arch repair</td>
<td>16.6</td>
</tr>
<tr>
<td></td>
<td>Hyperplastic left heart syndrome palliation</td>
<td>7.5</td>
</tr>
</tbody>
</table>
Veterinary SSI incidence:

There has been much less reporting of SSIs in small animal veterinary patients, yet studies of varying size and depth have described SSI rates as shown in Table 3.

Table 3: Veterinary surgical site infection rates according to various authors

<table>
<thead>
<tr>
<th>Author</th>
<th>Surgery Type</th>
<th>Overall surgical site infection rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gatineau et al, 2011</td>
<td>Tibial plateau leveling osteotomy</td>
<td>2.9</td>
</tr>
<tr>
<td>Etter et al, 2013</td>
<td>Tibial plateau leveling osteotomy</td>
<td>8.8</td>
</tr>
<tr>
<td>Savicky et al, 2013</td>
<td>Tibial plateau leveling osteotomy</td>
<td>3.1</td>
</tr>
<tr>
<td>Gallagher et al, 2010</td>
<td>Tibial plateau leveling osteotomy</td>
<td>7.4</td>
</tr>
<tr>
<td>Mayhew et al, 2012</td>
<td>Minimally invasive interventions into pleural and peritoneal cavitie</td>
<td>1.7</td>
</tr>
<tr>
<td>Eugster et al, 2004</td>
<td>All interventions except dental and ophthalmologic</td>
<td>3.0</td>
</tr>
<tr>
<td>Nicholson et al, 2002</td>
<td>All clean contaminated interventions</td>
<td>5.9</td>
</tr>
<tr>
<td>Beal et al, 2000</td>
<td>All interventions</td>
<td>4.8</td>
</tr>
<tr>
<td>Fitzpatrick et al, 2010</td>
<td>Tibial plateau leveling osteotomy</td>
<td>6.6</td>
</tr>
<tr>
<td>Brown et al, 1997</td>
<td>All interventions</td>
<td>5.5</td>
</tr>
<tr>
<td>Frey et al, 2010</td>
<td>Extracapsular lateral suture and tibial plateau leveling osteotomy</td>
<td>6.1</td>
</tr>
<tr>
<td>Vasseur et al, 1985</td>
<td>All clean interventions</td>
<td>0.8</td>
</tr>
</tbody>
</table>

These studies each illustrate a noteworthy SSI incidence, with varying number of procedures, and procedure types. The consequence of these incidence rates correlate with the overall burden of SSI in canine and feline patients, and although it is not possible to measure, it is likely that these consequences have a large impact. Existing studies have provided vital information on potential risk factors for developing an SSI and their rates;
[Eugster et al 2004, Nicholson et al, 2002, Beal et al, 2000, Fitzpatrick et al, 2010. Brown et al, 1997] however, these studies are often limited by sample size, reliance on retrospective study, the use of passive surveillance measures (i.e. medical record review), failure to use standard definitions for SSI and failure to differentiate infection from inflammation. Thus, while providing important information, they are suboptimal for thorough understanding of the problem, for establishing benchmarks, and for designing and interpreting interventional studies. One common limitation has been inconsistency or lack of clarity in definition of SSIs, specifically failure to use standard definitions as described above. Another is reliance on medical record data, with inherent issues of quality of medical records affecting the quality of the study data.

**Veterinary SSI Risk factors:**

Few comprehensive studies have been published that address the epidemiology and risk factors for SSIs in companion animals. There is an inherent risk of developing an infection with any type of surgery, [Barie, et al 2005, Eugster et al, 2004, Nicholson et al, 2002] but understanding factors that increase this risk is critical for development of preventive measures. The risk increases when factors such as virulence and size of inoculum of bacteria increase as well as a decrease in host resistance [Eugster et al, 2004].

Breed, and gender have shown to contribute to SSIs, but in somewhat conflicting studies and there has been no consistent evidence about predisposed breeds or genders. Gender may have a role in SSI for example, due to increase weight with varying gender.
According to a previous study, heavier dogs may be at a higher risk of SSI development [Eugster et al, 2004]

Patients with concurrent endocrinopathy have are said to be 8.2 times more likely to develop SSI as compared to those without concurrent endocrinopathy [Nicholson et al, 2002]. Examples of endocrinopathy include diabetes, hypoadrenocorticism, and hypothyroidism. Diabetes can cause a decrease in bactericidal activity and opsonic activity [Nicholson et al, 2002]. Hypoadrenocorticism are shown to have a decrease in natural killer (NK) and a decrease in T-lymphocytes. Hypothyroidism has conflicting views with some studies citing a decrease in SSI due to increased activation of T cells with decreased thyroid hormone, [Ohashi et al, 1994], while Nicholson et al mentions two cases of hypothyroidism that develop SSI, and hypothesize a species difference in the association between infection and thyroid function [Nicholson, et al, 2002].

Prophylactic antimicrobials are widely used, albeit with limited evidence of efficacy and optimal protocols. One study reported that the risk of developing infection is 5 times greater if surgery lasts for more than 4 hours and a 2 times greater if prophylactic antibiotics are given to the patient more than 1 hour before surgery [Shales et al, 2005]. Peri-operative prophylaxis is an area that is easy to implement and could be an important infection prevention tool, yet broad data for veterinary medicine are lacking [Whittem et al, 1999].

Duration of anesthesia and surgery have also been identified as a risk factors [Nicholson et al, 2002, Eugster et al, 2004] with an increased anesthesia and surgery time being associated with increased risk of infection. Suggested reasons for this include perioperative hypothermia and hypotension, which can lead to a decrease in immune
function, as well as hypothermia and hypoxia [Eugster et al, 2004]. Beal et al also suggests that vasoconstriction as a result of hypothermia can cause decrease oxygen delivery in the blood and decreased immune function [Beal et al, 2000]. Steps to decrease the chance of hypothermia include active re-warming which is a common practice in veterinary medicine. Active re-warming with devices such as bair huggers due to intraoperative hypothermia is associated with an increase risk of SSI development due to the possible re-circulation of bacteria-contaminated air around the surgical site [Beal et al, 2000]. Nicholson et al suggested that certain anesthetics such as halothane and enflurane can cause a decrease in the recruitment of neutrophils, due to a decrease production of respiratory burst products [Nicholson et al, 2012].

Specific surgical procedures have been shown to be associated with high rates of SSI in small animals thus are risk factors in themselves. Examples include orthopedic procedures such as tibial plateau leveling osteotomy (TPLO) [Fitzpatrick et al, 2010, Eugster, 2004, Nicholson et al, 2002, Gallagher et al, 2010, Gatineau et al, 2011, Etter et al, 2013, Savicky et al 2013]. Tibial plateau leveling osteotomy is associated with higher rates of SSI possibly because of the increased surgery time, further tissue dissection for the osteotomy, placement of implants, increased anesthesia time, and the invasive nature of this surgery [Gallagher et al, 2010, Fitzpatrick et al, 2010, Savicky et al, 2013].

Wound classification is a commonly used practice in human and veterinary medicine with dirty wounds producing the highest rate of surgical site infections, according to the CDC. There are many reasons as to why this may be the case including how dirty wounds are generally associated with existing infection, which increases the chances of sepsis, once surgery commences. According to Eugster et al, a patient is 5.6
times as likely to develop an SSI if they were classified as having a dirty wound (P=0.015)[Eugster et al, 2004].

Environmental factors such as operating room, surgical tools and re-warming devices could lead to increased risk of development of SSI due to the abundance of bacteria that could contaminate the surgical wound [Eugster et al, 2004], yet there has been limited study.

The use of propofol has been a common risk factor in the development of SSI in small animal patients. According to a previous study, propofol is a lipid based emulsion, which is capable of supporting rapid microbial growth. Patients can be up to 3 times as likely to develop SSI if they are given propofol [Heldmann et al, 1999].

Methods of skin closure have reportedly influenced the development of SSI in small animals. According to Etter et al, the use of staples has a decreasing effect on development of inflammation while Frey et al, suggested that the use of staples as opposed to nylon sutures has a positive effect on development of SSI. [Frey et al, 2010, Etter et al, 2013].

Duration of post-operative intensive care unit (ICU) stay has been associated with an increase in risk of SSI development [Eugster et al, 2004]. For every extra day that a patient is in ICU, the risk of developing an SSI is 1.16 times greater than if the patient was in step-down care [Eugster et al, 2004].

Minimally invasive surgery is becoming a popular choice for veterinary surgeons as it can be associated with shorter operative room time, smaller incisions and reduced risk for complication. Laparoscopic and thoracoscopic surgery are associated with a decrease in surgical site infections in one veterinary study (Mayhew et al, 2012) which
found minimally invasive surgical procedures has an SSI incidence that is 3.2 times less than open procedures [Mayhew et al, 2012]. Using this information, if a future procedure can be done using minimally invasive techniques such as laparoscopy and thoracoscopy in veterinary patients, it may lead to a decrease in SSI incidence.

Table 4: Risk factors for the development of surgical site infections for dogs according to various authors.

<table>
<thead>
<tr>
<th>Species</th>
<th>Risk factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eugster et al</td>
<td>Canine  Weight, dirty wound, antimicrobial prophylaxis, intensive care unit duration, post surgical drain</td>
</tr>
<tr>
<td>Nicholson et al</td>
<td>Canine  Sexually intact, gender, Endocrinopathy, hypoadrenocorticism hypothyroidism, surgical time, anesthesia time</td>
</tr>
<tr>
<td>Fitzpatrick et al</td>
<td>Canine  Weight, partial/complete rupture of cranial cruciate ligament, gender, postoperative antimicrobials</td>
</tr>
<tr>
<td>Brown et al</td>
<td>Canine/Feline  Duration of surgery, clipping prior to induction, antimicrobials</td>
</tr>
<tr>
<td>Frey et al</td>
<td>Canine  Post operative antimicrobial, surgical procedure, wound closure</td>
</tr>
<tr>
<td>Vasseur et al</td>
<td>Canine/Feline  Antimicrobial type</td>
</tr>
<tr>
<td>Etter et al</td>
<td>Canine  Use of staples for skin closure</td>
</tr>
<tr>
<td>Gallagher et al</td>
<td>Canine  Antimicrobial type</td>
</tr>
<tr>
<td>Mayhew et al</td>
<td>Canine/Feline  Duration of surgery, clipping time prior to surgery</td>
</tr>
<tr>
<td>Gatineau et al</td>
<td>Canine  None</td>
</tr>
<tr>
<td>Beal et al</td>
<td>Canine  Duration of anesthesia, clipping prior to induction</td>
</tr>
</tbody>
</table>
Human risk factor data

There has been much more extensive study of SSI risk factors in humans. Human literature specifically addresses scales such as the The American Society of Anesthesiology (ASA) score that uses a physical classification system for patients when they present for surgery [American Society of Anesthesiologists 2011]. This provides an indicator or score for patient susceptibility to infection and therefore patients can receive appropriate and timely treatment such as prophylaxis. Table 5 illustrates the ASA score for patients when they present for surgery. From the table it is clear that a patient with a low ASA score will be at less of a risk of developing SSI than will a patient with an ASA score of V [Bakkum-Games, J.N., et al, 2013].

Table 5: American Society of Anesthesiologists (ASA) scored for incoming surgery patients

<table>
<thead>
<tr>
<th>Score Description</th>
<th>ASA I</th>
<th>ASA II</th>
<th>ASA III</th>
<th>ASA IV</th>
<th>ASA V</th>
</tr>
</thead>
<tbody>
<tr>
<td>• A normal, healthy patient</td>
<td>• A patient with mild, systemic disease</td>
<td>• A patient with moderate systemic disease</td>
<td>• A patient with a severe systemic disease</td>
<td>• A patient with life threatening systemic disease</td>
<td></td>
</tr>
</tbody>
</table>

Propofol has been largely associated with an increased risk of surgical site infections in humans, due to its lack of oxidizing activity when compared to other anesthetics such as cevoflurane [Shimizu, et al 2010]. Cevoflurane generates reactive oxygen species (ROS) that are essential for immune functions to kill bacteria. Propofol scavenges ROS using hydrogen abstraction, which could impede the bactericidal immune function and increase the risk of SSI [Shimizu, et al 2010].
A variety of other human risk factors have been identified such as obesity, diabetes, age, hypothyroidism, hypoalbuminemia, smoking, emergency surgery, surgical access sites, immunosuppression, previous surgeries, chronic kidney infection, dialysis, corticosteroids, asthma, chemotherapy, number of blood transfusions, body mass index, high volume hospitals and bilateral procedures. [Ott et al, 2012, Tadros et al, 2013, Blackham et al, 2013, Namba et al, 2013]

Hyperglycemia as a result of diabetes can cause a decrease in leukocyte function, adherence, chemotaxis, and bactericidal activity [Erben, et al., 2013]. Hypoalbuminemia is associated with poor tissue healing, decreased collagen synthesis, as well as decreased granuloma synthesis at the site of surgery. Low serum albumin is also associated with a decrease in innate immunity such as decreased macrophage activity. Combined, these factors can predispose a patient to an increased risk of infection development [Hennessey, et al., 2010].

Preoperative hair removal one day before surgery has been associated with an increase in SSI development due to small cuts in the skin, which serve as reservoirs for bacteria to multiply prior to surgery, allowing for further colonization [Mangram et al, 1999].

Intraoperative ventilation conditions may contribute to an increase risk of SSI development as the air may be contaminated with dust, skin, or respiratory droplets that could come in contact with the surgical wound, colonize and further infect the patient [Mangram et al, 1999].

Minimally invasive surgery has become common practice in human medicine and according to Ee et al, they are associated with a decrease in SSI incidence. It is reported
that patients undergoing the same procedure are 5.5 times more likely to develop an SSI if the procedure was open, as opposed to minimally invasive [Ee et al, 2013].

**Microbiology of SSIs in dogs and cats**

Surgical site infections can be caused by a wide range of bacteria, overwhelmingly by opportunistic pathogens that form the commensal microbiota of the patient. External pathogens may also be involved, including those from the environment and from healthcare providers. A variety of factors facilitate development of an infection by endogenous and exogenous bacteria [Nienhoff, 2011]. Most if not all patients harbour opportunistic Gram-positive staphylococcal bacteria on the skin [Prescott et al, 2002]. When surgery is performed, this resident and transient microbiota must be kept from colonizing the surgical wound. During surgery, the opportunistic bacteria such as *Staphylococcus pseudintermedius* may be able to contaminate the wound during surgery, even with aseptic procedures in place [Leonard et al 2006, Borjesson, et al, 2012, Frank et al, 2012].

According to the National Nosocomial Infection Surveillance program sponsored by the U.S. CDC, the most common bacteria that are present in SSIs in humans include *E. coli*, *Staphylococcus aureus*, enterococci, *Pseudomonas aeruginosa* and coagulase negative staphylococci [NNIS 2008]. In veterinary medicine, Gram positive staphylococci such as *Staphylococcus pseudintermedius* are the most commonly reported bacteria found in SSIs in dogs and cats [Vasseur et al, 1988, Kadlec et al, 2010, Nienhoff, 2011, Osland, 2012, Frank et al, 2012,]. *Staphylococcus aureus* is a bacterium of interest as it is abundantly available on the skin of many patients [Morris et al, 2006, McLean et al,
2008] as well as their human contacts. One study observed a *Staphylococcus aureus* carriage rate in humans of 21.6% with an MRSA contamination rate of 2.1% [den Heijer, C.D.J., et al, 2013]. This gram positive opportunistic bacteria is a leading cause of SSI in humans and also causes infections in animals, ranging from skin and soft tissue infections to septicemia [Morris et al 2006]. Of particular concern in humans in methicillin-resistant *S. aureus* (MRSA), which is a leading cause of SSIs in many areas. [Anderson et al, 2007] Similarly, methicillin-resistant *S. pseudintermedius* (MRSP) has recently emerged as an important SSI pathogen in dogs (and to a lesser degree cats).[Borjesson et al 2012]. These are of concern because of the difficulties that can be encountered when treating these bacteria, which are often multidrug resistant.

**Impact of SSI**

The impact of surgical site infection is not well understood in veterinary medicine, but it is evident that SSIs can result in increased costs; health, economic and otherwise. Mortality rates of humans with surgical site infections have shown to be as high as 22% as compared to patients without a post surgical infection[Hollenbeak et al, 2000]. In veterinary practice, mortality rates have also shown significant increases with SSI [Eugster, et al, 2004]. In humans, patients with deep surgical site infections spent an average of 20 extra days in the hospital post surgery, resulting in substantial economic cost [Hollenbeak et al, 2000]. Similar veterinary data are lacking but it is reasonable to assume that the overall economic costs are substantial. As infections with multidrug resistant bacteria increase, the costs are likely to increase in a corresponding manner. The median cost for infections caused by methicillin-resistant *Staphylococcus aureus*
MRSA was 1.95 times greater than the median cost for infections caused by methicillin-susceptible Staphylococcus aureus (MSSA) [Capitano, et al, 2003]. Of added concern in veterinary medicine are SSIs caused by zoonotic pathogens such as MRSA, [Boerlin et al, 2001] which adds a component of risk to healthcare providers and owners. Beyond the patient, there can be a variety of negative impacts as shown in Table 6.


<table>
<thead>
<tr>
<th>Impact group</th>
<th>Patient</th>
<th>Hospital</th>
<th>Surgeon</th>
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| Description  | • Increased morbidity  
              • Increased mortality  
              • Further infection from endogenous microorganisms  
              • Removal of implant can cause further infection  
|              | • Increased cost for infection management  
              • Increased prevalence of infection in hospital  
              • Increased need for resources to manage infections  
              • Decreased hospital reputation  
|              | • Decreased reputation of the surgeon  
              • Diminished relationship between patient/owner and surgeon |

Surgical site infection surveillance

There does not exist a standard surveillance system for the detection of surgical site infections in veterinary medicine. This Study on the Efficacy of Nosocomial Infection Control (SENIC) has shown that a surveillance system effectively decreases the incidence of hospital associated infections (HAI) [Haley et al 1985]. The steps taken in the surveillance program include collection, analysis and feedback to surgeons about the
infections that were observed. The most important aspect of surveillance is to provide a benchmark for all hospitals to use a standard definition of what constitutes a surgical site infection. Using this benchmark, surgeons can adapt to new techniques and can allow for an ultimate decrease in infection rates, based on procedure [Haley et al, 1985]. Surveillance may also decrease infection rates due to the Hawthorne Effect, where aepsis protocols for example, are kept more accurately when one knows they are being surveied [Borer et al, 2001].

Surveillance is an effective tool to decrease rates of SSI; however, the short duration of hospitalization of most veterinary patients means that infection that is seen only in the hospital will underestimate the true prevalence of infection. Infections that occur following discharge therefore need to be identified during recheck visits, suture removal or other interactions with the pet owner. This may be simple in some situations but become more complicated when multiple different veterinary clinics or veterinarians are involved.

**Bacterial biofilms**

A biofilm is a complex community of bacteria that are encased in an extra polymeric substance (EPS) [Boles et al, 2012]. This substance is made up of proteins, extracellular DNA, and polysaccharides produced by the bacteria [Osland, 2012].

Bacteria within a biofilm behave differently than their planktonic counterparts. Previous studies suggest that bacteria within biofilms have a decreased growth rate due to down regulation of active cell processes, as well as altered gene transcription [Otto, 2008, Kong et al, 2006]. Bacterial communication is an essential component to biofilm
formation, and is said to be facilitated by quorum sensing [Schierle et al, 2009]. Staphylococcal quorum sensing is called \textit{agr} (accessory gene regulator), which uses signals known as autoinducers to promote it. [Kong et al, 2006]. Quorum sensing is also suggested to promote cell to cell adhesion within biofilms [Kong et al, 2006, Lorite et al, 2013]. Biofilm formation occurs on many different surfaces in five stages including reversible adsorption, irreversible attachment, growth and division, EPS/biofilm production, and attachment/detachment of other organisms to the formed biofilm [Osland et al 2012]. Figure 1 illustrates the stages of biofilm formation.

![Figure 1: Illustration of the stages of biofilm formation from attachment to detachment [Vuong et al, 2002].](image)

Initial adsorption can occur in a time span of seconds, while the final attachment of other organisms to the formed biofilm can occur in a time ranging from minutes to months, and in a repeating and dynamic fashion. Biofilm-embedded bacteria, because of
their altered metabolism and the physical structure of the biofilm, are relatively resistant to the effects of the immune system, desiccation, environmental forces (e.g. shearing) and antimicrobials.[Laverty et al, 2013]. Surface attachment is mediated by a variety of factors including those of the biofilm and the host that it will form on. Biofilms grow at a higher rate on hydrophobic materials than hydrophilic as well as rougher material than smooth[Laverty et al 2013].

Genes that form EPS can be expressed by different external stimuli such as stress signals, temperature and other physiological signals. These heterogeneous structures contain water and form water channels that deliver nutrients and oxygen to the cells within the biofilm. These bacteria can survive in the biofilm community while being resistant to antimicrobials. Detachment of bacteria from the underlying biofilm can cause systemic infection at any time.

Biofilm production is a potentially important virulence factor, particularly in device-associated and chronic infections. Yet, despite the potential importance of biofilm formation in SSIs, there has been limited investigation. In small animal practice, Staphylococcal biofilms have been shown to develop on post surgical implant devices [Arciola, et al, 2012].

Bacteria within biofilms may respond poorly to antimicrobials because of the physical protection of EPS, activation of endogenous virulence factors (e.g. efflux pumps) and altered growth and metabolism decreasing the efficacy of antimicrobials (e.g. antimicrobials that affect cell wall synthesis)[Maddox, 2011, Otto et al, 2006].

Several chemicals and enzymes have been isolated to try to permeate or disrupt the EPS, such as DispersinB [Ramasubbu et al 2005]. This is an enzyme of the bacterium
*Aggregatibacter actinomycetemcomitans.* DispersinB catalyzes the hydrolysis of poly-N-acetylglucosamine (PNAG), a core component of biofilm produced by some bacteria, causing interruption in intracellular adhesion [Ramasubbu et al., 2005] DispersinB is classified in the glycoside hydrolase family 20 as it has a specific site that it cleaves which in biofilm is the β 1-6-N-acetyl group[Brindle et al, 2011, Fazekas et al, 2012]. DispersinB is said to interrupt the intercellular linkages by removing the terminal N-acetyl glucosamine functional group at the non-reducing end, causing a softer and less consolidated biofilm cluster. [Fekete et al, 2011]. This is said to happen endolytically and low molecular weight oligosaccharides are a byproduct [Itoh et al, 2005]. A previous study indicates that the growth of biofilm was essentially completely inhibited in the presence of DispersinB with bacteria containing the ica gene, however DispersinB did not have the same effect on the planktonic forms of these bacteria. [Itoh et al, 2005].

Because DispersinB does not directly affect the bacteria within a biofilm, studies have combined DispersinB with antimicrobials in order to kill bacteria, once the biofilm structure has been interrupted, as antimicrobials cannot penetrate the biofilm structure themselves [Gawande et al, 2011]. These findings allow for further research into the prevention of biofilm growth on surgical instruments such as catheters and surgical drains that contribute to SSI and MD resistance.

**Current knowledge gaps**

The current literature is missing key factors in the prevention of SSIs in veterinary patients, including active surveillance with procedure specific risk assessment and standard SSI definitions. There has yet to be a study to actively follow a cohort of
patients in order to assess the risk for developing infection, and ultimately preventing the consequences of SSI mentioned above.

Previous studies have indicated that specific risk factors can contribute to an increase in SSI incidence;[Brown et al, 1997, Beal et al, 2000, Nicholson et al, 2002, Eugster et al, 2004, and Fitzpatrick et al, 2010, Etter et al, 2013] however, they do not define SSI in a standard approach, and therefore the term SSI is left ambiguous which leads to difficulty in its diagnosis, and subsequent risk factors.

Previous studies have also shown that biofilm is a current obstacle in the prevention of SSIs however methods to eradicate it have not been thoroughly investigated.
Thesis Objectives and Hypotheses:

The general purpose of this research is to provide information on prevention techniques of surgical site infections, as well as describe bacterial biofilms, their consequences, and treatment options. Specific objectives and hypotheses are outlined below.

Objectives:

• Identify overall and procedure specific SSI rates over a 10-month period in canine and feline patients undergoing surgery at the Ontario Veterinary College Health Sciences Centre.
• Identify factors associated with SSIs
• Describe the main type of bacteria isolated from infections
• Evaluate the effect of DispersinB on the formation of *Staphylococcus pseudintermedius* biofilm
• Evaluate the effect of DispersinB on eradication of *Staphylococcus pseudintermedius* biofilm, once formed.

Hypotheses:

• SSI incidence rates will be between 2 and 8%
• Surgery classifications, the use of implants, and surgical procedure types will be used as risk factors that increase the SSI rate
• The bacteria that will be most commonly isolated will be multidrug resistant staphylococci.
• DispersinB will significantly inhibit the formation of *Staphylococcus pseudintermedius* biofilm
• DispersinB will not significantly eradicate *Staphylococcus pseudintermedius* biofilm.
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CHAPTER 2

Prospective Surgical Site Infection Surveillance In Dogs And Cats

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Abstract

Objective - To 1) describe the incidence of SSI in dogs and cats undergoing surgical procedures at the Ontario Veterinary College Health Sciences Centre (OVCHSC) over a 10-month period, 2) describe and compare procedure-specific SSI rates, and 3) identify factors associated with development of SSI.

Design - Prospective, Cohort Study

Animals - Dogs (n=846), Cats (n=99) during 45 weeks from September 2010 to July 2011.

Methods - Follow-up telephone conversation with patient owners was performed 30 days post-operatively, with additional 1 yr follow-up performed for cases with surgical implants. A standardized questionnaire was administered to detect and characterize SSIs.

Results - SSIs were identified in 28 (3.0%) animals, 26 dogs and 2 cats. Eleven of 26 (42%) canine SSIs were classified as superficial SSI, while 13 were deep and 2 were organ/space. Of the confirmed canine SSI cases, only 17 (65%) were documented in the medical records of the patient. Hypotension (P=0.011), class of surgery (P=0.029) and the use of an implant (P=0.001) increased the risk of SSI. Cultures were submitted for 19/26 canine cases (73%) and of those, 74% were Staphylococci.

Conclusion - SSIs can have numerous deleterious effects on the health and well being of veterinary patients. While some risk factors such as hypotension are modifiable, others such as class of surgery are not. The most effective way to limit SSIs is through prevention methods, and using active surveillance.
Introduction

Surgical site infections (SSIs) are an inherent risk of any surgical procedure because of the inevitable compromise of the host’s protective barrier that ensues. 1 This complication can lead to increased patient morbidity relating to infection, mortality, prolonged hospitalization, increased treatment cost and emotional strain on the pet owner and medical caregiver alike. 1-7 While SSIs have been a problem since the inception of veterinary (and human) surgery, they are currently receiving more attention because of increases in antimicrobial resistance as well as more invasive procedures that occur in immunocompromised patients. 3,4,6 The threat of SSIs will be ever-present in veterinary surgery and measures to control them are critical to try to reduce their incidence and impact. A key component of this is a clear understanding of the incidence and risk factors for SSIs.

Previously published rates for SSIs in dogs and cats have ranged from ~3 - 10%. 1,2,4-7 While the overall health and economic burden of SSIs in dogs and cats has not been quantified, when one considers the large volume of surgery performed, the health and economic costs associated with SSIs must be substantial. Existing studies have provided critical information on potential risk factors for developing an SSI and their rates 1,2,4-7; however, these studies are often limited by sample size, reliance on retrospective design, the use of passive surveillance measures (i.e. medical record review), failure to use standard definitions for SSI and failure to differentiate infection from inflammation. 1,2,4-7 Thus, while providing important information, they are suboptimal for thorough understanding of the problem, for establishing benchmarks, and for designing and interpreting interventional studies. One common limitation has been
inconsistency or lack of clarity in definition of SSIs. The US Centers for Disease Control and Prevention (CDC) has established clear guidelines for defining SSIs in human medicine, including both clinical and timeframe criteria as shown in Table 1.\(^8\) However, prospective, active surveillance (use of direct post-operative follow-up with patient owners) using these definitions in veterinary surgery has not been previously reported.

The objectives of this study were to describe the incidence of SSIs in dogs and cats undergoing surgical procedures at the Ontario Veterinary College Health Sciences Centre (OVCHSC) over a 10-month period, to describe and compare procedural-specific SSI rates, and to identify factors associated with the development of SSIs.

**Materials and Methods:**

**Study Population**

A prospective, cohort study design was used, involving all dogs and cats that underwent surgical procedures under general anesthesia (excluding dental, ophthalmologic and interventional radiologic procedures) at the OVCHSC between September 2010 and August 2011.

**Data collection**

Animals were identified via the surgical case log, and data from this log, the surgical report, anesthetic report and other components of the medical record were used to obtain patient, (e.g. age, breed, gender, weight, co-morbidities) and procedure information including: type of surgery, elective or emergency, surgical classification, surgeon(s), antimicrobial prophylaxis, duration of surgery and anesthesia (categorical: greater or less than 1.5 hrs, intraoperative hypoxia, hypothermia (<37°C) and/or
hypotension (<60 mmHg), use of an active re-warming system, type of deep tissue suture used, type of subcutaneous suture used, type of skin closure used, American Society of Anesthesiologists (ASA) score, and whether an implanted medical device was placed. Procedures were classified as soft tissue, orthopedic, and neurological.

_Surgical site infection surveillance_

Active 30d follow-up was performed. One author (RT) contacted owners of each patient by telephone and the patient’s status was evaluated using a standard, pre-tested questionnaire developed by the authors. This questionnaire queried the presence of any surgical site abnormalities with specific signs of redness, swelling, inflammation, heat, or purulent drainage from the wound, and when present, obtained specific details to allow for differentiation of infection and inflammation, and SSI classification. SSI was defined using CDC definitions as illustrated in Table 1. Owners were also asked if other clinical abnormalities (e.g. diarrhea) were noted and whether the patient was seen to disturb (e.g. lick/chew) the surgical incision. If signs of inflammation or infection were reported by the owner, further queries were made as to whether they had taken their animal to their primary care veterinarian or any veterinary clinic apart from the OVCHSC for evaluation of the surgical site and whether any diagnostic testing had been performed. All information obtained was cross-referenced with the patient’s OVCHSC medical record. One year post-operative surveillance assessment was performed in an identical manner for patients that underwent procedures involving an implanted medical device as defined by the CDC.
**Classification**

Surgical sites were classified as normal, inflamed but not infected, superficial SSI, deep SSI or organ/space SSI using standard SSI definitions (Table 1).\(^8\)

**Statistical analysis**

Statistical analysis was performed using STATA 10\(^A\) (A- Stata Corp LP. College Station, Texas, United States of America). The dependent variable was development of SSI. The independent variables were the patient and procedural factors described above. Chi square tests and Fisher’s exact tests were used to test associations between categorical and continuous variables, as part of the univariable analysis. All dependent variables with a P value of <0.2 were selected for multivariable analysis. Multivariable analysis was conducted using a backwards-stepwise elimination technique where all terms were initially present in the model, and taken out to determine their overall effect. A P value of <0.05 was considered significant and insignificant variables were removed from the multivariable model unless they were deemed to be a confounder. Confounding variables were tested for by removing one variable from the multivariable model and observing the coefficient change in other terms. If there was a change of more than 20%, the variable was considered confounding and was included automatically in the multivariable model. Two-way interaction terms were created manually and tested with the appropriate terms. If the interaction term was significant, it remained in the model.

Once the final multivariable model was constructed, Pearson residuals were evaluated to identify outliers which outliers were examined to exclude data entry errors. Because of the small number of cats that were represented in the study population, statistical analysis of feline data was not performed.
Results

Descriptive statistics

One thousand forty eight animals, 947 dogs and 101 cats, underwent a surgical procedure during the study period. One hundred three (11%) animals, 101 dogs and two cats, were excluded from the study because their owners could not be contacted despite repeated attempts. Complete follow-up information was obtained for 846 dogs and 99 cats. Twenty-eight SSIs were documented, 26/846 (3.1%) dogs and 2/99 (2.0%) cats, for an overall rate of 3.1%. An additional 49 (5.2%) patients had incision abnormalities that were classified as inflammation, not infection. This corresponds to a total abnormal incision (infection and inflammation) rate of 8.1%. The incidence of SSI per month of study and SS abnormality per month of study are illustrated in figure 1 and 2 respectively.

One-year post-operative surveillance data was obtained for all patients with implanted medical devices. No additional SSIs were identified.

Eleven of 26 (42%) canine SSIs were classified as superficial SSI, while 13/26 (50%) were deep and 2/26 (8%) were organ/space. For cats, there was one each of superficial (50%) and deep SSI (50%). All deep infections were implant associated and ten (36%) required further surgery. Among orthopedic procedures, the SSI rate for tibial plateau leveling osteotomy (TPLO) was 7/93 (7.5%).

The attributable mortality rate for SSI was 0%. One canine patient developed an incisional hernia and SSI following resection of a grade II mast cell tumour in the inguinal region. Euthanasia was elected because of the hernia; however, whether the SSI lead to herniation was unclear and difficult to definitively determine.
All further data pertain only to dogs. Only two of 26 (8%) SSIs were diagnosed before the patient was discharged from hospital. Of the confirmed SSI cases, 17/26 (65%) were documented in the medical record of the patient. The remaining nine cases were presented to their primary care veterinary hospital for SSI diagnosis and treatment according to the owners, with information either not being reported to the OVCHSC or reported information not being entered into the OVCHSC medical record.

A sample was submitted for bacterial culture and susceptibility testing in 19/26 (73%) SSI cases and bacteria were isolated from 14/19 (74%) (Figure 3). Staphylococci accounted for 14/19 (74%) of the culture-confirmed cases. Methicillin-resistant *S. pseudintermedius* (MRSP) was most commonly found (9/14 (64%)), followed by methicillin-resistant *S. aureus* (MRSA) (3/14 (21%)) and methicillin-susceptible *S. pseudintermedius* (2/14 (15%).

Univariable data are presented in Table 4. Implant placement, TPLO, hypotension, surgical type (soft tissue: orthopedic), the presence of incisional drains, surgical classification (clean: dirty) and hypoalbuminemia were included in the multivariable analysis. The final model included implant placement, intraoperative hypotension, and surgical classification (Table 5). A Hosmer-Lemshow test confirmed that the model fit the data (P=0.24)

**Discussion**

This study identified a clinically important incidence of SSIs in dogs and cats undergoing a range of surgical procedures at a tertiary care referral hospital. The use of standard definitions and prospective, active surveillance has not been reported in the
veterinary literature and should provide a more accurate assessment of SSI incidence.\textsuperscript{8,11,12} This is highlighted by the large percentage of SSIs (35%) that would not have been determined if there was reliance on medical record data only. Various reasons could account for this, including owners taking animals to their primary care veterinarian for SSI diagnosis and treatment (with no subsequent notification of OVCHSC surgeons) or incomplete medical record keeping. This highlights the need for effective communication between primary care facilities and surgical facilities to ensure that optimal SSI reporting occurs. It also highlights the need for effective record keeping and the potential benefit of methods that allow for easier categorization and reporting of SSIs in medical record systems. Whether this under-reporting bias is applicable to other institutions is unclear but it is a point that must be considered when interpreting other studies, designing future SSI surveillance studies and for hospitals relying on passive, medical record-based surveillance for infection control purposes.\textsuperscript{13,14}

These data suggest that active surveillance is important (at least in some institutions) but the time required may be problematic for routine surveillance or large-scale research studies. Determination of factors associated with incomplete passive SSI reporting is important because of the labour-intense nature of active surveillance. Improving the accuracy of passive surveillance, including measures such as communication with clinicians, facilitating electronic reporting of SSIs and emphasizing the need for referring veterinarians to report SSIs should be taken as a priority.

The overall rates of SSI in dogs (3.1\%) and cats (2.0\%) identified in this study were similar to other studies\textsuperscript{1,2,4-7}, but, as expected, there was variation in SSI rates between different procedure types. Overall, 20\% of dirty procedures resulted in SSI
compared to only 3.2% of clean surgeries. This makes sense from a biological standpoint and has been previously reported in both humans and animals.\textsuperscript{1,2,5,8} However, a statistically significant difference was only evident for the extremes of the surgical wound classification system (clean vs dirty). There were no differences between those classifications and clean-contaminated or contaminated procedures. It has been previously suggested that wound classifications are not sufficient themselves as predictors of SSI risk due to the variability of surgical procedures within the same classification\textsuperscript{1}, something that is not surprising because of the multifactorial nature of SSIs.

The risk of developing SSI for those patients with implanted medical devices was 5.6 times that of patients who did not have implants surgically placed (P=0.001). Despite all efforts of surgical asepsis, surgical implants can become colonized with bacteria and are a recognized risk factor for SSI.\textsuperscript{15,16} Additionally, implant surfaces can act as substrates for bacterial biofilm formation.\textsuperscript{17} Bacteria embedded in biofilms are more able to evade the host immune response and antimicrobial therapy, thereby complicating elimination of infection.\textsuperscript{17,18} Recent studies have shown that \textit{S. pseudintermedius}, the most common SSI pathogen identified in this study, produces biofilm which may play an important role in pathophysiology of SSI.\textsuperscript{19,20} There were no additional cases of SSI found in patients one year after they received an implant during surgery. This suggests that the 30-day follow up time will identify the majority of SSI cases, even though late onset of infection can occur. Recently, the CDC has released an updated guideline that reduced the 1-year post-operative period for SSI development following implant
placement to 90-days following surgery for selected procedures, further demonstrating that a 1-year period may not be required.\textsuperscript{8}

Patients in this study were 27 times as likely to develop an SSI if they experienced hypotension (P=0.01) as compared to patients that did not experience hypotension (95% CI: 2.14-358.32) (defined by mean arterial pressure < 60 mm Hg and systolic arterial pressure of less than 80-90 mm Hg\textsuperscript{21}) at any point intra-operatively. This has not been previously reported in dogs and is accompanied by a very broad confidence interval but there is a biological basis to this finding. Intraoperative hypotension could cause a reduction in oxygenated blood flow to the surgical wound, as well as a decrease in phagocytic activity, potentially leading to a decreased ability to clear bacterial contamination in the surgical wound.\textsuperscript{1} It is also possible that hypotension is a proxy for some other factor that was not adequately studied, particularly as an indicator of disease severity or an unstable patient during surgery. This association requires more study; however, hypotension is a risk factor that can be easily monitored prior to and during surgery, and measures can be taken to reduce the likelihood of this potential risk factor.

The predominance of staphylococci was expected since \textit{S. pseudintermedius} and \textit{S. aureus} are common opportunistic pathogens and leading cause of SSI in animals.\textsuperscript{20,22-26} Methicillin-resistant \textit{S. pseudintermedius} was the most commonly diagnosed cause of SSI in this study, a finding that was not surprising given the remarkable increase in the prevalence of this bacterium in dogs and cats and its increasing role in opportunistic infection.\textsuperscript{22-26} These infections occurred sporadically throughout the study and no outbreaks were reported. Therefore, this likely reflects the endemic nature of MRSP. This is of concern because MRSP is inherently resistant to virtually all beta-lactam
antimicrobials and typically resistant to numerous other drug classes.\textsuperscript{24,25} Therefore, MRSP is resistant to drugs typically used for peri-operative antimicrobial prophylaxis and empirical treatment, and few viable options may be available for some SSIs. Methicillin-resistant \textit{S. aureus} was found less commonly but is still of concern because of many of the same issues as for MRSP, plus additional concerns about zoonotic transmission.\textsuperscript{20,26}

While TPLO had a relatively high SSI rate in our study at 7/93 (7.5\%), a rate that is consistent with other studies\textsuperscript{4,6,16,27,28,31}, this procedure did not remain as a significant variable in the final model. This suggests that the presence of an implant might be the driving factor associated with TPLO SSIs. Given the commonness of cranial cruciate ligament injuries and this procedure, understanding factors that influence TPLO SSIs is critical.

The types of bacteria that were found bear consideration. While the presence of multidrug resistant bacteria is of concern, the low rate of SSI pathogens susceptible to commonly used peri-operative antimicrobials is, in some ways, encouraging. Cephalosporins accounted for the vast majority of peri-operative antimicrobial prophylaxis and would not have efficacy against MRSA or MRSP, as well as enterococci. Therefore, only 5/19 (26\%) of culture-confirmed SSIs were caused by pathogens that were susceptible to peri-operative drugs. Whether this can be used to infer possible mechanisms of SSI is debatable but in some ways it could be taken as an indication that current practices (both antimicrobial and overall infection control) are rather effective for the prevention of infections caused by bacteria that are susceptible to those antimicrobials. This should not be taken as an indication to change peri-operative
prophylaxis regimens (although that may bear consideration) since the decision making process must involve more than just SSI pathogen distribution. Indeed, it could be of debate whether a facility is better off having a higher SSI rate caused by susceptible (and therefore more easily treatable) pathogens versus a lower SSI rate caused by difficult-to-treat multidrug resistant pathogens (e.g. MRSP).

Factors that have previously been reported as associated with SSI in dogs and humans but which were not significant in this study included number of clinicians in the operating room and method of surgical wound closure. This could be because these are not true risk factors or that aspects of this study (e.g. statistical power, differences between practices at different facilities, homogeneity of practices within facilities) precluded detection of a role in SSI development. Similarly, the failure to identify peri-operative antimicrobials as protective for SSI likely reflects the rather homogenous practices at this facility, with the vast majority of cases receiving similar peri-operative regimens. It was particularly interesting that both duration of anesthesia and duration of surgery were not significantly associated factors with developing SSI, as many previous studies have indicated otherwise. One reason for this could be that duration of anesthesia and surgery are confounding variables for other risk-associated factors such as intraoperative hypotension.

Several limitations to our study exist. Since patients were not commonly examined by a veterinarian on day 30 (or after 1 yr for implant cases), there was reliance on owner assessment of the surgical wound. While the use of a simple standardized approach seemed to be effective in having owners provide data to identify normal versus inflamed versus infected surgical sites, owners are not trained at assessing surgical sites
and some degree of misinterpretation is possible. The fact that no animals classified as inflamed subsequently (within the 30d or 1y observation periods) required treatment for SSI supports the fact that incisions classified as abnormal and not infected were probably correctly classified. It is possible that mild infection was present and was self-limiting; however, there is probably little clinical relevance of such a mild infection, if it occurred.

Inflammation is an inevitable response to a surgical incision and post-operative inflammation can vary greatly between patients in the absence of infection. In this study, while 49 patients had incisional abnormalities, only 28 were classified as SSI. While there is no guarantee that all abnormal incisions were properly classified as infected or non-infected, the fact that none of the animals with incisions that were classified as inflamed but not infected subsequently required treatment (as per owner reporting and medical record review) supports their classification as non-infected, although self-limiting, mild infections could not be ruled out.

Misidentification of uninfected but inflamed incisions as SSI is a possibility but given the rather simple signs that were being queried and the apparent ease with which owners provided information, this was considered to be of limited concern. In order to minimize this subjectivity and potential for error, future approaches could include providing owners with illustrations and simple lessons in recognizing these signs prior to discharge of their pet, to facilitate any future discussions. The use of a single hospital also limits broader interpretation of the data. A study encompassing many veterinary hospitals from different geographical regions would minimize this bias, although large-scale multicenter studies can be logistically challenging, particularly given the time commitment required for active surveillance.
Analyzing SSI risk factors for a diverse surgical population can also limit some conclusions by missing procedure-specific factors associated with SSI. Larger scale study of specific procedures might allow for identification of other risk factors. However, whenever procedures are relatively homogenous (even though standardization is not always based on evidence) there can be limited ability to evaluate risk factors. Therefore, while factors such as suture type, peri-operative antimicrobial therapy, implant material, operating room design and patient preparation practices could influence SSI risk, if virtually all procedures are performed in the same manner, identifying the role of these factors in SSI will be impossible. Prospective, randomized controlled trials are needed for assessment of certain potential risk factors, yet these can be difficult to design and implement, particularly when certain practices are accepted as standard of care in the absence of objective evidence.

**Conclusion**

Surgical site infections can be a devastating complication in veterinary surgery and a better understanding of their impact and causes is required. Understanding SSI rates is an important part of the infection control program and this study has provided important information regarding SSI rates at this facility using an active surveillance approach to attempt to obtain optimal sensitivity and specificity. While some SSI risk factors that were identified here are not easily modifiable (i.e. use of an implant, dirty vs clean procedure) there may be ways to impact others (i.e. intraoperative hypotension). Further, identifying risk factors can help provide a better understanding of the pathophysiology of SSI and support the need for measures such as modified implant
design, assessment of perioperative antimicrobial practices and improvement of general infection control and surveillance activities.

References:


10) American Society of Anesthesiologists. ASA Physical Status Classification. Park Ridge, Illinois, United States of America (http://www.asahq.org/)


12) Centers for Disease Control and Prevention. Surgical Site Infection. Atlanta, Georgia, United States of America (http://www.cdc.gov/hicpac/SSI/table7-8-9-10-SSI.html)


**Tables and Figures**

**Table 1: Surgical site infection definitions[^8][^12]**

<table>
<thead>
<tr>
<th>Criteria</th>
<th></th>
</tr>
</thead>
</table>
| **Superficial SSI** | - Within 30 days.  
- Skin and/or subcutaneous tissues  
- 1 or more of:  
  Pus  
  Bacteria  
  Heat, redness, pain OR localized swelling AND incision reopened by surgeon UNLESS culture negative. |
| **Deep SSI** | - Within 30 days, 1 year if implant is present.  
- Deep soft tissues of the incision  
- 1 or more of:  
  Pus  
  Spontaneous dehiscence of deeper incision OR incision is deliberately opened when patient has fever, localized pain OR tenderness UNLESS culture negative  
  Abscess of other evidence of infection on imaging or histology. |
| **Organ/Space SSI** | - Within 30 days, 1 year if implant is present.  
- Involves any part of the body, excluding the skin incision, fascia, or muscle layers, that is opened or manipulated during the operative procedure  
- 1 or more of |
<table>
<thead>
<tr>
<th>Pus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
</tr>
<tr>
<td>Abscess or other evidence of infection on exam, re-operation, histology or imaging.</td>
</tr>
</tbody>
</table>
Table 2: Number of canine patients and number and rate of SSI for differing procedural type including orthopedic, soft tissue (including cardiothoracic and ophthalmologic) and neurological.

<table>
<thead>
<tr>
<th>Type</th>
<th>Number of patients</th>
<th>SSI rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orthopedic(^a)</td>
<td>310</td>
<td>16 (5.2%)</td>
</tr>
<tr>
<td>Soft tissue(^b)</td>
<td>435</td>
<td>10 (2.3%)</td>
</tr>
<tr>
<td>Neurological(^c)</td>
<td>101</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^a\) Includes bone, tendon and ligament procedures.

\(^b\) Includes all areas of gastrointestinal system such as resection and anastomosis as well as reproductive systems such as ovariectomy, respiratory system such as lung lobectomy, and cardiothoracic system such as thoracotomy.

\(^c\) Includes all spinal surgery such as pediculectomy, hemilaminectomy.
**Table 3:** Number of canine patients and the number rate of SSI for differing categories of surgical procedure including clean, clean-contaminated, contaminated and dirty.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Number of patients</th>
<th>SSI number and rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clean</td>
<td>656</td>
<td>21 (3.2%)</td>
</tr>
<tr>
<td>Clean-contaminated</td>
<td>156</td>
<td>6 (3.8%)</td>
</tr>
<tr>
<td>Contaminated</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td>Dirty</td>
<td>5</td>
<td>1(20%)</td>
</tr>
</tbody>
</table>
**Table 4**: Univariable analysis of factors potentially associated with surgical site infection in dogs (n=846)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number of procedures per risk factor</th>
<th>Number of SSI cases per</th>
<th>Odds Ratio</th>
<th>Confidence Interval</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPLO</td>
<td>Yes: 93 No: 753</td>
<td>7 (7.5%) 18 (23%)</td>
<td>3.123</td>
<td>1.276-7.643</td>
<td>0.013</td>
</tr>
<tr>
<td>Large canine (&gt;20Kg)</td>
<td>Yes: 331 No: 515</td>
<td>N/A</td>
<td>1.134</td>
<td>0.514-2.500</td>
<td>0.755</td>
</tr>
<tr>
<td>Other infection</td>
<td>Yes: 18 No: 828</td>
<td>1(5.6%) 25(3.0%)</td>
<td>1.878</td>
<td>0.240-14.669</td>
<td>0.548</td>
</tr>
<tr>
<td>Active rewarm</td>
<td>Yes: 254 No: 592</td>
<td>6(2.4%) 20(3.4%)</td>
<td>0.686</td>
<td>0.272-1.729</td>
<td>0.424</td>
</tr>
<tr>
<td>ASA I: ASA II</td>
<td>N/A</td>
<td></td>
<td>0.990</td>
<td>0.214-4.595</td>
<td>0.990</td>
</tr>
<tr>
<td>ASA I: ASA III</td>
<td>N/A</td>
<td></td>
<td>0.810</td>
<td>0.141-4.645</td>
<td>0.813</td>
</tr>
<tr>
<td>Emergency</td>
<td>Yes: 198 No: 648</td>
<td>7(3.5%) 19(2.9%)</td>
<td>1.203</td>
<td>0.498-2.907</td>
<td>0.680</td>
</tr>
<tr>
<td>Hypoalbuminemia</td>
<td>Yes: 6 No: 8406</td>
<td>1(16.7%) 25(3.0%)</td>
<td>6.480</td>
<td>0.730-57.531</td>
<td>0.093</td>
</tr>
<tr>
<td>Condition</td>
<td>Yes</td>
<td>No</td>
<td>%</td>
<td>p-value</td>
<td>CI</td>
</tr>
<tr>
<td>------------------------------------------------</td>
<td>-----------</td>
<td>-----------</td>
<td>-------</td>
<td>---------</td>
<td>--------</td>
</tr>
<tr>
<td>Preoperative antimicrobials</td>
<td>802</td>
<td>44</td>
<td>24(3.0%)</td>
<td>0.571</td>
<td>0.130-2.501</td>
</tr>
<tr>
<td>Licking wound</td>
<td>173</td>
<td>673</td>
<td>6(3.5%)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Surgical Site inflammation noted in hospital</td>
<td>17</td>
<td>829</td>
<td>0(0%)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Hypothermia</td>
<td>3</td>
<td>843</td>
<td>0(0%)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Asepsis breach</td>
<td>0</td>
<td>846</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Previous pyodermas</td>
<td>0</td>
<td>846</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>5</td>
<td>841</td>
<td>0(0%)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Active pyoderma</td>
<td>1</td>
<td>845</td>
<td>0(0%)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1</td>
<td>845</td>
<td>0(0%)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Hypotension</td>
<td>3</td>
<td>843</td>
<td>1(33%)</td>
<td>10.827</td>
<td>1.088-</td>
</tr>
<tr>
<td>Implant</td>
<td>255</td>
<td>591</td>
<td>16(6.3%)</td>
<td>3.856</td>
<td>1.723-8.619</td>
</tr>
<tr>
<td>Description</td>
<td>Yes:</td>
<td>No:</td>
<td>%</td>
<td>Value (CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>------</td>
<td>------</td>
<td>-------</td>
<td>------------</td>
<td>---------</td>
</tr>
<tr>
<td>Propofol</td>
<td>568</td>
<td>278</td>
<td>2.6%</td>
<td>0.646</td>
<td>0.280</td>
</tr>
<tr>
<td>ICU stay</td>
<td>121</td>
<td>725</td>
<td>5.0%</td>
<td>1.826</td>
<td>0.206</td>
</tr>
<tr>
<td>Pain Diffusion Catheter</td>
<td>28</td>
<td>816</td>
<td>3.6%</td>
<td>1.124</td>
<td>0.910</td>
</tr>
<tr>
<td>Duration of Anesthesia All</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>1.064</td>
<td>0.852</td>
</tr>
<tr>
<td>Duration of Surgery</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>1.002</td>
<td>0.991</td>
</tr>
<tr>
<td>Incisional Drain</td>
<td>34</td>
<td>812</td>
<td>8.8%</td>
<td>3.399</td>
<td>0.062</td>
</tr>
<tr>
<td>Soft tissue: Orthopedic</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>0.433</td>
<td>0.042</td>
</tr>
<tr>
<td>Neurological: Orthopedic</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Clean-contaminated: clean</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>1.053</td>
<td>0.919</td>
</tr>
<tr>
<td>Dirty: Clean</td>
<td>N/A</td>
<td>N/A</td>
<td>7.9</td>
<td>0.844-73.916</td>
<td>0.070</td>
</tr>
</tbody>
</table>
Table 5: Multivariable analysis of risk factors contributing to surgical site infection in dogs (n=846).

<table>
<thead>
<tr>
<th></th>
<th>SSI Odds ratio</th>
<th>SSI Confidence Interval</th>
<th>SSI P-Value</th>
<th>SSabnorm Odds ratio</th>
<th>SS abnorm Confidence Interval</th>
<th>SSabnorm P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPLO</td>
<td>1.37</td>
<td>0.50-3.79</td>
<td>0.542</td>
<td>1.52</td>
<td>0.61-3.84</td>
<td>0.372</td>
</tr>
<tr>
<td>Implant</td>
<td>5.61</td>
<td>2.01-15.63</td>
<td>0.001</td>
<td>1.46</td>
<td>0.69-3.07</td>
<td>0.323</td>
</tr>
<tr>
<td>Hypotension</td>
<td>27.67</td>
<td>2.14-358.32</td>
<td>0.011</td>
<td>8.64</td>
<td>.074-100.45</td>
<td>0.085</td>
</tr>
<tr>
<td>Class (Clean:dirty)</td>
<td>14.60</td>
<td>1.31-162.15</td>
<td>0.029</td>
<td>4.77</td>
<td>0.51-44.29</td>
<td>0.170</td>
</tr>
<tr>
<td>Class (Clean:Clean contaminated)</td>
<td>2.15</td>
<td>0.66-7.00</td>
<td>0.204</td>
<td>1.30</td>
<td>0.60-2.84</td>
<td>0.504</td>
</tr>
</tbody>
</table>
Figure 1: Incidence of canine and feline SSI cases per month during the study period of September 2010 to July 2011.
Figure 2: Incidence of canine and feline SSI cases per month during the study period of September 2010 to July 2011.
Figure 3: Bacteria isolated from surgical site infections from 19 canine patients. MRSP: methicillin-resistant \textit{Staphylococcus pseudintermedius}, MSSP: methicillin-susceptible \textit{Staphylococcus pseudintermedius}, MRSA: methicillin-resistant \textit{Staphylococcus aureus}. 
CHAPTER 3

In vitro evaluation of DispersinB on methicillin-resistant Staphylococcus pseudintermedius biofilm

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Abstract

Methicillin-resistant Staphylococcus pseudintermedius (MRSP) is an important canine pathogen that has been shown to produce biofilm in vitro. Biofilm production may be an important virulence factor and methods to eliminate biofilm-associated infections are required. An enzyme of Aggregatibacter actinomycetemcomitans, DispersinB, has been shown to degrade the extracellular matrix in the biofilm of a variety of bacteria including Staphylococcus aureus and Staphylococcus epidermidis. The objective of this study was to determine the effect of DispersinB on MRSP biofilm production and eradication in vitro.

A quantitative microtitre plate assay was used to assess the impact of DispersinB on 30 MRSP isolates from dogs. While DispersinB did not have any effect on MRSP growth (P=0.98), it reduced biofilm formation (P=0.0002) and degraded established biofilm (P=0.0001). These data indicate that in vivo study of the effect of this enzyme is indicated to determine if it may be a useful treatment option for MRSP biofilm-associated infections.

Key Words: Staphylococcus pseudintermedius, antimicrobial resistance, biofilm
Introduction

*Staphylococcus pseudintermedius* is a canine-adapted coagulase positive *Staphylococcus* sp that is a leading cause of pyoderma, otitis and surgical site infections in dogs [Eugster et al., 2004; Borjesson et al., 2012; Eckholm et al., 2013;]. Recently, methicillin-resistant *S. pseudintermedius* (MRSP) has emerged as an increasingly important cause of opportunistic infections in dogs, and to a lesser degree cats [Eckholm et al., 2013; Eugster 2004; Nicholson et al. 2002]. The emergence of MRSP has complicated management of many infections because of the extensively resistant nature of many isolates [van Duijkeren et al., 2011].

Surgical site infections (SSIs) are an important problem in veterinary medicine, with infections occurring in approximately 2% - 10% of all small animal surgeries [Eugster, 2004; Nicholson et al. 2002; Fitzpatrick et al., 2010; Beal et al., 2000; Frey et al., 2010]. The pathophysiology of SSIs is complex and multifactorial, and there is increasing concern about the role of bacterial biofilms in both the development of SSIs and as a complicating factor in the treatment of SSIs. [DiCicco et al., 2012.]

Biofilms are complex communities of bacteria that are encased in a self-produced matrix known as extracellular polymeric substance (EPS). This substance is composed of extracellular DNA, proteins, and polysaccharides [Lorite et al., 2013]. Biofilm-associated bacteria have differing properties than their planktonic counterparts, with different growth rates and resistance capabilities to antimicrobials, thereby creating difficulties for elimination of infections [Osland, 2012]. The ability of biofilm-embedded bacteria to evade the immune system and antimicrobial therapy is a potentially important factor in the pathophysiology of SSIs and biofilm formation by *S. pseudintermedius*. 
DispersinB is an enzyme produced by the bacterium *Aggregatibacter actinomycetemcomitans*. DispersinB catalyzes the hydrolysis of poly-N-acetylglucosamine (PNAG), a core component of biofilm, causing interruption in intercellular adhesion [Ramasubbu et al., 2005]. This results in physical detachment of the staphylococcal biofilm from surfaces and facilitates eradication of infection by the immune system and antimicrobial therapy [Ramasubbu et al., 2005]. Degradation of biofilm has also been observed with *Staphylococcus epidermidis* and *Staphylococcus aureus* [Boles, et al., 2011], but there has been no study of *S. pseudintermedius*. Accordingly, the objectives of this study were to assess the impact of DispersinB on MRSP biofilm formation and eradication, as well as MRSP growth.

**Materials and Methods**

A convenience sample of 30 MRSP isolates from dogs was selected for study. Isolates were from dogs with MRSP infection (n=18) or colonization (n=12). Isolates had been previously classified by dru typing [Goering et al., 2008] and consisted of dru types dt5i (n=1), dt6r (n=1) dt7ac (n=2), dt7d (n=1), dt9a (n=13), dt9av (n=1), dt10a (n=1), dt10h (n=3), dt10bm (n=1), dt11a (n=5), dt11av (n=1).

Evaluation of the impact of DispersinB on MRSP growth; To ensure that DispersinB did not have a direct impact on bacterial growth, nine isolates were grown in pure culture on Columbia blood agar for 24h at 35°C. A MacFarland 1.0 suspension of each isolate was prepared and 100 ul was inoculated into 500ul Tryptic Soy Broth (TSB) (control) or 500ul TSB with 20 ug/ml DispersinB. After
24h of aerobic incubation at 35°C, serial 10-fold dilutions (100 ul into 900 ul PBS) were performed and 100 ul was inoculated onto Columbia blood agar. Colonies were counted on plates containing between 20 and 200 distinct colonies. Testing was performed in triplicate.

Evaluation of the impact of DispersinB on MRSP biofilm production;

Thirty isolates were grown in pure culture for 24h at 35°C. MacFarland 1.0 suspensions were created in TSB with 1% glucose. 100ul of suspension was added to a 96-well polystyrene, flat-bottom microtitre plate with either 100 ul of TSB with 1% glucose or 100 ul of TSB with 1% glucose and 40 ug/ml DispersinB (to achieve a final concentration of 20 ug/ml DispersinB). Plates were rotated gently for five minutes, and then incubated aerobically for 18 hours at 35°C. Well contents were discarded and wells were washed 3 times with PBS. The remaining adhered (biofilm-embedded) bacteria were heat fixed for 60 minutes at 60°C. The attached cells were dyed through the addition of approximately 200 ul of 0.1% (w/v) crystal violet for 15 minutes, After washing, plates were air dried at room temperature. Approximately 200 ul of 95% ethyl alcohol was added to re-solubilize the crystal violet and the optical density (OD) at 570 nm (OD$_{570}$) was measured using a microtitre plate reader. The mean of the triplicate measurements was calculated and the mean OD$_{570}$ of the triplicates of the appropriate negative control was subtracted from the mean OD$_{570}$ of the triplicates of the samples.

Eradication of MRSP biofilm by DispersinB;

The 30 isolates used in part 2 were grown as described above. To produce
biofilm, MacFarland 0.5 suspensions were created in TSB with 1% glucose and 200 ul was added to microtitre plate wells. Six wells were prepared for each isolate. Plates were incubated aerobically for 18 hours at 35°C without shaking. Well contents were aspirated and 100 ul of TSB + glucose was then added to three wells per isolate while 100 ul of TSB + glucose + 20 ug/ml of DispersinB was added to the other three wells. Six negative controls, three consisting of un-inoculated TSB with 1% glucose and three consisting of TSB with 1% glucose and 20 ug/ml DispersinB were included on each plate. Plates were then incubated for 4 hours at 35°C. Well contents were then discarded and plates were processed as described above to quantify the amount of remaining biofilm.

Statistical analysis

Data were compared by paired t-test using STATA 10 statistical software. 

Evaluation of the impact of DispersinB on biofilm formation and eradication was performed using a paired t-test. Additionally, analysis was performed using paired t test individually on three different isolate groups, dru cluster 9a (n=13, associated with sequence type (ST) 71), dru cluster 11a (n=9, associated with ST68) and a combination of all other types (n=8)[Weese et al, 2012]. A P value of <0.05 was considered significant.

1- StataCorp LP

Results

Impact of Dispersin B on MRSP growth
There was no significant difference in MRSP growth with (7.23 \pm 0.16 \log_{10} \text{cfu/ml, mean \pm SD}) or without (8.48 \pm 0.11 \log_{10} \text{cfu/ml}) DispersinB (P=0.98) as demonstrated in Figure 1.

Impact of DispersinB on MRSP biofilm formation

There was a significant difference in \( \text{OD}_{570} \) between groups; 0.64 \pm 0.87 (mean \pm SD) for the treatment group versus 0.97 \pm 0.80 for the control group (P=0.0002) as seen in Figure 2. In comparing differing lineage types, a significant difference of the effect of DispersinB was seen in dru cluster 9a (p=0.033) and other types (p=0.012) but not for dru cluster 11a types (p=0.071).

Impact of DispersinB on MRSP biofilm eradication

Overall, there was a significant impact of DispersinB, with an \( \text{OD}_{570} \) of 1.37 \pm 0.82 for the control group vs. 0.67 \pm 0.24 for the treatment group (P=0.001) as seen in Figure 3. In comparing differing lineage types, a significant effect was noted for dru cluster 11a type (p=0.013), dru cluster 9a type (p=0.006) and other types (p=0.034).

Discussion

The combination of antimicrobial resistance and biofilm formation may have a serious impact on the ability to treat MRSP infections, and methods to address biofilm are required. This study indicates that the enzyme DispersinB has the ability to both inhibit biofilm formation and reduce the amount of established biofilm \textit{in vitro}. These properties suggest that DispersinB could be useful for prevention or treatment of biofilms
by MRSP *in vivo*. As expected, there was no impact on MRSP growth, which indicates that the effects on MRSP biofilm are related to influencing establishment and persistence of biofilm, not because of alteration in MRSP growth or viability. This was not surprising because this enzyme targets the PNAG component of biofilm and not the microorganism itself.

DispersinB was effective at reducing, but not eliminating, MRSP biofilm formation *in vitro*. This indicates that while DispersinB may aid in controlling biofilm-mediated surgical site infection, it cannot be expected to completely prevent the establishment of biofilm. This, in addition to the lack of an antibacterial effect of DispersinB, indicates that this enzyme would not be used as a monotherapy but should be evaluated as an adjunctive treatment along with antimicrobial therapy and potentially other options such as surgical intervention. The effect of DispersinB on biofilm eradication was present in both of the main MRSP lineages, as well as a combination of other types, suggesting that there is limited variation in the impact of this enzyme amongst different MRSP lineages. However, while there was an impact of biofilm prevention for the ST71-associated and other groups, there was not a statistically significant difference for ST68-associated isolates. This might indicate some inter-strain variability but considering the results for biofilm eradication, the P value (0.071) and the small sample size, this most likely reflects inadequate statistical power rather than a true biological difference. The presence of an effect on established biofilm was encouraging and the most clinically relevant finding as a compound such as this would more likely be used after infection was established and when biofilm was already present.
As with any study, some limitations exist. Multiple genetic lineages of MRSP were investigated, including 10 isolates consistent with sequence type (ST) 68 and 14 with ST71, the two predominant clones in North America and Europe [Perreten et al., 2010]. However, it is possible that there are strain-specific variations in susceptibility that were not explored here that should be investigated in future research to observe susceptibility in differing geographical locations. Further, while microtitre plate assays are widely used for assessment of biofilms, they cannot replicate the complex in vivo environment; so proper in vivo testing is required. Methicillin susceptible S. pseudintermedius (MSSP) is another pathogen commonly found on dogs, however the literature is lacking on the nature of MSSP and its reactivity with DispersinB and biofilm formation [van Duijkeren et al., 2011]. According to a recent study, the biofilm formation of MSSP and MRSP was the same, however the statistical power may have been low due to low numbers of MSSP, and further study is justified [Singh et al, 2013].

Development of any new treatment should be accompanied by considerations of potential resistance mechanisms. Since DispersinB does not inhibit MRSP itself, exposure to this enzyme would not be expected to create selection pressure for emergence of resistant clones. DispersinB resistance would presumably require profound alteration in biofilm production by MRSP through development of a PNAG-independent mechanism, something that is perhaps unlikely.

**Conclusion**

These data suggest that in vivo study of the efficacy of DispersinB for the treatment and prevention of MRSP biofilm would be beneficial. Approaches such as the use of enzymes to prevent or degrade biofilm may be critical tools to prevent or treat
infections, particularly device-associated infections that can be difficult to treat. If DispersinB can degrade biofilm once it has fully formed in vivo, it may be used in future to treat device-associated infections in a more efficient way as a multimodal approach to eliminate biofilm in conjunction with antimicrobial therapy.

Acknowledgements

This study was not funded by any funding source. DispersinB® was provided by Kane Biotech.
References


Figure 1: Growth by methicillin-resistant *Staphylococcus pseudintermedius* isolates from dogs (n=9)
Figure 2 – Biofilm formation by methicillin-resistant *Staphylococcus pseudintermedius* isolates (including dru type) from dogs (n=30) incubated with (blue) and without (red) DispersinB
Figure 3- Eradication of biofilm formed by methicillin-resistant *Staphylococcus pseudintermedius* isolates (with dru type) (n=30) incubated with (blue) and without (red) DispersinB
CHAPTER 4

General Discussion

Fortunately, surgical site infections (SSIs) in veterinary medicine are relatively uncommon, yet they remain an ever present risk and can result in negative impacts on patient health such as, increased treatment cost, increased need for hospital resources, and overall negative perceptions of the hospital and/or personnel involved. Infection can arise in any patient following any type of surgical procedure, and SSI surveillance is a key component of SSI prevention measures in human medicine. However, a similar intensity of surveillance is lacking in veterinary medicine, particularly studies using standard definitions and active surveillance methods. This makes establishing prevention and surveillance programs challenging. Active surveillance is an effective way of implementing methods and ideas in order to prevent SSIs from forming, as it allows veterinary personnel to assess patient, treatment, and environmental risk factors in order to decrease the risk of SSI development.

The main objectives of this research project included describing incidence rates of SSIs in small animal surgery, describing and comparing procedure specific SSI rates, and identifying patient, treatment, and environmental risk factors. These are important because of the limitations in existing data and the need to properly understand a problem (in this case SSIs) to develop measures to reduce the incidence and impact of that problem.

The SSI rate identified in this study (3.0%) is within the range of that reported elsewhere. However, this study used a novel, approach of active surveillance. Further, it used standard definitions established by the US Centers for Disease Control and
Prevention (CDC) to try to maximize the data quality. This combination is novel in veterinary medicine and allows for collection of the most accurate SSI data. This is important for both understanding the incidence of SSIs (both as a whole and for specific procedures) but also to ensure that appropriate data are used for risk factor analysis. This type of active surveillance is essential for progression of the field of infection control and for implementation of programs to decrease SSI rates. The main limitation of this approach is that this is a very time consuming process that took a year to gain appropriate data. Also, only one institution was used to collect data whereas if more than one institution was used it would provide a more accurate sample of the diversity of patients and practices throughout the region.

Risk factor analysis identified a few important findings. Multivariable analysis illustrated that implants, surgical class and hypotension influenced the risk of developing SSI. The association of implant and SSI was not surprising as this is well established in humans. Therefore further steps such as preoperative antimicrobials, surgical site asepsis, and implant asepsis should be taken with all implant related procedures, so as to minimize the risk of infection. While these are widely applied already, there are other measures that could be considered, including use of implants with lower bioreactivity, implants that are coated with antimicrobials or biocides and other novel approaches to reduce establishment of infection. Taking these precautionary measures could yield to a significant decrease in the incidence of SSIs. Hypotension was an interesting risk factor, as it has not been previously reported as a risk factor for developing SSI in animals. There is a plausible pathophysiological basis to this and this might be an important finding since this is an area that can potentially be addressed. While the results illustrate a
low number of cases of hypotension (3/846), careful attention to the patient’s condition before and during surgery is warranted.

While veterinary personnel are essential in promoting patient health, the patient owner has an equal responsibility in ensuring their pets are recovering appropriately, recognizing signs of infection or other complications, and reporting any surgical site abnormalities to the surgeon or their primary care veterinarian. Since almost all SSIs occur following discharge from hospital, owners are the first line of surveillance and play a critical role in early identification. Communication between owners and veterinarians is a vital component in the prevention of SSI. Therefore, education of owners about the importance of early detection of SSIs and how to evaluate the surgical site is needed. This could be facilitated by providing an informational sheet containing images of normal and abnormal surgical sites, which includes easily recognized signs such as redness, purulent drainage and swelling, and information about what to do in response to surgical site abnormalities.

A concerning result from a surveillance standpoint was the large percentage (35%) of SSIs that were not indicated in the OVCHSC medical record. This occurred because owners elected to take their pets to local veterinarians for treatment. While College of Veterinarians of Ontario regulations dictate that there must be communication between veterinarians in such scenarios, either the primary care veterinarians failed to notify OVCHSC veterinarians or OVCHSC personnel failed to record that information in the medical record.

Surgical site infections can become complicated by bacterial biofilms, which often lead to chronic/persistent infections. This is of particular concern with implant-
associated infections, which were not uncommon in this study. There is a paucity of veterinary data on biofilms, particularly biofilms associated with the main canine SSI pathogen (*S. pseudintermedius*). Therefore, a study was undertaken to evaluate the *in vitro* efficacy of a potential anti-biofilm enzyme. This is an important area of study because when a biofilm forms, the standard antimicrobials used to treat SSIs in veterinary patients may no longer be effective. While prevention of SSIs and subsequent biofilm formation is critical, biofilm-associated SSIs will continue to develop and effective adjunctive measures to eradicate biofilm are needed. This study determined that an enzyme that has been previously shown to have effects against biofilm produced by some other bacteria was effective against *S. pseudintermedius* biofilms. This is important because this is one of the most common bacteria isolated in SSIs in dogs and cats. Results of this study are promising and indicate that in vivo assessment of this enzyme is indicated. Care should be taken, though, to ensure that proper study is done because of the limitations in *in vitro* biofilm assessment and the need to evaluate its effect in the more complex and dynamic in vivo environment.

One of the major limitations in the first part of this study is pet owner recall bias due to their lack of understanding of SSIs and the signs to look for, even though a comprehensive and standardized set of questions was used to identify incision abnormalities and characterize them as SSI or inflammation. Both under-estimation and over-estimation of SSI and inflammation could have occurred based on different peoples’ recalls, observations and interpretations. Direct observation by a veterinarian would have been optimal but is impractical as a routine surveillance tool. These data also only involve one institution and some factors may vary between facilities. Further, a study
such as this cannot assess all potential risk and protective factors. For example, the impact of antimicrobial prophylaxis was virtually impossible to assess because of the relatively homogenous practices. Similarly, some comorbidities (e.g. diabetes mellitus) are well-recognized risk factors in humans but were not adequately assessed here because of the small number of animals with those disorders. Thus, it must be remembered that failing to identify something as a risk factor does not mean that it is not potentially relevant.

Future studies must emphasize to patient owners what to look for when discussing infections, through the use of diagrams or notes for example. This would decrease recall bias, especially in patient owners that were called one year following implant-associated procedures. While recall bias is a concern, it is unlikely that owners would forget important consequences of SSI, since a large percentage of implant-associated SSIs require long term antimicrobials, frequent veterinary evaluation and often removal of the implant. Therefore, recall bias is unlikely to have accounted for significant under-reporting. Also, patients from many different institutions should be sampled, so as to account for differing surgical staff, environments, resources and protocols.

Limitations of the biofilm study largely relate to questions about how well a simple in vitro biofilm model represents the complex in vivo situation, as is mentioned above. The use of explant models or animal models could be considered, along with clinical study. Future research could test the results seen here in vivo by coating an implant device with DispersinB, for example, and observing whether an SSI forms after a month and subsequent year of surgery, as compared to a control.
Overall, the general aims and themes of this study were recognition and active surveillance of SSIs and their associated risk factors, and a method to inhibit or remove bacterial biofilm. This study has provided insight into the epidemiology of SSIs, important information for understanding endemic rates, detection of changes in rates, risk factor analysis and development of potential interventions ultimately to promote patient health, decrease cost to patient owners and improve quality of service in all veterinary institutions.
Appendix A

Script for calling patients for SSI

Hello is Mr. or Mrs. ______ there please?
Hi my name is ____ and I am a graduate student at the University of Guelph. About a month ago, my records show that your pet has had (Procedure), and I just wanted to ask you a couple of questions for my research. Would you have a few minutes to answer a few questions?
My research is looking how the surgical sites of dogs and cats heal after surgery. I have most of the information I need about the procedure with me, but the remaining information I need from you.
Would you mind me asking a couple of questions about your -------?
  1) Did you notice any problems with your pet’s surgical site, such as oozing, redness, tenderness, or pain?

  If YES –
  When did you notice the problems?
  What specifically did you see (Note: may need a list of specific questions to help categorize inflammation/infection/ and infection type)?
  If yes, when was this?
  Which veterinarian did you see?
  Were any treatments prescribed? (If so, what was given?)
  Did your pet have to be admitted to a veterinary clinic (at least overnight) for treatment?
  Was there any need for further surgery?
  Has the problem resolved? (Should be able to be categorize on the spreadsheet cases at 30d as either no problems ever, SS inflammation but resolved, SSI but resolved, SSI ongoing, dead because of SSI, dead because of other problem)
Currently, are there any problems with the surgical site.
  If yes, describe.
  Did you see your pet licking or rubbing the surgical site before you noticed any abnormalities?
  Was your pet wearing an Elizabethan collar after surgery?
  Did you have to take your pet to a vet because of this?

  If NO,
  Did you see your pet licking or rubbing the surgical site before you noticed any abnormalities?
  Was your pet wearing an Elizabethan collar after surgery?
  2) Did your pet experience any other problems after surgery, such as diarrhea, coughing or decreased appetite?
   If yes, what problems were encountered and when did they occur?
Thank you for your time. If you have any further questions about my research, please feel free to call me at ________.